UNIVERSIDADE DE LISBOA
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Insights into the epigenetic and transcriptional response of grapevine to *P. viticola* infection

"Documento Definitivo"

Doutoramento em Biologia

Especialidade em Biologia de Sistemas

Vanessa Sophia Saavedra Azevedo

Tese orientada por:

Professora Doutora Andreia Figueiredo

Doutora Fiammetta Alagna

Professor Doutor Rui Malhó

Documento especialmente elaborado para a obtenção do grau de doutor

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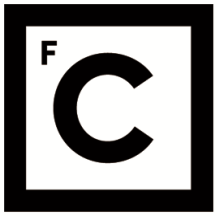

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“The measure of intelligence is the ability to change”
“In the middle of the difficulty lies opportunity”

Albert Einstein

Resumo

O cultivo da videira e todo o processo subjacente desde a obtenção de uvas até à produção de vários derivados transformou a viticultura, de um recurso de sobrevivência, para uma das atividades agro-económicas mais prolíferas mundialmente. *Vitis vinifera* L. é a única espécie utilizada na viticultura europeia e é suscetível a vários patógenos como o *Plasmopara viticola* (Berk. & Curtis) Berl. & De Toni, um oomicete biotrófico obrigatório que causa uma das doenças mais preocupantes na vinha, o míldio da videira.

O míldio da videira atinge todos os tecidos verdes da planta. O seu desenvolvimento é favorecido por temperaturas amenas e humidade elevada. Ao entrar em contato com a água, o *P. viticola* liberta vários zoósporos flagelados que usam o filme de água para migrar até aos estomas onde se fixam e encistam. Desenvolvem então um tubo germinativo que penetra a cavidade subestomática e a partir do qual se desenvolve uma vesícula subestomática. Desta vesícula desenvolve-se uma hifa primária que dá origem a um micélio que prolifera nos espaços intercelulares do parênquima esponjoso e, em última instância, forma haustórios que penetram na parede celular do hospedeiro para obter os nutrientes da planta necessários ao seu desenvolvimento. Os sinais identificadores de míldio na videira são descolorações amareladas ou manchas de óleo nas folhas.

O míldio da videira é atualmente controlado de forma preventiva, através da aplicação sistemática de fungicidas durante a época de cultivo. No entanto, diretrizes recentes da União Europeia apontam para a obrigatoriedade da redução da utilização de pesticidas na viticultura e por consequência a necessidade de encontrar novas medidas de controle de doenças provocadas por diferentes patógenos. Uma alternativa ao uso de fungicidas surge através do foco na resistência/tolerância natural das plantas. Para tal, a comunidade científica tem efetuado vários estudos na área do transcritoma, proteoma e metaboloma de videira de forma a identificar moléculas associadas à imunidade da videira, que possam ser utilizadas em estratégias de controlo da doença (quer em programas de melhoramento, pela utilização de novas técnicas genómicas (edição génica), quer pela identificação de moléculas com potencial biocida).

O sistema de defesa das plantas está intrinsecamente relacionado com o desenvolvimento dos patógenos e numa constante evolução para a adaptação e sobrevivência da planta à colonização. A imunidade das plantas é constituída por um sistema de reconhecimentos inicial de moléculas específicas dos patógenos (*Pathogen Associated Molecular Patterns* (PAMP)) e/ou posteriormente de efetores de patógenos, ambos libertados em diferentes fases de infecção.

Todo este método de proteção é regulado por diferentes processos moleculares e de sinalização. A adaptação do processo imunológico ao nível genómico pode levar um sistema de defesa transitória ou criação de "memórias" de defesa, denominado de priming, para uma ação mais rápida e precisa contra a colonização realizada na planta.

Estudos em plantas modelo demonstraram evidências da ação de outros processos de regulação a auxiliar/modelar o sistema de defesa e imunitário das plantas, a regulação epigenética sem alteração genética da planta. A regulação epigenética nas plantas é composta por processos de metilação do DNA, modificações das histonas, rearranjo da cromatina e ação de complexos de RNA, que induzem e reprimem a expressão génica. A importância da compreensão do papel da regulação epigenética na imunidade das plantas surge como uma abordagem promissora para o desenvolvimento de novas abordagens de controle de doenças, nomeadamente através de *epibreeding*.

Atualmente pouca informação existe acerca da modulação destes mecanismos durante a interação da videira com *P. viticola*. Desta forma, o objetivo deste trabalho é aumentar o conhecimento acerca da regulação epigenética bem como do seu impacto no sistema de defesa da videira na presença do *P. viticola*. Para tal foi realizada uma avaliação fenotípica de híbridos de cruzamento provenientes do programa de melhoramento de uva de mesa do Instituto CREA-VE (Itália). Dois híbridos de cruzamento, N20/020 e N23/018, foram selecionados por apresentarem suscetibilidade contrastante (suscetível e tolerante, respetivamente). Foram efetuadas inoculações controladas em N20/020 e N23/018 e recolhidas amostras às 6 e 24 horas após infeção (hpi). Foi então avaliada a modelação do transcritoma em ambos os genótipos bem como a metilação do DNA. O genótipo N20/020 apresentou uma ativação mais tardia da resposta de defesa em comparação com genótipo N23/018. Foi verificada uma diminuição da metilação global das citosinas C5 nas primeiras horas de inoculação em N23/018, compatível com a ativação da transcrição verificada. Ao nível da transcritómica, genes relacionados com defesa, reconhecimento e sinalização estavam diferencialmente expressos em ambos genótipos analisados. N20/020 apresentou genes relacionados com a defesa e a sinalização predominantemente num período mais tardio contrariamente a N23/018.

Ao analisar a metilação nos dois genótipos observou-se que existiu uma diminuição de metilação no tolerante e visto que a metilação tem como papel de repressão da expressão génica é congruente que os genes do híbrido tolerante não estão a ser reprimidos e assim atuar com maior rapidez contra o patógeno invasor. Ao nível da regulação epigenética, genes relacionados com a metilação do DNA, com a estrutura da cromatina e *small* RNA's estão diferencialmente

expressos durante a interação especificamente no híbrido tolerante numa fase inicial da colonização do *P. viticola*.

Palavras-chave: *Vitis vinifera*; defesa; epigenética; trancritomica; *Plasmopara viticola*

Abstract

Downy mildew, caused by the biotrophic oomycete *Plasmopara viticola*, is one of the most economically significant grapevine diseases worldwide. Current strategies to cope with this threat rely on the massive use of chemical compounds during each cultivation season. The economic costs and negative environmental impact associated with these applications increased the urge to search for alternative strategies for sustainable disease control. Improved knowledge of plant mechanisms able to counteract pathogen infection may allow the development of alternative strategies for plant protection.

To confirm the different response to *Plasmopara viticola* infection evaluated in preliminary studies, a leaf disc assay and an artificial inoculation test on potted plants (*in planta* assay) were performed on *Vitis vinifera* crossing hybrids selected from breeding activities for their contrasting level of susceptibility to the pathogen. Results of both experiments reveal a different susceptibility between bunches and leaves to *P. viticola* infection for most of the genotypes analysed. The genotypes N20/020 and N23/018 were the most contrasting genotypes regarding disease incidence (more susceptible (S) and tolerant (T), respectively) and therefore selected for molecular studies.

Epigenetic regulation, in particular DNA methylation, is emerging as a key factor in the context of plant-pathogen interactions, associated with the expression modulation of defense genes. To improve our understanding of the genetic and epigenetic mechanisms underpinning grapevine response to *P. viticola*, we studied the modulation of both 5-mC methylation and gene expression at 6- and 24-hours post-infection (hpi). Following pathogen infection, we found variations in the 5-mC methylation level and in the gene expression profile. The results indicate a genotype-specific response to pathogen infection. The tolerant genotype (N23/018) at 6 hpi exhibits a lower methylation level compared to the susceptible one (N20/020), and it shows an early modulation (at 6 hpi) of defense and epigenetic-related genes during *P. viticola* infection. These data suggest that the timing of response is an important mechanism to efficiently counteract the pathogen attack.

Keywords: *Vitis vinifera*; defense; epigenetics; trancriptomics; *Plasmopara viticola*

Declaration

Part of the results presented on this dissertation has been published and the chapters/sections are differently structured from the following publication:

- Azevedo, V., Daddiego, L., Cardone, M.F., Perrella, G., Sousa, L., Santos, R. B., Malhó, R., Bergamini, C., Marsico, A. D., Figueiredo, A., Alagna, F. (2022) Transcriptomic and methylation analysis of susceptible and tolerant grape genotypes following *Plasmopara viticola* infection. Physiol Plantarum 2022 Sep;174(5):e13771. doi: 10.1111/ppl.13771

The data that support the findings of this study are openly available in Gene Expression Omnibus repository (GEO) at https://www.ncbi.nlm.nih.gov/geo/, reference number GSE206244.

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Abbreviations, symbols and units

µg	Microgram
µg/ml	microgram per millilitre
♀	Female
♂	Male
Γ	Gamma
ΔCt	Delta Ct
2-ΔΔCt	delta delta Ct
5-mC	5-methylcytosine
6mA	N6-methyladenine
ABA	Abscisic Acid
ACT	Actin
AR	Acquired Resistance
ATP	Adenosine Triphosphate
Bp	Base Pairs
Ct	Threshold Cycle
C-	Negative Control
C+	Positive Control
cDNA	complementary DNA
CpG	Cytosine Base followed by Guanine
CREA-VE	Consiglio per la Ricerca in Agricoltura e l'analisi dell'economia Agraria, Centro di Ricerca Viticoltura ed Enologia
CRC	Chromatin-remoddleing complexes
Ct	Threshold Cycle
DAMP	Self-Damage Associated Molecular Pattern
DEG's	Differentially Expressed Genes
DI	Disease Incidence
DNA	Deoxyribonucleic acid
Dot-Blot	Dot blot
dpi	days post infection
DR-DEG's	Defense Related Differentially Expressed Genes
DS	Disease Severity

EDS	Enhanced Disease Suceptibility-1
ELISA	Enzyme-Linked Immunosorbent Assay
epiQTL	Epigenetic Quantitative Trait Loci
epiRIL	Epigenetic Recombinant Inbred Lines
ER-DEG's	Epigenetic Related Differentially Expressed Genes
ET	Ethylene
ETI	Effector Triggered Immunity
ETS	Effector Triggered Susceptibility
FDR	False Discovery Rate
GA	Gibberellins
GO	Gene Ontology
HAT	Histone Acetyltransferase
HAMP	Herbivores-Associated Molecular Pattern
hc-siRNA	heterochromatic siRNA
HDAC	Histone Deacetylase
HKDM	Histone Lysine Demethylases
HKMT	Histone Lysine Methyltransferases
Hpi	hours post inoculation
HR	Hypersensitive response
IIS	Innate Immune System
INIAV	Instituto Nacional de Investigação Agrária e Veterinária
JA	Jasmonic Acid
KEGG	Kyoto Encyclopaedia of Genes and Genomes
LncRNA	Long non-coding RNA
nat-siRNA	natural antisense siRNAs
MAMP	Microbe-Associated Molecular Pattern
MAPK	Mitogen-Activated Protein Kinase
MeV	Multiple Experiment Viewer
miRNA	micro-RNA
NCBI	National Center for Biotechnology Information
Ng	Nanogram
NLR	nucleotide-binding site–leucine-rich repeat
Nm	Nanometer

ºC	Degrees Celsius
OIV	International Organization of Vine and Wine
P	p-value
PAMP	Pathogen-Associated Molecular Pattern
PCA	Principal Component Analysis
PCD	Programmed Cell Death
PCR	Polymerase Chain Reaction
phsiRNA	phased secondary siRNA
PR	Pathogenesis-related proteins
PRR	Pattern Recognition Receptors
PTGS	Post-Transcriptional Gene Silencing
PTI	PAMP-Triggered Immunity
PTM	Post-Translational Modification
PVP40	Polyvinylpyrrolidone 40
QTL	Quantitative Trait Loci
R genes	Resistance genes
R.G.	Red Globe
R.S.	Regal Seedless
RdDM	RNA-directed DNA methylation
RNA	Ribonucleic acid
RNAi	RNA interference
RP	Rank Products
RPV	Resistance of *P. viticola*
qPCR	quantitative real-time PCR
S	Susceptible
Σ	Sum
SA	Salicylic Acid
SAR	Systemic Acquired Resistance
sRNAs	small RNAs
siRNA	Short interfering RNA
tasiRNA	trans-acting RNA
TE	Transposable Elements
TF	Transcriptional Factors

v/v	volume/volume
vsiRNA	viral siRNA

Chapter I - General Introduction

I.1. The interaction between Plant and Pathogens at the immunity level

Plants are sessile organisms that developed physical, biochemical and immunological strategies to avoid and tolerate stresses, either from environmental conditions (abiotic) or due to pathogens (biotic) (Capriotti et al. 2020). Plant immunity is composed of a multilayered innate immune system (IIS) that either regulates or is regulated by a variety of important components to identify, activate the defense and supress any intruder (Wilkinson et al. 2019). The first step of IIS is the pattern-triggered immunity (PTI) which starts with the recognition of the molecular signals from pathogens by receptor kinases and receptor like-proteins therefore called pattern recognition receptors (PRRs) (Camborde et al. 2019; Judelson and Ah-Fong 2019; Wilkinson et al. 2019; Huang et al. 2022b; Koledenkova et al. 2022). PRR identify several different associated molecular patterns from pathogens (PAMPs), microbes (MAMPs), herbivores (HAMPs) and self-damage (DAMPs) (Mauch-Mani et al. 2017; Wilkinson et al. 2019; Capriotti et al. 2020; Huang et al. 2022b; Koledenkova et al. 2022). A cascade of signalling pathways (Jasmonic Acid (JA) and Salicylic Acid (SA)) is activated and regulates the rest of the defense strategy (Judelson and Ah-Fong 2019; Wilkinson et al. 2019; Koledenkova et al. 2022). For an efficient PTI approach other plant defense mechanisms are involved, including cell wall alteration, metabolites accumulation and the production of specific proteins (pathogenesis-related proteins (PR)) (Wilkinson et al. 2019; Koledenkova et al. 2022). Some pathogens to survive and overcome the host defense system developed specific molecules known as effector proteins, as well as metabolites or small RNAs (sRNAs) that overpower the host PTI (Wilkinson et al. 2019; Koledenkova et al. 2022). To avoid this new adaptive strategy of the pathogens, plants create their own new countermeasure with specific genes being activated, the R genes, generally linked to the nucleotide-binding site–leucine-rich repeat (NLR) receptor proteins and leading to the establishment of Effector triggered immunity (ETI) (Camborde et al. 2019; Wilkinson et al. 2019; Capriotti et al. 2020; Koledenkova et al. 2022; Shilpa et al. 2022). The establishment of ETI can lead to the establishment of the hypersensitive response (HR) where programmed cell death (PCD) occurs (Wilkinson et al. 2019; Capriotti et al. 2020). Jones and Dangl were able to create and propose a representative model of the IIS while pathogen interaction, identified as the Zig-Zag model (Jones and Dangl 2006; Zvereva and Pooggin 2012). With time this model has been improved showcasing the several steps previously referred, as well as the evolution of pathogens to the defense system of the host

creating alternative methods of attack (Figure I. 1) (Jones and Dangl 2006; Zvereva and Pooggin 2012).

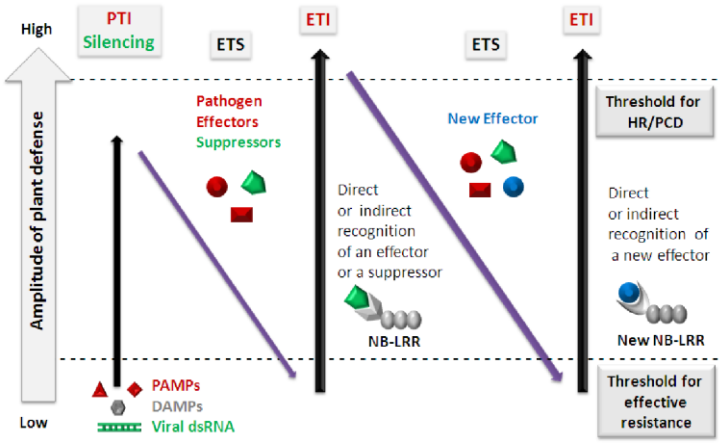


Figure I. 1 - The Plant immunity against pathogen interaction is represented by a zig-zag model. The detection of specific pathogens molecules (PAMPs and DAMPS) by PRRs lead to a PTI phase. Pathogen afterwards release effectors, creating an effecter-triggered susceptibility phase (ETS), which are recognized by receptor proteins (NB-LRR) culminating on the ETI phase. A second ETI phase could occur in the presence of new modified pathogen effectors which through the recognition can create the HR and PCD if the threshold for resistance is surpassed (From (Zvereva and Pooggin 2012)) .

I.2. Plant: *Vitis vinifera* L.

Viticulture is one of the most successful agricultural activities worldwide (Buonassisi et al. 2017). Grapes have been present in the human dietary, agriculture, religion and cultural activities for thousands of years (This et al. 2006; Zhou et al. 2017; Taskesenlioglu et al. 2022).

The process of domestication of this crop from its wildtype progenitor was essentially focused on the berry, bunch and seed morphology, sugar content, plant sex modification and/or mutations as vegetative dissemination occurs (This et al. 2006; Zhou et al. 2017; Magris et al. 2021; Margaryan et al. 2021). The origin and time frame of grapevine domestication by humans is still a topic of debate and research (Zhou et al. 2017). Furthermore, historically multiple key domestication selection events have been reported throughout decades taking in account human needs (Zdunić et al. 2013). Archaeological analysis showed that the relationship between domestication process and wine production was reported as far as approximately 6 000 to 8 000 years ago (Myles et al. 2011; Zdunić et al. 2013; Zhou et al. 2017; Grassi and De Lorenzis 2021). At this time period, southern Caucasus (Near East) has been pinpointed to be the presumably original focal point for grapevine domestication because of the observed archaeological artefacts related to wine productions apparatus and seed analysis performed in different countries of that region, although it is still unclear what preceded this activity (This et

al. 2006; Zdunić et al. 2013; Zhou et al. 2017; Grassi and De Lorenzis 2021; Magris et al. 2021; Margaryan et al. 2021; Taskesenlioglu et al. 2022). Grapevine expansion for human consumption further migrated to the Middle East area known as the fertile crescent (approximately 5 000 years ago) and subsequently to western Europe specially the Mediterranean (approximately 2 800 years ago) (This et al. 2006; Myles et al. 2011; Zhou et al. 2017; Margaryan et al. 2021). Roman civilization followed by the Catholic Church were the main promoters for the dissemination of *Vitis vinifera* seeds since trade commerce activities and/or religion expansion played an important part on plant dissemination throughout these regions (This et al. 2006).

With this worldwide distribution, an ecogeographical classification on the grapevines was attributed (Grassi and De Lorenzis 2021; Magris et al. 2021). From Prole orientalis (Central Asia and Caspian Sea), Prole occidentalis (central and western Europe) to Prole pontica (eastern Europe, Georgia and Turkey), these attributions are in accordance with grapes ancestral, geographical and phenotypic similarities (Zdunić et al. 2013; Grassi and De Lorenzis 2021; Magris et al. 2021). Overall, the chronology of grapevine domestication and colonization is still difficult to grasp because of the wild vegetative material possibly scattered across different continents (Europe, Africa and Asia) (Grassi and De Lorenzis 2021; Laurell et al. 2021; Magris et al. 2021; Sivan et al. 2021). However, new cultivated grapevines allowed to create small clusters of genetic diversity (Grassi and De Lorenzis 2021; Magris et al. 2021; Sivan et al. 2021) as observed by Bacilieri and colleagues in the Iberia and Italian Peninsula (Bacilieri et al. 2013). In general, the segregation occurred throughout decades which allowed the categorization in the main subspecies *Vitis vinifera L.* ssp. *sylvestris* (C.C. Gmel.) Hegi (known as ancestral/wild grapevines) and *Vitis vinifera L.* ssp. *vinifera* (or *sativa*) (Figure I. 2) (This et al. 2006; Zhou et al. 2017; Magris et al. 2021; Margaryan et al. 2021).

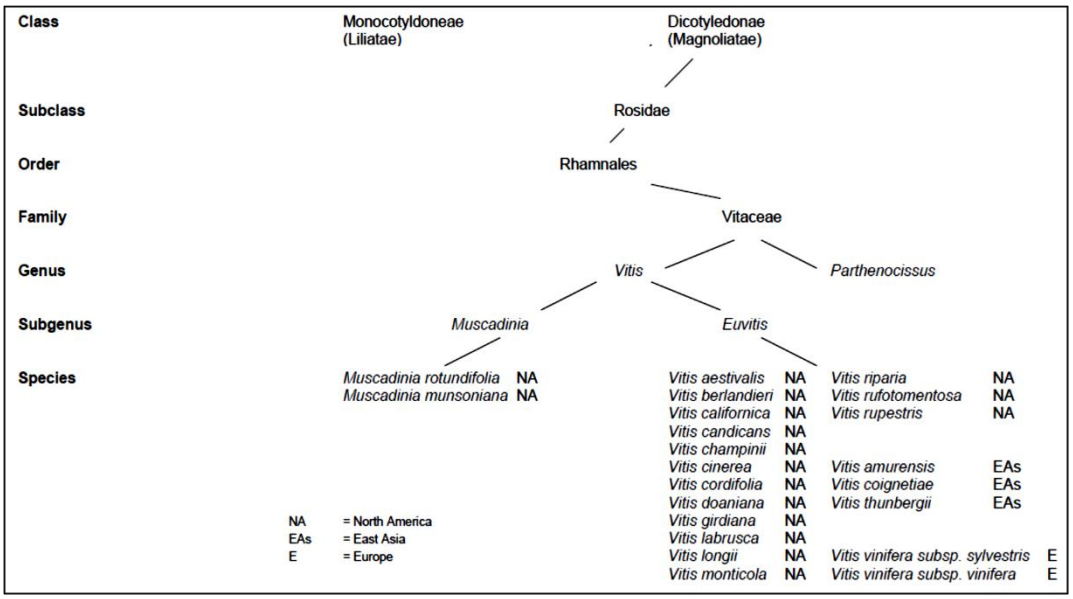


Figure I. 2 - Taxonomic tree of the *Vitis vinifera L.* (adapted from Maul et al. (2022): Vitis International Variety Catalogue - https://www.vivc.de/index.php?r=aboutvivc%2Ftaxonomictree (accessed August 2022))

V. vinifera L. spp *vinifera* is the main species used in viticulture, and the human consumption product is the main cultivar classifier (This et al. 2006; Zhou et al. 2017). Also, there is a wide variety of grapevine cultivars nowadays because of the mutation created by either natural or human intent (This et al. 2006; Myles et al. 2011). This molecular alteration led to a reduced genetic diversity because of the needs of improvement/selection of berries for a more globalized market (This et al. 2006; Myles et al. 2011). Zhou and colleagues have reported that approximately 2 500 years ago a separation between wine and table grape domestication could have occurred (Zhou et al. 2017). Throughout the years it has become more evident the phenotypic and genotypic disparity between table and wine cultivars (This et al. 2006; Magris et al. 2021).

Nowadays, the most commonly used *Vitis vinifera* cultivars for wine and table grape production are heavily affected by pests and diseases that influence plants and its derived products (Micheloni 2017). Farmers apply extensive preventive fungicide treatments to control downy mildew and other diseases, leading to important concerns associated with pesticide residues and environmental and health risks (Micheloni 2017). Given that, there is a high European Union demand for the reduction of pesticide and fungicide use in viticulture (Micheloni 2017). Thus, in the past few years, producers and researchers have tried to find alternative solutions to control these biotic stresses (Espinas et al. 2016). It has been reported the introgression hybrids construction with the use of resistant varieties (North American) backcrossing with susceptible varieties (Eurasian) for a genetic transfer essentially important in breeding programs (Vezzulli

et al. 2018; Bove and Rossi 2020; Atak and Şen 2021). Interestingly, Morales-Cruz and authors have analysed the North American wild grapes where they report that introgression between grapes showed some intersection on the geographical area and climate-pathogen dependency impacting the transfer of adaptability traits against microbe stress (Morales-Cruz et al. 2021).

I.3. Pathogen: *Plasmopara viticola*

Plasmopara viticola (Berk. et Curt.) Berl. & de Toni is a biotrophic oomycete which affects grapevines causing the disease known as downy mildew (Brilli et al. 2018; Santos and Figueiredo 2021; Peng et al. 2024). This pathogen affects all green parts of the plant (Santos and Figueiredo 2021; Peng et al. 2024), reducing the fruit quality and leading to severe economic losses (Santos and Figueiredo 2021). *P. viticola* is original from the eastern US and colonized the European continent through cuttings introduced in France to fight the *Phylloxera* pandemics that destroyed entire vineyards (Clippinger et al. 2024).

After its introduction, *P. viticola* spread affecting all European vineyards (Armijo et al. 2016; Fontaine et al. 2021; Koledenkova et al. 2022; Clippinger et al. 2024; Peng et al. 2024). The life of *P. viticola* presents two different reproduction stages (Figure I. 3) (Buonassisi et al. 2017; Koledenkova et al. 2022; Peng et al. 2024). During spring and summer, upon contact with water due to rain or increased air moisture and warm temperatures, *P. viticola* sporangia release numerous flagellate zoospores that swim within the water film on the lower surface of the leaf. When the zoospores encounter the stomata, they encyst and germinate which allow to develop a germ tube that penetrates the substomatal cavity. A vesicle is formed, and a primary hypha emerges developing a mycelium that spreads within the leaf tissue, extending mainly into the intercellular spaces of the spongy parenchyma. An haustoria is then formed, penetrating the plant cell wall and invaginating the plasma membrane (Armijo et al. 2016; Buonassisi et al. 2017; Santos and Figueiredo 2021; Koledenkova et al. 2022; Clippinger et al. 2024). After 5 to 10 days sporangiophores emerge from the stomata (Figure I. 3). New zoospores can be released leading to the establishment of secondary infections (Armijo et al. 2016; Buonassisi et al. 2017; Santos and Figueiredo 2021; Koledenkova et al. 2022; Clippinger et al. 2024). For the sexual reproduction stage in late summer, oogonia and antheridia are formed by meiosis. The oogonia is fertilized by the antheridia inside the leaf tissue, resulting in an oospore (Armijo et al. 2016; Buonassisi et al. 2017; Santos and Figueiredo 2021; Koledenkova et al. 2022; Clippinger et al.

2024). These oospores are then released from decaying leaves on the vineyard floor and will remain within the decomposing foliage during the winter.

The colonization of grapevine by *P. viticola* leads to phenotypic symptoms on the leaves, green-yellow lesions known as oil-spots (Nascimento et al. 2019; Santos and Figueiredo 2021; Clippinger et al. 2024). Moreover, the phenotypic manifestations comes from a cascade of defense responses that are triggered from deposition or synthesis of lignin, callose, chitinases glucanases, peroxidases and stilbenes which further led to the process of lack of tissue development (Koledenkova et al. 2022). Overall, the life cycle of *P. viticola* with the optimal conditions ranges from 5 to 10 days (Koledenkova et al. 2022).

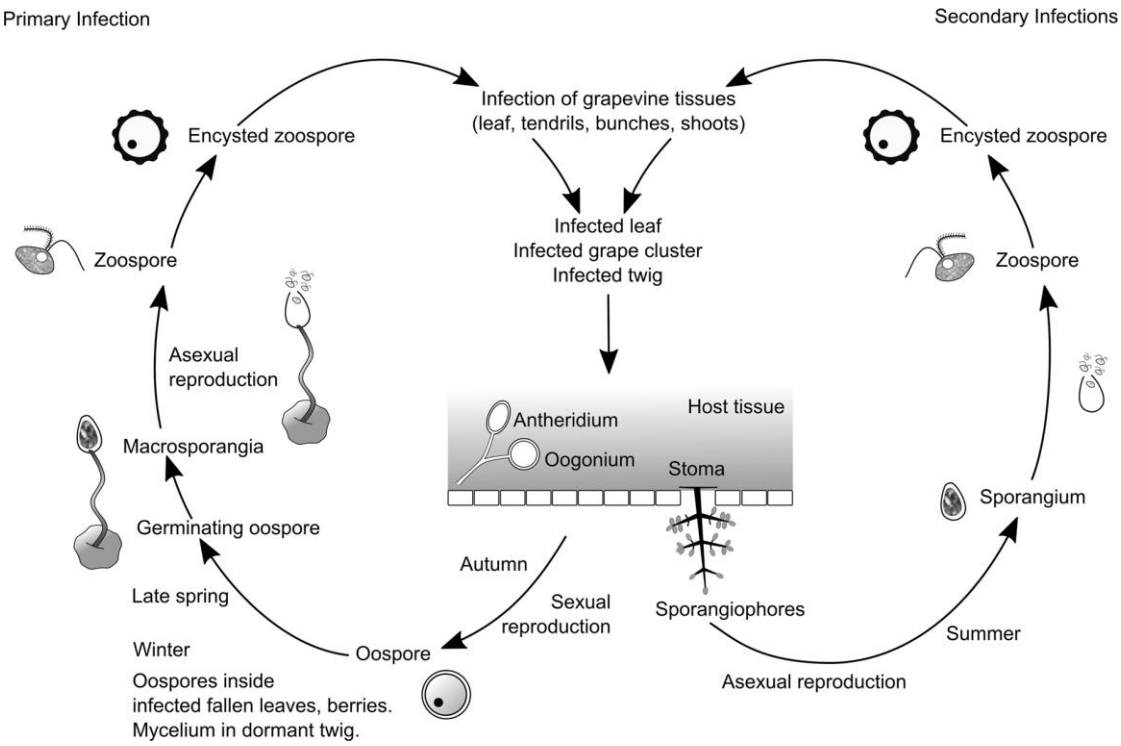


Figure I. 3 - Different stages of *Plasmopara viticola* life, from sexual to asexual reproduction and different infections steps (primary and secondary) occur in *V. vinifera.* The primary stage of infection initiates when oospores germinate to form macrosporangium. This structure creates zoospores that are released when environmental conditions are favourable (rain and wind). Grapevines leaves are structures which zoospores swim and encyst. Within the leave tissue, in the stomata, sporangiophores form if the environmental conditions. Afterwards there is the release of sporangium which liberates zoospores that similarly with the primary infection conditions, can again infect grapevines. At this stage the oospores are form through the sexual reproduction between antheridium and oogonium (From (Buonassisi et al. 2017)).

The *P. viticola* genome was recently sequenced (2016 (Dussert et al. 2016) and 2017 (Yin et al. 2017)) and our knowledge on *P. viticola* pathogenicity, effectors and virulence has increased (recently reviewed in (Gouveia et al. 2024)). Several different effectors have been

identified with the role on plant immunity as the RxLR effector family (Santos and Figueiredo 2021; Peng et al. 2024). A study performed by Yin and colleagues has identified 100 *P. viticola* RxLR (PvRxLR) effectors in the pathogen genome (Yin et al. 2017). Some examples of RxLR functions are the RxLR111 that was described as a manipulator of plant susceptibility through host WRKY transcription factor 40 (VvWRKY40) modulation and RxLR76 influences the pathogen pathogenicity (Ma et al. 2021). RxLR50253 effector was also shown to affect the *V vinifera* immunity through its repression via *V. piasezkii binding partner 1 of accelerated cell death 11* (VpBPA1) stabilization (Yin et al. 2022).

I.4. Transcriptomics in *V. vinifera*-*P. viticola* interaction

Grapevine plants affected by *P. viticola* present transcriptional reprograming correlated with the phenotypic plasticity and the defense machinery (Polesani et al. 2010; Malacarne et al. 2011; Buonassisi et al. 2017; Faize and Faize 2018; Koledenkova et al. 2022). Several different studies have shown that genes involved in photosynthesis-related processes, genes encoding pathogenesis related protein, transcription factors, metabolic processes (viniferins, lignin, anthocyanins, flavonoid and phenolic compounds) and specific enzymes (Malacarne et al. 2011; Perazzolli et al. 2012; Li et al. 2015b; Fröbel et al. 2019; Liu et al. 2020b) are modulated upon pathogen challenge. The phytohormone signalling pathway has been observed to be an intricate part of the *V. vinifera- P. viticola* interaction specially regarding jasmonic acid (JA) and salicylic acid (SA) pathways (Guerreiro et al. 2016; Rossarolla et al. 2021). Guerreiro and colleagues observed that at an initial timeframe after *V. vinifera* infection by *P. viticola* both phytohormones signalling pathways were activated in an *Resistance of P. viticola* 3 (RPV3) tolerant genotype (Guerreiro et al. 2016). In agreement with this study, a research performed by Rossarolla and colleagues showcased that both phytohormone pathways were activated upon infection (Rossarolla et al. 2021). Also, genes encoding the stilbenes biosynthesis pathway were shown to be activated, particularly in tolerant genotypes (Rossarolla et al. 2021). Liu and colleagues also shown that secondary metabolism related genes were upregulated in a resistant genotype in the presence of the pathogen (Liu et al. 2020a). Also, the hormonal signalling pathways related genes appear to have a higher expression in the resistant cultivars specially related with the JA, SA and ABA after *P. viticola* inoculation (Chitarrini et al. 2020; Liu et al. 2020b). Transcriptional factors such as WRKY and MYB family were shown to be highly

expressed in the resistant genotypes upon inoculation (Guerreiro et al. 2016; Fröbel et al. 2019; Chitarrini et al. 2020), genes from both families were then identified (WRKY75 and MYB4) in the quantitative trait loci (QTL) identified has *Resistance of P. viticola* 10 (RPV10) (Fröbel et al. 2019). The pathogenesis related (PR) genes, particularly *pathogenesis related protein 10* (PR10) were pinpointed as markers of grapevine response to *P. viticola* (Fröbel et al. 2019).

Furthermore, Gong and authors have also observed that ROS, lipids, flavonoids and signalling pathways (specially MAPK and calcium) seemingly are part of the triggering effect of the resistant response after *P. viticola* infection (Gong et al. 2022). Also, some receptors (R genes) could be identified for their role of recognition of effectors as well as the impact on the defense signalling pathways and hypersensitivity response (Gong et al. 2022).

Moreover, callose has been reported to be part of the *V, vinifera* defense response against *P. viticola*. Yu and colleagues observed that genes from the callose synthase family (*CalS1* and *CalS10*) play a role in the defense response after this pathogen interaction as well as possible regulatory interaction with plant hormones ABA and gibberellin (GA) (Yu et al. 2015).

I.5. Epigenetics, an emergent player in Plant defense

I.5.1. An overview of the epigenetic machinery

Nowadays the concept of Epigenetics world widely used entails the study of molecular mechanisms with reversable capabilities that regulates gene expression with possible heritability impact without altering the DNA sequence (Kumar et al. 2017; Topart et al. 2020; Ospelt 2022; Tompkins 2022). Additionally, the organisms environment surrounding has been reported to impact the epigenetic machinery however the phenotypic effect as well as a possible transgenerational inheritance correlated with epigenetics is still unclear (Kumar et al. 2017; Topart et al. 2020; Ospelt 2022; Tompkins 2022).

Different epigenetic mechanisms and pathways contribute to epigenetic state stability and modification in plant cells and tissues in response to the stimuli. The most well-known epigenetic marks are DNA modification, histone post-translational modifications, RNA based modifications and chromatin rearrangement with influence of chromatin remodelling complexes (Figure I.4) (Ueda and Seki 2020; Kakoulidou et al. 2021; Chachar et al. 2022). Specific enzymes with different functions act on these specific marks being classified according

to the modification imposed: specific molecules added which releases catalysis/signs (writers), interpreters/recognizers of the chemical signals (readers) and removers of the chemical modifiers (erasers), that further allows gene expression modification without sequence manipulation as well as triggering important pathways (Figure I. 4) (Saldívar-González et al. 2018; Crispo et al. 2019; Topart et al. 2020; Chachar et al. 2022). These modifications and associated enzymes enables epigenetic marks to have a reversible capability that allows to influence either genomic and metabolic developments (Crispo et al. 2019; Ueda and Seki 2020)

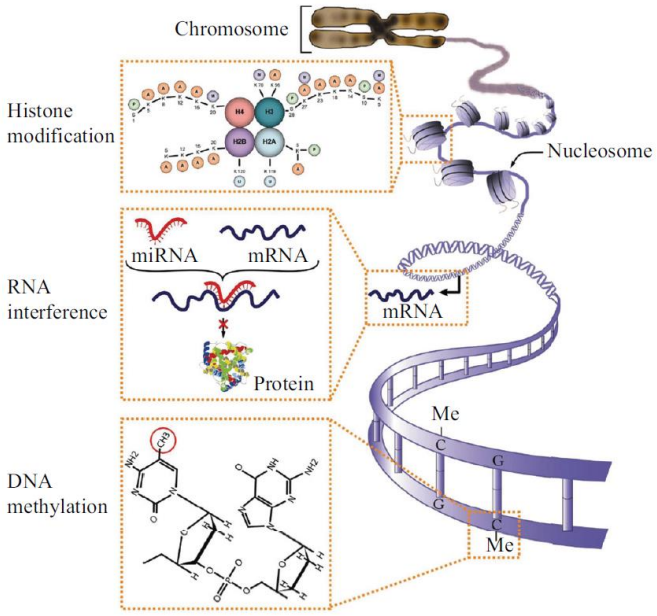


Figure I. 4 - Representation of the epigenetic machinery (Adapted from: (Kim et al. 2011))

DNA methylation

DNA methylation of the 5-methylcytosine (5-mC) is known to be an epigenetic repressor of gene expression (promoter and transposable elements) (Kumar et al. 2018; Kumar and Mohapatra 2021; Chachar et al. 2022; Guarino et al. 2022; Shaikh et al. 2022).The DNA methylation process is only possible though key enzyme known as DNA methyltransferases. Within DNA methyltransferases in plants there are: METHYLTRANSFERASE 1 (MET1), CHROMOMETHYLTRANSFERASE2 (CMT2) and CMT3 (Figure I.5) (Brocklehurst et al. 2018; Sudan et al. 2018; Zhang et al. 2018; Shin et al. 2022). MET1 can act before and after DNA replication through the methylation of the hemi-methylated CG dinucleotides and the basic cytosines in new strands, respectively (Figure I.5) (Brocklehurst et al. 2018; Li et al. 2018; Kumar and Mohapatra 2021; Samantara et al. 2021; Shin et al. 2022). Also, MET1 is important for DNA methylation conservation on the non-symmetric sequence through the interaction with

SU(VAR)3-9 homolog 2 (SUVH2) and *SU(VAR)3-9* homolog 9 (SUVH9) that allows the involvement of the RNA-directed DNA methylation (RdDM) pathway (Zhang et al. 2018).

Also in plants, CMT2 and CMT3 were identified to be associated to CHG and CHH methylation (Figure I.5) (Deleris et al. 2016; Neto et al. 2016; Niederhuth et al. 2016; Hoang et al. 2018; Zhang et al. 2018; Raju et al. 2019; Shin et al. 2022). CMT3 is part of CHG methylation maintenance with interactive capabilities with specific histone methylation modification such as H3K9me1, H3K9me2 and H3K9me3 through CMTs specific binding structures (bromo-adjacent homology (BAH) and CHRomatin Organization MOdifier (CHROMO) domains) (Figure I.5) (Ashapkin et al. 2016, 2020; Niederhuth et al. 2016; Lanciano and Mirouze 2017; Zhang et al. 2018; Raju et al. 2019; Shaikh et al. 2022). Furthermore, CMT2 is mostly known to maintain the methylation status on the CHH context and in lower capacity on the CHG context (Figure I. 5) (Niederhuth et al. 2016; Li et al. 2018; Zhang et al. 2018; Wendte et al. 2019; Kumar and Mohapatra 2021; Shaikh et al. 2022).

In addition to the reported enzymes, DRM family has been observed to be key players in the *de novo* DNA methylation as well as have a role in the maintenance of the methyl group in the non-symmetric CHH methylation (Figure I. 5). Overall, throughout RdDM pathway the plant-specific RNA Pol V branch mediates this pathway whereas the most known enzyme from this family, DRM2 which plays a role in catalysation of this pathway (Li et al. 2018; Sudan et al. 2018; Zhang et al. 2018; Kumar and Mohapatra 2021; Shaikh et al. 2022; Shin et al. 2022).

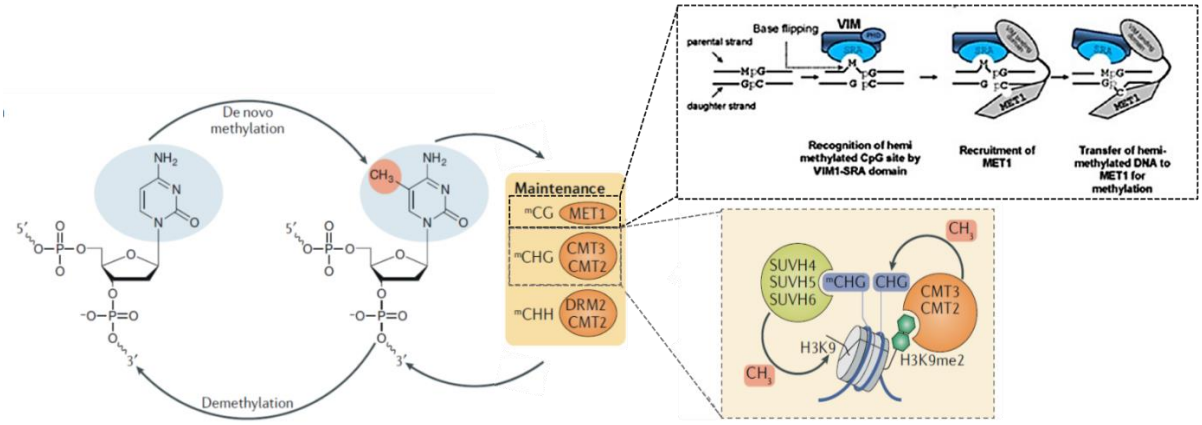


Figure I. 5 - Schematic representation of the maintenance DNA methylation process on the CG and CHG site with the recruitment of the DNA methyltransferase MET1 and CMT3/CMT2 respectively. Also, DRM2 is a DNA methyltransferase that besides playing role in DNA maintenance also is a key player in the *de novo* DNA methylation (Adapted from (Yao et al. 2012; Zhang et al. 2018)

For an equilibrium of DNA methylation, there are also enzymes that remove the methyl groups from DNA. DNA demethylases harbour DEMETER (DME), Repressor of Silencing1 (ROS1),

and DME-like 2 and 3 (DML2, DML3) (Kumar and Mohapatra 2021; Samantara et al. 2021; Guarino et al. 2022; Gui et al. 2022; Huang et al. 2022b; Parker et al. 2022; Shaikh et al. 2022).

Histone modification

The nucleosomes core is composed essentially by octomer histones (H2A, H2B, H3 and H4) and their N-terminal tails (Annacondia et al. 2018; Yan et al. 2019; Samantara et al. 2021; Guarino et al. 2022). Besides the referred histones, there is also Histone 1 (H1) that is not a direct component of the nucleosome core but is a linker which allows the separation between the nucleosomes and stabilizes the chromatin (Ageeva-Kieferle et al. 2019; Guarino et al. 2022).

The histone modification landscape varies depending upon the group that binds to the N-terminal tail of the histones which can go from methyl to acetyl or other groups (Samantara et al. 2021; Shin et al. 2022). Therefore, the histone modification can vary from methylation, acetylation, ubiquitination, phosphorylation and SUMOylation, however the most studied is the histone methylation and acetylation (Annacondia et al. 2018; Shin et al. 2022; Tao et al. 2023). Nevertheless, these modifications are only possible with the contribute of specific proteins that can write (histone methyltransferase and histone acetyltransferase) or erase (histone demethylases and histone deacetylases) these modifications (Annacondia et al. 2018; Yan et al. 2019; Shin et al. 2022). The histone composition and modifications are important for the transcription machinery, chromatin conformation (open or closed) and gene regulation (Figure I. 6) (Annacondia et al. 2018; Samantara et al. 2021; Shin et al. 2022).

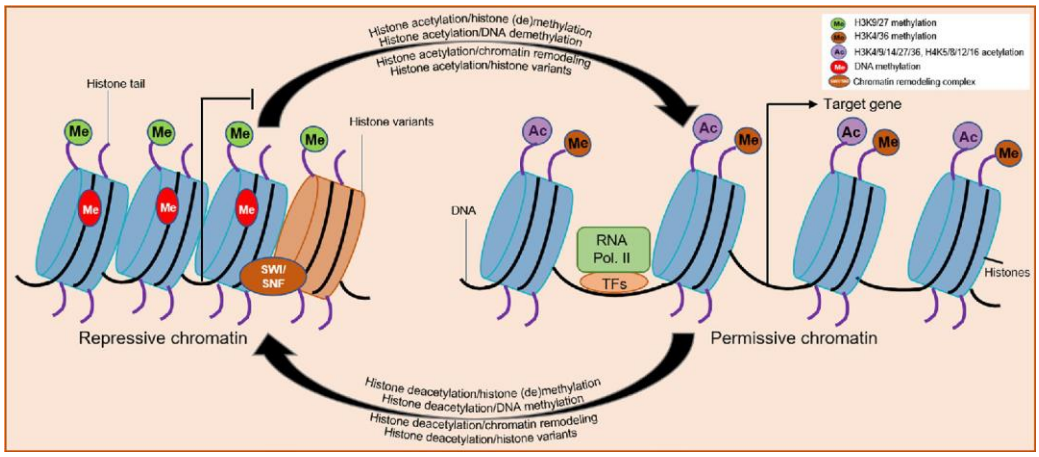


Figure I. 6 - Histone modification landscape and the effect on the chromatin structure. (From (Kumar et al. 2021))

Histone methylation can occur through the addition of one or more methyl groups (mono-, di-, or trimethylation) on the lysine or arginine locations (Ramirez-Prado et al. 2018b; Zarreen and Chakraborty 2020; Guarino et al. 2022; Shin et al. 2022). There are histone methylation events that can be associated with either repression (H3K27me2, H3K9me, H3K27me, and H4K20me) or activation (H3K9me2, H3K4me, H3K36me and H3K79me) of gene expression (Ramirez-Prado et al. 2018b; Yan et al. 2019; Zarreen and Chakraborty 2020; Shin et al. 2022). Whereas different enzymes have been registered has catalysers (KRYPTONITE (KYP) also known as SU(VAR)3–9 homolog (SUVH4), SUVH5 and SUVH6) therefore recognised has histone lysine methyltransferases (HKMTs) (Samantara et al. 2021; Shin et al. 2022). Also, other domains have been identified as recognizers of histone lysine methylation such as PHD, chromo, WD40, Tudor, double/tandem Tudor, MBT, Ankyrin Repeats, zf-CW, and PWWP (Zarreen and Chakraborty 2020). On the other hand, removal enzymes of the histone methylation are known as histone lysine demethylases such as lysine-specific demethylase 1 (KDM1/LSD1) homologues, Jumonji C domain-containing (JMJC) and PAD4/PADI4 (Zarreen and Chakraborty 2020; Samantara et al. 2021; Shin et al. 2022).

The histone acetylation, occurs through adding a acetyl group to the lysine residue in the histone tail which allows chromatin to be relaxed (Hu et al. 2019; Samantara et al. 2021; Guarino et al. 2022; Shin et al. 2022). Oppositely chromatin condensation can occur through the histone deacetylation process (Guarino et al. 2022). Histone acetylation marks impacts the transcriptional process (active gene expression) and with regulatory influence in plant development as well as environmental or pathogen impact (Samantara et al. 2021; Guarino et al. 2022; Shin et al. 2022). The reported histone acetylation landscape in plants has been identified at the H3K9, H3K14, H3K18, H3K23, H3K27, H4K5, H4K8, H4K12, H4K16 and H4K20 sites (Samantara et al. 2021; Guarino et al. 2022; Shin et al. 2022). The presence of specific enzymes that adds (histone acetyltransferase - HAT) or removes (histone deacetylase - HDAC) the acetyl group allows to antagonistically regulate the transcriptional process (Samantara et al. 2021; Guarino et al. 2022; Shin et al. 2022). In plants, HAT were divided in different classes: GCN5-related N terminal-acetyltransferase (GNAT), MOZ/Ybf2/Sas2/Tip60 (MYST), CREB-binding protein (CBP/p300), to TFII250-TATA-binding protein-associated factor 1(TAF1) (Hu et al. 2019; Kumar et al. 2021; Samantara et al. 2021; Wang et al. 2021; Guarino et al. 2022; Shin et al. 2022). Histone deacetylases (HDAC) families/classes have been also classified from HD2 family, Reduced Potassium Dependency 3 (RPD3)/HDA1 family to Sir2 family) (Hu et al. 2019; Kumar et al. 2021; Samantara et al. 2021; Shin et al. 2022).

Chromatin remodelers

The chromatin modifications are also important epigenetic regulators that are comprised by: chromatin remodeler complexes (CRC), enzymes that affect the modification of histone and DNA, and epigenetic related RNA (Kim 2021). Chromatin remodelling complexes (CRC) can occur through the presence of chromatin structural components forming specific complexes that are preponderant for the chromatin modification at the histone and its variants level with the help of ATP hydrolysis (Figure I. 7) (Bhadouriya et al. 2021; Tao et al. 2023).

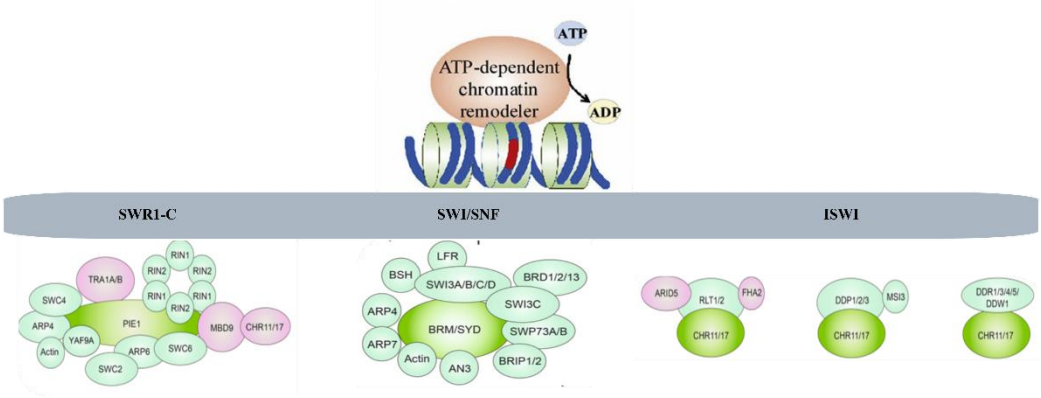


Figure I. 7 - Chromatin remodeler complexes with its subunits. (Adapted from (Chen et al. 2017; Shang and He 2022)

The CRCs vary in four class families which have been identified present in several different stress signals in plants. The CRC is encoded in SWItch/Sucrose Non-Fermentable (SWI/SNF), IMITATION SWITCH (ISWI), chromodomain and helicase-like domain (CHD) and Inositol Requiring 80 (INO80)/SWR1-C complexes that regulate specific regions of protein-coding genes (Bhadouriya et al. 2021; Kim 2021; Parker et al. 2022; Tao et al. 2023). Throughout decades it has been observed that CRC is involved in plants development, in the plant immunity system, epigenetics and defense against biotic and abiotic stresses (Bhadouriya et al. 2021; Kim 2021; Parker et al. 2022).

RNA mediated epigenetic regulation

Non-coding RNAs are an essential part of the epigenetic regulation. The most relevant are micro RNAs (miRNA), short interference RNAs (siRNA) and long ncRNA (lncRNA). Micro RNAs are transcribed from *MIR* genes and are key players in the post-transcriptional gene silencing (PTGS) pathway (Dong et al. 2022). On the other hand, siRNAs can originate from both endogenous and exogenous sources. These can be further grouped into phased secondary siRNA (phasiRNA) and their subtype trans-acting RNA (tasiRNA), natural antisense siRNAs

(nat-siRNA) and heterochromatic siRNA (hc-siRNA), are responsible for not only PTGS but also serve as guides for RdDM (Liu et al. 2020c; Sanan-Mishra et al. 2021; Waheed et al. 2021).

I.5.2. Epigenetic regulation in the context of plant-pathogen interactions

After the 1990s, epigenetic regulation was associated to plant defense and priming "memory" (Annacondia et al. 2018; Parker et al. 2022). The links between plant immunity, epigenome and disease resistance are being analysed and key players from the *de novo* DNA methylation (RdDM), DNA methylation, chromatin and histone modifications (Huang et al. 2022b; Parker et al. 2022; Shilpa et al. 2022) were pinpointed.

DNA methylation was shown to be associated to susceptibility or tolerance to pathogens as hypo or hypermethylation stages lead to transcription activation/repression (Annacondia et al. 2018; Parker et al. 2022; Tao et al. 2023). The impact of DNA methylation modification after pathogen attack can affect promoters, gene bodies, and TEs which leads to the need to increase the understanding of the correlation between the regulation of gene expression and host defense (Tirnaz et al. 2022). This has been shown in several plants as Arabidopsis, rice, tobacco, tomato, maize, grapevine, wheat, cucumber, Nicotiana and others where DNA methylation modulation occurs after pathogen infection (Castellano et al. 2016; Huang et al. 2022b; Pereira et al. 2022; Shilpa et al. 2022; Tirnaz et al. 2022).

In plant-bacteria interactions *Pseudomonas syringae* pv. *tomato* strain DC3000 (*Pto* DC3000) (a hemibiotrophic bactéria) has been one of the most reported bacteria to be involved with a diverse number of epigenetic mechanisms (DNA methylation, DNA demethylation and RdDM pathway) that afterwards impact the immune defense response of plants (Annacondia et al. 2018; Huang et al. 2022b). Studies reported that the *Pst DC3000* impacts host DNA methylation/demethylation machinery (through ROS1 manipulation) influencing the plant susceptibility/resistance (Dowen et al. 2012; Yu et al. 2013; Hewezi et al. 2018; Alonso et al. 2019; Ashapkin et al. 2020; Cambiagno et al. 2021; Ramos-Cruz et al. 2021; Huang and Jin 2022; Huang et al. 2022b; Parker et al. 2022; Saeed et al. 2022; Tao et al. 2023). Consequently, TE regions with altered DNA methylation status (hypomethylation) are linked with biotic stress effect on the immune system of the host (triggered) which leads to repression of gene transcription of other epigenetic components (from RdDM pathway) and the DNA demethylation (ROS1) of TE is important for defense activation (Dowen et al. 2012; Yu et al.

2013; Hewezi et al. 2018; Alonso et al. 2019; Ashapkin et al. 2020; Zhi and Chang 2021; Parker et al. 2022; Saeed et al. 2022). Complementary, other studies in Arabidopsis-*Pto* DC3000 interaction indicate that ROS1 affects the RdDM pathway through an antagonistic behaviour against *Dicer-like 2* (*DCL2)* and *Dicer-like 3 (DCL3),* as well as induce genes at the promoter level that trigger the immune defense system (specifically PAMP triggered immunity (PTI)) such as *TNL RESISTANCE METHYLATED GENE 1* (*RMG1*), contributing therefore to the basal resistance (Halter et al. 2021). Therefore, these several different studies have indicated DNA demethylation to be involved with locating/promoting basal resistance against different pathogens, especially the DNA demethylase ROS1, and with the host priming immunity status (Halter et al. 2021; Huang et al. 2022b; Tao et al. 2023).

Besides ROS1 also other DNA demethylases have been reported to be important for plant-pathogen interaction as shown by Zeng and colleagues in Arabidopsis (Zeng et al. 2021). Within the analysis of different *dme* mutants after pathogen infection (*Pst DC3000* and *V. dahlia*), both methylome and transcriptome analysis was able to verify that DME plays an important role in the response to biotic stresses through the report of phenotypic hypersensitivity which indicate importance on resistance to pathogens (Zeng et al. 2021). Also, while the analysis of defense-related genes after bacterial infection connected to DNA demethylation, DME was reported to regulate the expression of AtPRX34, SOC3 and gene encoding a TIR-NBS-LRR protein which are overall important in the PTI and ETI immune stages (Zeng et al. 2021). In *Populus* genotypes with contrasting resistance against *Lonsdalea populi* hypomethylation was linked to a higher tolerance (Xiao et al. 2021). Therefore, a specific relationship seems to be brewing specially with the CHH context since in agreement with other studies hypomethylation of the CHH context can be important for immune response against pathogens (Xiao et al. 2021). Also, the importance of the promoter region is conveyed in the Xiao and colleagues work for DNA methylation in regard to plant stress response (Xiao et al. 2021). Accordingly, in mulberry infection by phytoplasma, host hypermethylation occurred after pathogen challenge (Liu et al. 2021).

In plant-fungus interactions, studies in the interaction of *Fusarium oxysporum* with Arabidopsis indicated that DNA demethylases play a role on the pathogen resistance as well as RdDM-DNA methylation pathways relationship (CHH sequence) throughout the activation of genes related to stress response (Le et al. 2014; Hewezi et al. 2018; Tao et al. 2023). Tirnaz and colleagues focused on the analysis of either susceptible or resistant plant of canola to *Leptosphaeria maculans* and showed that a DNA hypomethylation occurred in the resistant genotypes, (Tirnaz

et al. 2020). This and other studies in fungus interaction with plants leads to a better understanding of the epigenetic machinery in the defense response as observed also in the relationship between tomato and *Trichoderma harzianum* observed by Palma and colleagues (De Palma et al. 2019). Their study showed that a bimodal pattern for the global DNA methylation occurred at 24 and 48/72 hours after inoculation (hpi) from hypo to hypermethylation observation, respectively (De Palma et al. 2019). In grapevine after *P. viticola* infection of two different grapevine cultivars (Regent - tolerant and Trincadeira - susceptible) a bimodal behaviour of global DNA methylation stage was reported (specially at 6 and 48 hpi) and both *DNMT* and *CMT* genes expression correlated with this modulation (Pereira et al. 2022).

Besides DNA methylation, histone modifications are also important in plant-pathogen interactions (Ramirez-Prado et al. 2018a; Ayyappan et al. 2019; Yan et al. 2019). In *Phaseolus vulgaris* infection by *Uromyces appendiculatus*, host histone modification at H3K9me2 and H4K12ac occurred (Ayyappan et al. 2015; Yan et al. 2019). H3K27me3, H3K9me2 and H3K9ac histone modifications were also reported after *Meloidogyne graminicola* inoculation (Atighi et al. 2021). Also in *Cucurbita pepo* infection by *Podosphaera xanthii* H3K4me3 and H3K27me3 occurred after inoculation with the pathogen (Margaritopoulou et al. 2022). The histone modifications and the H3K4me3/H3K27me3 ratio before and after inoculation allowed to showcase in the susceptible line the role of activation of chromatin (through increased ratio) in the defensive strategy of the plants. At *Paulownia fortune-phytoplasma* interaction H3K4me3, H3K36me3 or H3K9ac were highlighted as presenting a role in defense (Yan et al. 2019). Also, infection by *B. cineria* infection led to different histone modifications H3K4me3 and H3K9ac increased while H3K27me3 reduced (Crespo-Salvador et al. 2018).

Histone demethylation was shown to play a role in the plant-pathogen interaction. The Jumanji domain-containing (JMJ) family, a histone demethylase family that in several cases has been identified as having a crucial role in the defense pathway and plant phenotype after pathogen infection (*Pst DC3000*) such as JMJ14, JMJ27, JMJ704 and JMJ705 (Tao et al. 2023). This family of demethylases has been identified has the removal proteins especially of H3K4me2, H3K4me3, H3K27me3, H3K9me, H3K9me2 and H3K9me3 (Ramirez-Prado et al. 2018a). As an example JMJ27 analysis though mutant showed a susceptible phenotype in Arabidopsis plants after *Pst DC3000* with genes related with the defense response to be upregulated (WRKY25 and WRKY33) (Ramirez-Prado et al. 2018a; Tao et al. 2023). Also, JMJ705 has been observed in rice-*Xanthomonas oryzae pv. oryzae* (Xoo) interaction analysis through

overexpression and loss of this genes which an indication of opposite phenotypic patterns (resistance and susceptibility, respectively) was reported, and the histone methylation related mark (H3K27me3) was lesser in specific defense related genes in the presence of *JMJ705* overexpression (Ramirez-Prado et al. 2018b; Tao et al. 2023). Therefore, this histone demethylase of H3K27me2 and H3K27me3 is important for plants defense and immune strategies against the biotic stresses (Ramirez-Prado et al. 2018b; Tao et al. 2023). The histone demethylase *INCREASE IN BONSAI METHYLATION 1* (IBM1) was shown to negatively regulate DNA methylation sequence (CHG) with impact on genes related with the defense system in case of bacterial infection (Chan and Zimmerli 2019). Also, histone landscape was altered in the *ibm1* mutants after bacterial infection (H3K9me2 and H3K4me3 marks) therefore histone demethylase IBM1 has a role of maintenance of the activation histone mark (H3K4me3) (Chan and Zimmerli 2019). The H3K27me3 was shown to be regulated by *Phytophthora sojae* effectors (Wang et al. 2020). Hence, the defense strategy of plants in any stage of the response highlights the activity of either histone methylation and specific enzymes (HKMT and HKDM) affecting gene inhibition or activation upon pathogen colonization (Ramirez-Prado et al. 2018b).

Another histone tails modification, known as histone acetylation affect the transcription process as well as post-transcriptional modification therefore being important for the transcriptional reprograming (Kang et al. 2022). At a broader scope histone acetylation and deacetylation enzymes are involved with plant defense and immunity in the presence of pathogens infection with compelling evidence of the possible relationship with several different signalling pathways (Kim et al. 2021). HATs play a role in pathogens such as reported by Kong and authors that observed and characterize *Fusarium graminearum* HATs, where they play a key role in the metabolism, development, and pathogenicity of this pathogen (Kong et al. 2018). From the HATs known, the CBP/p300-family are known to be an important part of the plants development however the HAC1 and HAC5 from the five known Arabidopsis genes from this family were described to play a role in the defense pathway against pathogens through the impact on the signalling pathways (SA) and on the pathogenesis-related genes (Jin et al. 2018; Ramirez-Prado et al. 2018b; Hu et al. 2019; Tao et al. 2023). Also the General Control Non-repressed 5 protein (GCN5) family seems to be involved with the SA pathway has reported by analysis of *gcn5* mutant after infection with *Pst DC3000* (Kim et al. 2021; Kumar et al. 2021; Wang et al. 2021). The GNAT family (HAG1) was also shown to be an important regulator

within the wheat defense system helping the resistance against *Blumeria graminis*, with impact in important signalling pathways (Tao et al. 2023).

The histone deacetylase known as HDA19 (RPD3-like family) was shown to impact JA, ET and SA pathway and defense related genes such as some WRKY (Ramirez-Prado et al. 2018b; Kumar et al. 2021; Tao et al. 2023). Other studies corroborated the impact of HDA19 in the signalling pathways has observed on Arabidopsis after *Pst DC 3000* triggering defense related genes (*PR1* and *PR2*) as well as impacting specifically the SA signalling pathway (Choi et al. 2012; Ramirez-Prado et al. 2018a, 2018b; Yan et al. 2019; Kumar et al. 2021). In *Triticum aestivum* after *Blumeria graminis f. sp. tritici* (*Bgt*) infection a correlation between defense system and histone deacetylation enzymes was shown (Liu et al. 2019; Wang et al. 2021). The RPD3 type deacetylase known as histone deacetylase 6 (HDA6) was shown to interact with WD40-repeat protein HOS15, that plays a role in the regulation of the phenotypical response (*PR1, PR2, PR5* and *WRKY45*) (Liu et al. 2019; Samantara et al. 2021; Wang et al. 2021; Tao et al. 2023). Interestingly, the suppression of genes linked with plant response against pathogens attacks such as *Pst* DC3000 have been associated with HDA6 (Wang et al. 2017; Liu et al. 2019; Tao et al. 2023). Researchers indicate that wheat defense is fine-tuned by the antagonistic behaviour of both histone acetylation and deacetylation enzymes in the presence of *Bgt* infection (Liu et al. 2019; Samantara et al. 2021). Another complex formed by HOS15 and HDA9 has been identified to play a role in plant-pathogen interaction though expression modification of defense-related genes. In a *Pst DC3000* infection *NOD-LIKE RECEPTOR (NLR*) and *SUPPRESSOR OF npr1-1, CONSTITUTIVE 1* (*SNC1*) gene suppression can occur through modification of H3K9ac to a deacetylation status (Yang et al. 2020; Kumar et al. 2021; Tao et al. 2023). HDA9 *per si* has been observed to be part of the immune defense system through the transcriptional process while *Pst DC3000* attack (Ramirez-Prado et al. 2018a; Yang et al. 2020; Wang et al. 2021). Also is involved with the PR and SA related genes after pathogen infection (Ramirez-Prado et al. 2018a; Yang et al. 2020; Wang et al. 2021).

Furthermore, HDT701, an HDAC from the HD2 family was shown to be involved in H4K5 and H4K16 acetylation (Hu et al. 2019). Ding and colleagues pinpointed that the phenotypic susceptibility/resistance variability, defense related triggers (PRR and genes) and H4 acetylation modification were correlated and therefore indicated that this histone deacetylase impacts negatively the immunity of rice after infection (Ding et al. 2012; Ramirez-Prado et al. 2018b; Yan et al. 2019; Kumar et al. 2021; Wang et al. 2021). Zhi and colleagues shown in the *T. aestivum* after *Bgt* infection that HDT70 is a suppressor of the plants defense machinery (Zhi

et al. 2020; Kumar et al. 2021). Also, a conjoint work with the complex HOS15-HDA6 with HDT701 have been observed since they behave similarly has reported previously in the same plant-pathogen interaction (Zhi et al. 2020; Kumar et al. 2021). A possible role of recruitment by either HOS15 and HDT701 of the HDA6 to the needed locations of the defense-related genes, the stabilization capabilities of HDT701 to this complex and alteration of other histone modifications indicate the complex HDT701-HDA6-HOS15 role in the defense response genes at their chromatic level to overcome their expression with histone deacetylation activation (Zhi et al. 2020; Kumar et al. 2021).

Interestingly not only other histone modifications have been observed to collaborate with histone acetylation/deacetylation but also DNA methylation after host infection (Wang et al. 2018). As an example of this is the HDA6 which has been reported to be involved with MET1 (Wang et al. 2018; Zarreen and Chakraborty 2020). A study performed by Wang and colleagues analysed the TYLCV V2 protein in *Nicotiana benthamiana* host where an interaction between this protein and the HDA6 was reported (Wang et al. 2018; Zarreen and Chakraborty 2020). Furthermore, they observed that both V2 protein and MET1 competitively combine with HDA6 which doesn't allow the other component to recruit, respectively (Wang et al. 2018; Zarreen and Chakraborty 2020).Therefore, transcriptional gene silencing is reduced because of the interaction of viral proteins and HDA6 and lack of methylation interaction as well as an altered phenotype (susceptibility improvement) while plant-pathogen interaction (Wang et al. 2018; Zarreen and Chakraborty 2020; Kumar et al. 2021). Other HDACs and complementary components have been identified in plant diseases, such as after *Pst DC3000*, the HD2B impacts the plant immunity and the phenotype (Ramirez-Prado et al. 2018a, 2018b; Kumar et al. 2021; Wang et al. 2021). Also, SRT2 has been observed in the same pathosystem with impact on the host defense genes pathway (PR1, PAD4, EDS5 and SID2) and phenotype (Ramirez-Prado et al. 2018a, 2018b; Wang et al. 2021; Tao et al. 2023).

Moreover the stresses stimuli make the chromatin to change its conformation therefore chromatin modifications can occur which can make the DNA accessible or not (Bhadouriya et al. 2021). The CRCs have been identified has an intricate part of this process affecting the transcriptional reprograming as well as at the plant-pathogen interaction (Kang et al. 2022; Tao et al. 2023). Studies has shown the CRCs families impact in the plant-pathogen interaction such as the SWI/SNF complex that has been reported to have a key role in the chromatin structure/modification, gene transcriptional process and an intricate connection with signalling pathways (such as ABA, SA, JA, auxin and GA) and RNAi pathway (AGO1). Therefore, an

impact on plant development, responses and defense has been observed. Nonetheless, SWI/SNF has been best known to be involved in the response to abiotic stresses and, more recently, several studies have observed the impact of this CRC against different biotic stress (Bhadouriya et al. 2021; Jian et al. 2021; Parker et al. 2022; Tao et al. 2023). To be able to achieve this responsibilities, the SWI/SNF family is composed by SWI2/SNF2 subfamily that showcase important proteins that play a role in the defense/imune system against pathogens through the action of its subunits such as BRM, SPLAYED (SYD), SWI/SNF-ASSOCIATED PROTEINS 73 (SWP73A) and Decrease in DNA methylation 1 (DDM1) (Bhadouriya et al. 2021; Parker et al. 2022; Tao et al. 2023).

According with Walley and authors, SYD is a CRC with a role in the defense against *B. cineria* at a resistance level and with positioning in the signalling pathway (JA and ET) (Walley et al. 2008; Bhadouriya et al. 2021; Parker et al. 2022). Other infections in plants such as *M. oryzae* have been analysed with this CRC impact where different genes related with the defense response had their expression changed throughout their connection with different related histone variants (H2A.X and H2B.7) (Parker et al. 2022). Also, according with Johnson and colleagues SYD interacts with specific immune receptors involved in different stages of plant immunity (Johnson et al. 2015; Song et al. 2021). Also, the immune receptor Suppressor of NPR1 Constitutive 1 (SNC1) has been correlated with defense response and its regulation is possible with influence of SYD with other chromatin components (Jian et al. 2021). DDM1 is an ATP dependent SWI2/SNF2 chromatin remodeler important for the DNA methylation process since it is important for the maintenance process of methylation on cytosines (Bhadouriya et al. 2021; Song et al. 2021). Also, it has been observed that DDM1 plays a role in histone H1 and in the immune receptor SNC1 expression through its negative regulation (Bhadouriya et al. 2021; Song et al. 2021). Another CRC implicated with the defense system of plants against pathogens was observed through the study with Arabidopsis immune response against *Pst DC 3000* (Jian et al. 2021; Huang et al. 2022a; Parker et al. 2022; Tao et al. 2023). Meanwhile, BIT-responsive Histone interacting SNF2 ATPase 1 (BRHIS1) was shown to participate in CRC and mono-ubiquitination process of H2A and H2B variants. This leads to BRHIS1 localization to specific regions of defense related genes not allowing their expression while in the rice-*Magnaporthe oryzae* interaction (Li et al. 2015a; Jian et al. 2021; Kim 2021; Song et al. 2021).

The Swi2/Snf2-related 1 CHROMATIN REMODELER COMPLEX (SWR1-C) is one of the most studied CRCs and is formed by several different important subunits (PHOTOPERIOD-

INDEPENDENT EARLY FLOWERING1 (PIE1), ACTIN-RELATED PROTEIN6 (ARP6), SWR1 COMPLEX 6 (SWC6) and SWC4 that regulates/interacts with histone chaperones (NUCLEOSOME ASSEMBLY PROTEIN-RELATED PROTEIN (NRP)) or variants (H2A. Z) which therefore play a role in the plant defense system against abiotic different pathogens stresses (Jakada et al. 2019; Bhadouriya et al. 2021; Song et al. 2021; Parker et al. 2022; Tao et al. 2023). Signalling pathways such as JA and SA have been identified to be connected with SWR1-C (Bhadouriya et al. 2021; Song et al. 2021; Parker et al. 2022; Tao et al. 2023). Besides the observation of SWR1-C has a whole, also the subunits have been analysed independently with its own functions in the plant immune and defense system (Jakada et al. 2019). Also, a close relationship has been reported between SWR1-C and the histone variant H2A.Z specially at the defense response against biotic stresses (Parker et al. 2022). Mutation of the subunits of SWR1-C, specially PIE1, ARP6, and SWC6, has impacted the histone variant deposition as well as the phenotype (resistant behaviour) in the presence of different pathogens therefore indicating a role in the regulation of defense related genes (Berriri et al. 2016; Jakada et al. 2019; Parker et al. 2022). As example, the pathogen *Sclerotinia sclerotiorum* interacted with pineapple SWC6 mutant leaves where is possible to observe a resistance on the mutant to this pathogen (Jakada et al. 2019).

The PIE1 catalytic activity impacted the Arabidopsis genes expression at normal and plant disease conditions which lead to a resistance phenotype after *Pst DC3000* in plant mutants (Song et al. 2021). PIE1 and ARP6 were observed after *Sclerotinia sclerotiorum infection* where *ARP6* was show to have an indirect role in plant immunity (at the deposition of H2A.Z at specific defense related genes - *WRKY33* and *YDD*) (Cai et al. 2021). The interaction between CRC (SWR1-C), histone variants (H2A.Z) and defense related chaperons (NRP1 and NRP2) create a pathway of plant defense at the gene impact and transcription at a biotic stress (Parker et al. 2022).

CRC and plant immunity were also related through the interaction between SNC1, a plant immune receptor, and CHR5, a CHD subfamily, that plays a regulatory role to this receptor, affects its transcription process, autoimmunity, nucleosome occupancy and impact genes related with histone modification (H3K27me3) repression (Zou et al. 2017; Kim 2021; Song et al. 2021). Other components besides CRC that have been correlated plant immunity throughout the decades is *Microrchidia (MORC)* ATPase family such as *MORC1* or *MORC6* that has been observed in Arabidopsis (Bhadouriya et al. 2021; Parker et al. 2022). These proteins have presented a role in the gene silencing (Bhadouriya et al. 2021; Parker et al. 2022). In normal

conditions, in different plant species MORC was reported with different roles although specially within chromatin modification, structure and components such as transposable elements (Parker et al. 2022). In addition, a role in the DNA-SA cooperation can be observed in analysed mutants (Parker et al. 2022). Meanwhile, in different biotic stresses (*Phytophthora infestans*, *N. benthamiana*, *Turnip crinkle virus* (TCV), *Pst DC3000* and *Hyaloperonospora arabidopsidis*), *MORC1* aka CTR1 showed an important role as a regulator in different stages of immunity (ETI and PTI) (Bhadouriya et al. 2021; Parker et al. 2022). Another CRC related is the chromatin remodeler ATPase-like protein MORPHEUS MOLECULE 1 (MOM1) which in the presence of *Pst DC3000* was observed to be associated with defense related genes (NLR/PRR) (Cambiagno et al. 2018; Parker et al. 2022). Also the close relationship with siRNA at a epigenetic level indicates a strong evidence of epigenetic memory aka prime effect at the defense related genes level in relation with RNA (Parker et al. 2022).

Within the RNA silencing, the DCL proteins are important for the biogenesis of siRNA and microRNA (miRNA) and RdDM pathway (Andika et al. 2019; Kaushal et al. 2021). The main function of these siRNAs is to be part of the transcriptional and post-transcriptional gene silencing via the mediated/induction of siRNA in *de novo* DNA methylation pathway (DNA methylation/RdDM) and RNA-induced silencing complexes (RISC) complex, respectively (Borges and Martienssen 2015; Zhu et al. 2019; Ali et al. 2020; Guarino et al. 2022; Saeed et al. 2022). Also, siRNA play a role in the defense against plant diseases through different ways such as effectors released by bacteria which in plants influences the siRNA synthesis (Ali et al. 2020). Several different studies have shown a variety of siRNA interconnected with genes related with plant defense through RNA interference (RNAi) mechanisms and also in some pathogens their siRNA affects also the development and pathogenicity (Ali et al. 2020).

Specially on the host and viral pathogen, PTGS and TGS were described to impact plant-virus interaction as DCL impacts negatively on the replication and symptomatology of the *C. parasitica* after Arabidopsis infection (Andika et al. 2019). Further analysis on DCL mutants, after *Botritis cineria* infection highlight the existence of pathogen sRNA (Cai et al. 2018). Haile and authors analysed *Plasmopara viticola* (Pv)DCL1 and PvDCL2 dsRNA on grapevine showing that RNAi machinery is involved in the colonization of the pathogen to the host therefore could be a new method for the disease control (Haile et al. 2021).

Besides DCL also other components important for RNA silencing have been observed in *B.napus* and *S.sclerotiorum* colonization shows the involvement of miRNAs interacting with

Argonauts (AGO1 and AGO2) with a role on the susceptibility shown on mutant hosts (Cao et al. 2016).

siRNAs accumulation was observed in the plant-virus interaction leading to viral to RNA silencing and therefore acting as an antiviral protection (Huang et al. 2016).

miRNA play a role on the DNA methylation pathway (Huang et al. 2016; Hou et al. 2019; Raju et al. 2019; Panigrahi et al. 2021; Guarino et al. 2022; Huang and Jin 2022). The target of miRNA can go from the R genes which influences the host phenotype in the presence of the pathogen, to the auxin response factor (ARF) proteins, and to RNA Dependent RNA polymerases (RDR1, RDR2, RDR6) an important antiviral pathway component (Seo et al. 2013; Huang et al. 2016; Hoang et al. 2018).

The miRNA impacts the defense system of plants infected with different types of pathogens from bacteria, fungus, viruses and nematodes (Khraiwesh et al. 2012). Also, miRNA has been reported as part of some pathogens and helps on their pathogenicity therefore colonize the hosts with the use of their defense system such as *Botritis cineria* (Dal Bosco et al. 2018).

In plants, the first miRNA to be associated with an imune defense strategy was miR393 (Khraiwesh et al. 2012). Also, miR156, miR158, miR159, miR160, miR161, miR164, miR167, miR171, miR172c, miR396, miR398a, miR399, miR408, miR444, miR482, miR773, miR825, miR827, miR1885 miR2118 and miR7695 have been identified and with different expression upon the plant-pathogen interaction (Khraiwesh et al. 2012; Huang et al. 2016; Prakash et al. 2017; Islam et al. 2018; Tang et al. 2021).

I.6. Main aim and thesis layout

Scarce information on the role of the epigenetic machinery in the grapevine immune system led to the aim of this project which is to increase this knowledge specifically in the defence responses of *V. vinifera* to infection of *P. viticola*. Also, we aim to define the tolerance and/or susceptibility determinants of *Vitis* to downy mildew disease at a molecular level. To accomplish these aims, several approaches were followed:

- Analysis of the level of susceptibility and/or tolerance to downy mildew in different varieties/crossing populations of Italian table grape through a phenotypic characterization of the inoculated grapevine plants with *P. viticola*.

- Characterization of the *V. vinifera* epigenetic variation after *P. viticola* inoculation through studying the changes in DNA methylation pattern during grapevine-*P. viticola* interaction.

- Characterization of transcriptome modulation after *P. viticola* infection. In particular, differentially expressed genes involved in plant-pathogen interaction, plant defense response and in the epigenetic regulation will be searched.

I.7. References

Ageeva-Kieferle A, Rudolf EE, Lindermayr C (2019) Redox-dependent chromatin remodeling: A new function of nitric oxide as architect of chromatin structure in plants. Front Plant Sci 10: 1–12

Ali M, Javaid A, Naqvi SH, Batcho A, Kayani WK, Lal A, Sajid IA, Nwogwugwu JO (2020) Biotic stress triggered small RNA and RNAi defense response in plants. Mol Biol Rep 47: 5511–5522

Alonso C, Ramos-Cruz D, Becker C (2019) The role of plant epigenetics in biotic interactions. New Phytol 221: 731–737

Andika IB, Kondo H, Suzuki N (2019) Dicer functions transcriptionally and posttranscriptionally in a multilayer antiviral defense. Proc Natl Acad Sci U S A 116: 2274–2281

Annacondia ML, Magerøy MH, Martinez G (2018) Stress response regulation by epigenetic mechanisms: changing of the guards. Physiol Plant 162: 239–250

Armijo G, Schlechter R, Agurto M, Muñoz D, Nuñez C, Arce-Johnson P (2016) Grapevine Pathogenic Microorganisms: Understanding Infection Strategies and Host Response Scenarios. Front Plant Sci 7: 1–18

Ashapkin V V., Kutueva LI, Aleksandrushkina NI, Vanyushin BF (2020) Epigenetic mechanisms of plant adaptation to biotic and abiotic stresses. Int J Mol Sci 21: 1–32

Ashapkin V V., Kutueva LI, Vanyushin BF (2016) Plant DNA methyltransferase genes: Multiplicity, expression, methylation patterns. Biochem 81: 141–151

Atak A, Şen A (2021) A grape breeding programme using different Vitis species. Plant Breed 140: 1136–1149

Atighi MR, Verstraeten B, De Meyer T, Kyndt T (2021) Genome-wide shifts in histone modifications at early stage of rice infection with Meloidogyne graminicola. Mol Plant Pathol 22: 440–455

Ayyappan V, Kalavacharla V, Thimmapuram J, Bhide KP, Sripathi VR, Smolinski TG, Manoharan M, Thurston Y, Todd A, Kingham B, Mantovani R (2015) Genome-wide profiling of histone modifications (H3K9me2 and H4K12ac) and gene expression in rust (uromyces appendiculatus) inoculated common bean (Phaseolus vulgaris L). PLoS One 10: 1–29

Ayyappan V, Sripathi VR, Kalavacharla VK, Saha MC, Thimmapuram J, Bhide KP, Fiedler E (2019) Genome-wide identification of histone methylation (H3K9me2) and acetylation (H4K12ac) marks in two ecotypes of switchgrass (Panicum virgatum L.). BMC Genomics 20: 1–15

Bacilieri R, Lacombe T, Le Cunff L, Di Vecchi-Staraz M, Laucou V, Genna B, Péros JP, This P, Boursiquot JM (2013) Genetic structure in cultivated grapevines is linked to geography and human selection. BMC Plant Biol 13: 1–14

Berriri S, Gangappa SN, Kumar SV (2016) SWR1 Chromatin-Remodeling Complex Subunits and H2A.Z Have Non-overlapping Functions in Immunity and Gene Regulation in Arabidopsis. Mol Plant 9: 1051–1065

Bhadouriya SL, Mehrotra S, Basantani MK, Loake GJ, Mehrotra R (2021) Role of Chromatin Architecture in Plant Stress Responses: An Update. Front Plant Sci 11: 1–22

Borges F, Martienssen RA (2015) The expanding world of small RNAs in plants. Nat Rev Mol Cell Biol 16: 727–741

Bove F, Rossi V (2020) Components of partial resistance to Plasmopara viticola enable complete phenotypic characterization of grapevine varieties. Sci Rep 10: 1–12

Brilli M, Asquini E, Moser M, Bianchedi PL, Perazzolli M, Si-Ammour A (2018) A multi-omics study of the grapevine-downy mildew (Plasmopara viticola) pathosystem unveils a complex protein coding and noncoding-based arms race during infection. Sci Rep 8: 1–12

Brocklehurst S, Watson M, Carr IM, Out S, Heidmann I, Meyer P (2018) Induction of epigenetic variation in Arabidopsis by over-expression of DNA METHYLTRANSFERASE1 (MET1). PLoS One 13: 1–22

Buonassisi D, Colombo M, Migliaro D, Dolzani C, Peressotti E, Mizzotti C, Velasco R, Masiero S, Perazzolli M, Vezzulli S (2017) Breeding for grapevine downy mildew resistance: a review of "omics" approaches. Euphytica 1–21

Cai H, Huang Y, Chen F, Liu L, Chai M, Zhang M, Yan M, Aslam M, He Q, Qin Y (2021) ERECTA signaling regulates plant immune responses via chromatin-mediated promotion of WRKY33 binding to target genes. New Phytol 230: 737–756

Cai Q, Qiao L, Wang M, He B, Lin F, Palmquist J, Jin H (2018) Pathogen To Silence Virulence Genes. Science (80-) 360: 1126–1129

Cambiagno DA, Nota F, Zavallo D, Rius S, Casati P, Asurmendi S, Alvarez ME (2018) Immune receptor genes and pericentromeric transposons as targets of common epigenetic regulatory elements. Plant J 96: 1178–1190

Cambiagno DA, Torres JR, Alvarez ME (2021) Convergent Epigenetic Mechanisms Avoid Constitutive Expression of Immune Receptor Gene Subsets. Front Plant Sci 12: 1–11

Camborde L, Raynaud C, Dumas B, Gaulin E (2019) DNA-Damaging Effectors: New Players in the Effector Arena. Trends Plant Sci 24: 1094–1101

Cao JY, Xu YP, Li W, Li SS, Rahman H, Cai XZ (2016) Genome-wide identification of dicer-like, argonaute, and RNA-dependent RNA polymerase gene families in brassica species and functional analyses of their arabidopsis homologs in resistance to sclerotinia sclerotiorum. Front Plant Sci 7: 1–17

Capriotti L, Baraldi E, Mezzetti B, Limera C, Sabbadini S (2020) Biotechnological approaches: Gene overexpression, gene silencing, and genome editing to control fungal and oomycete diseases in grapevine. Int J Mol Sci 21: 1–29

Castellano M, Martinez G, Marques MC, Moreno-Romero J, Köhler C, Pallas V, Gomez G (2016) Changes in the DNA methylation pattern of the host male gametophyte of viroid-

infected cucumber plants. J Exp Bot 67: 5857–5868

Chachar S, Chachar M, Riaz A, Shaikh AA, Li X, Li X, Guan C, Zhang P (2022) Epigenetic modification for horticultural plant improvement comes of age. Sci Hortic (Amsterdam) 292: 1–8

Chan C, Zimmerli L (2019) The Histone Demethylase IBM1 Positively Regulates Arabidopsis Immunity by Control of Defense Gene Expression. Front Plant Sci 10: 1–10

Chen W, Zhu Q, Liu Y, Zhang Q (2017) Chromatin Remodeling and Plant Immunity. Adv Protein Chem Struct Biol 106: 243–260

Chitarrini G, Riccadonna S, Zulini L, Vecchione A, Stefanini M, Larger S, Pindo M, Cestaro A, Franceschi P, Magris G, Foria S, Morgante M, Di Gaspero G, Vrhovsek U (2020) Two-omics data revealed commonalities and differences between Rpv12- and Rpv3-mediated resistance in grapevine. Sci Rep 10: 1–15

Choi SM, Song HR, Han SK, Han M, Kim CY, Park J, Lee YH, Jeon JS, Noh YS, Noh B (2012) HDA19 is required for the repression of salicylic acid biosynthesis and salicylic acid-mediated defense responses in Arabidopsis. Plant J 71: 135–146

Clippinger JI, Dobry EP, Laffan I, Zorbas N, Hed B, Campbell MA (2024) Traditional and Emerging Approaches for Disease Management of Plasmopara viticola, Causal Agent of Downy Mildew of Grape. Agriculture 14: 1–24

Crespo-Salvador Ó, Escamilla-Aguilar M, López-Cruz J, López-Rodas G, González-Bosch C (2018) Determination of histone epigenetic marks in Arabidopsis and tomato genes in the early response to Botrytis cinerea. Plant Cell Rep 37: 153–166

Crispo F, Condelli V, Lepore S, Notarangelo T, Sgambato A, Esposito F, Maddalena F, Landriscina M (2019) Metabolic dysregulations and epigenetics: A bidirectional interplay that drives tumor progression. Cells 8: 1–23

Dal Bosco D, Sinski I, Ritschel PS, Camargo UA, Fajardo TVM, Harakava R, Quecini V (2018) Expression of disease resistance in genetically modified grapevines correlates with the contents of viral sequences in the T-DNA and global genome methylation. Transgenic Res 27: 379–396

Deleris A, Halter T, Navarro L (2016) DNA Methylation and Demethylation in Plant Immunity. Annu Rev Phytopathol 54: 579–603

Ding B, Bellizzi M d. R, Ning Y, Meyers BC, Wang G-L (2012) HDT701, a Histone H4 Deacetylase, Negatively Regulates Plant Innate Immunity by Modulating Histone H4 Acetylation of Defense-Related Genes in Rice. Plant Cell 24: 3783–3794

Dong Q, Hu B, Zhang C (2022) microRNAs and Their Roles in Plant Development. Front Plant Sci 13: 1–17

Dowen RH, Pelizzola M, Schmitz RJ, Lister R, Dowen JM, Nery JR, Dixon JE, Ecker JR (2012) Widespread dynamic DNA methylation in response to biotic stress. Proc Natl Acad Sci U S A 109: E2183–E2191

Dussert Y, Gouzy J, Richart-Cervera S, Mazet ID, Delière L, Couture C, Legrand L, Mestre MCPP, Delmotte F (2016) Draft genome sequence of Plasmopara viticola, the grapevine downy mildew pathogen. Genome Announc 4: 4–5

Espinas NA, Saze H, Saijo Y (2016) Epigenetic Control of Defense Signaling and Priming in Plants. Front Plant Sci 7: 1–7

Faize L, Faize M (2018) Functional analogues of salicylic acid and their use in crop protection. Agronomy 8: 1–20

Fontaine MC, Labbé F, Dussert Y, Delière L, Richart-Cervera S, Giraud T, Delmotte F (2021) Europe as a bridgehead in the worldwide invasion history of grapevine downy mildew, Plasmopara viticola. Curr Biol 31: 2155-2166.e4

Fröbel S, Dudenhöffer J, Töpfer R, Zyprian E (2019) Transcriptome analysis of early downy mildew (Plasmopara viticola) defense in grapevines carrying the Asian resistance locus Rpv10. Euphytica 215: 1–21

Gong P, Kang J, Sadeghnezhad E, Bao R, Ge M, Zhuge Y, Shangguan L, Fang J (2022) Transcriptional Profiling of Resistant and Susceptible Cultivars of Grapevine (Vitis L.) Reveals Hypersensitive Responses to Plasmopara viticola. Front Microbiol 13: 1–17

Gouveia C, Santos RB, Paiva-Silva C, Buchholz G, Malhó R, Figueiredo A (2024) The pathogenicity of Plasmopara viticola: a review of evolutionary dynamics, infection strategies and effector molecules. BMC Plant Biol 24: 1–11

Grassi F, De Lorenzis G (2021) Back to the origins: Background and perspectives of grapevine domestication. Int J Mol Sci 22: 1–20

Guarino F, Cicatelli A, Castiglione S, Agius DR, Orhun GE, Fragkostefanakis S, Leclercq J,

Dobránszki J, Kaiserli E, Lieberman-Lazarovich M, Sõmera M, Sarmiento C, Vettori C, Paffetti D, Poma AMG, Moschou PN, Gašparović M, Yousefi S, Vergata C, Berger MMJ, Gallusci P, Miladinović D, Martinelli F (2022) An Epigenetic Alphabet of Crop Adaptation to Climate Change. Front Genet 13: 1–18

Guerreiro A, Figueiredo J, Sousa Silva M, Figueiredo A (2016) Linking Jasmonic Acid to Grapevine Resistance against the Biotrophic Oomycete Plasmopara viticola. Front Plant Sci 7: 1–7

Gui X, Liu C, Qi Y, Zhou X (2022) Geminiviruses employ host DNA glycosylases to subvert DNA methylation-mediated defense. Nat Commun 13: 1–11

Haile ZM, Gebremichael DE, Capriotti L, Molesini B, Negrini F, Collina M, Sabbadini S, Mezzetti B, Baraldi E (2021) Double-Stranded RNA Targeting Dicer-Like Genes Compromises the Pathogenicity of Plasmopara viticola on Grapevine. Front Plant Sci 12: 1–7

Halter T, Wang J, Amesefe D, Lastrucci E, Charvin M, Rastogi MS, Navarro L (2021) The arabidopsis active demethylase ros1 cis-regulates defense genes by erasing dna methylation at promoter-regulatory regions. Elife 10: 1–62

Hewezi T, Pantalone V, Bennett M, Neal Stewart C, Burch-Smith TM (2018) Phytopathogen-induced changes to plant methylomes. Plant Cell Rep 37: 17–23

Hoang TV, Vo KTX, Hong WJ, Jung KH, Jeon JS (2018) Defense Response to Pathogens Through Epigenetic Regulation in Rice. J Plant Biol 61: 1–10

Hou N, Cao Y, Li F, Yuan W, Bian H, Wang J, Zhu M, Han N (2019) Epigenetic regulation of miR396 expression by SWR1-C and the effect of miR396 on leaf growth and developmental phase transition in Arabidopsis. J Exp Bot 70: 5217–5229

Hu Y, Lu Y, Zhao Y, Zhou DX (2019) Histone Acetylation Dynamics Integrates Metabolic Activity to Regulate Plant Response to Stress. Front Plant Sci 10: 1–9

Huang C, Rangel DS, Qin X, Bui C, Li R, Jia Z, Cui X, Jin H, Haya E, Sciences P (2022a) The chromatin remodeling protein BAF60/SWP73A regulates the plant immune receptor NLRs. Cell Host Microbe 29: 425–434

Huang CY, Jin H (2022) Coordinated Epigenetic Regulation in Plants: A Potent Managerial Tool to Conquer Biotic Stress. Front Plant Sci 12: 1–11

Huang J, Yang M, Zhang X (2016) The function of small RNAs in plant biotic stress response. J Integr Plant Biol 58: 312–327

Huang M, Zhang Y, Wang Y, Xie J, Cheng J, Fu Y, Jiang D, Yu X, Li B (2022b) Active DNA demethylation regulates MAMP-triggered immune priming in Arabidopsis. J Genet Genomics 49: 796–809

Islam W, Noman A, Qasim M, Wang L (2018) Plant responses to pathogen attack: Small rnas in focus. Int J Mol Sci 19:

Jakada BH, Aslam M, Fakher B, Greaves JG, Li Z, Li W, Lai L, Ayoade OA, Cheng Y, Cao S, Li G, Hu JM, Qin Y (2019) Identification of SWI2/SNF2-related 1 chromatin remodeling complex (SWR1-C) subunits in pineapple and the role of pineapple SWR1 complex 6 (ACSWC6) in biotic and abiotic stress response. Biomolecules 9: 1–18

Jian Y, Shim W-B, Ma Z (2021) Multiple functions of SWI/SNF chromatin remodeling complex in plant-pathogen interactions. Stress Biol 1: 1–15

Jin H, Choi SM, Kang MJ, Yun SH, Kwon DJ, Noh YS, Noh B (2018) Salicylic acid-induced transcriptional reprogramming by the HAC-NPR1-TGA histone acetyltransferase complex in Arabidopsis. Nucleic Acids Res 46: 11712–11725

Johnson KCM, Xia S, Feng X, Li X (2015) The Chromatin Remodeler SPLAYED Negatively Regulates SNC1-Mediated Immunity. Plant Cell Physiol 56: 1616–1623

Jones JDG, Dangl JL (2006) The plant immune system. Nature 444: 323–329

Judelson HS, Ah-Fong AMV (2019) Exchanges at the plant-oomycete interface that influence disease. Plant Physiol 179: 1198–1211

Kakoulidou I, Avramidou E V., Baránek M, Brunel-muguet S, Farrona S, Johannes F, Kaiserli E, Lieberman-lazarovich M, Martinelli F, Mladenov V, Testillano PS, Vassileva V, Maury S (2021) Epigenetics for crop improvement in times of global change. Biology (Basel) 10: 1–46

Kang H, Fan T, Wu J, Zhu Y, Shen WH (2022) Histone modification and chromatin remodeling in plant response to pathogens. Front Plant Sci 13: 1–14

Kaushal R, Peng L, Singh SK, Zhang M, Zhang X, Vílchez JI, Wang Z, He D, Yang Y, Lv S, Xu Z, Morcillo RJL, Wang W, Huang W, Paré PW, Song CP, Zhu JK, Liu R, Zhong W, Ma P, Zhang H (2021) Dicer-like proteins influence Arabidopsis root microbiota

independent of RNA-directed DNA methylation. Microbiome 9: 1–20

Khraiwesh B, Zhu JK, Zhu J (2012) Role of miRNAs and siRNAs in biotic and abiotic stress responses of plants. Biochim Biophys Acta - Gene Regul Mech 1819: 137–148

Kim GH, Ryan JJ, Marsboom G, Archer SL (2011) Epigenetic mechanisms of pulmonary hypertension. Pulm Circ 1: 347–56

Kim JH (2021) Multifaceted chromatin structure and transcription changes in plant stress response. Int J Mol Sci 22: 1–25

Kim S, Piquerez SJM, Ramirez-Prado JS, Mastorakis E, Veluchamy A, Latrasse D, Manza-Mianza D, Brik-Chaouche R, Huang Y, Rodriguez-Granados NY, Concia L, Blein T, Citerne S, Bendahmane A, Bergounioux C, Crespi M, Mahfouz MM, Raynaud C, Hirt H, Ntoukakis V, Benhamed M (2021) GCN5 modulates salicylic acid homeostasis by regulating H3K14ac levels at the 5' and 3' ends of its target genes. Nucleic Acids Res 48: 5953–5966

Koledenkova K, Esmaeel Q, Jacquard C, Nowak J, Clément C, Ait Barka E (2022) Plasmopara viticola the Causal Agent of Downy Mildew of Grapevine: From Its Taxonomy to Disease Management. Front Microbiol 13: 1–29

Kong X, van Diepeningen AD, van der Lee TAJ, Waalwijk C, Xu J, Xu J, Zhang H, Chen W, Feng J (2018) The Fusarium graminearum histone acetyltransferases are important for morphogenesis, DON biosynthesis, and pathogenicity. Front Microbiol 9: 1–16

Kumar S, Chinnusamy V, Mohapatra T (2018) Epigenetics of Modified DNA Bases: 5-Methylcytosine and Beyond. Front Genet 9: 1–14

Kumar S, Mohapatra T (2021) Dynamics of DNA Methylation and Its Functions in Plant Growth and Development. Front Plant Sci 12: 1–17

Kumar S, Singh AK, Mohapatra T (2017) Epigenetics: History, present status and future perspective. Indian J Genet Plant Breed 77: 445–463

Kumar V, Thakur JK, Prasad M (2021) Histone acetylation dynamics regulating plant development and stress responses. Cell Mol Life Sci 78: 4467–4486

Lanciano S, Mirouze M (2017) Dna methylation in rice and relevance for breeding. Epigenomes 2: 1–14

Laurell C, Berglund T, Ohlsson AB (2021) Transcriptome analysis shows nicotinamide seed

treatment alters expression of genes involved in defense and epigenetic processes in roots of seedlings of Picea abies. J For Res doi.org/10.1007/s11676-021-01410-z

Le TN, Schumann U, Smith NA, Tiwari S, Khang Au PC, Zhu QH, Taylor JM, Kazan K, Llewellyn DJ, Zhang R, Dennis ES, Wang MB (2014) DNA demethylases target promoter transposable elements to positively regulate stress responsive genes in Arabidopsis. Genome Biol 15: 1–18

Li J, Li C, Lu S (2018) Identification and characterization of the cytosine-5 DNA methyltransferase gene family in Salvia miltiorrhiza. PeerJ 6: 1–21

Li X, Jiang Y, Ji Z, Liu Y, Zhang Q (2015a) BRHIS 1 suppresses rice innate immunity through binding to monoubiquitinated H2A and H2B variants . EMBO Rep 16: 1192–1202

Li X, Wu J, Yin L, Zhang Y, Qu J, Lu J (2015b) Comparative transcriptome analysis reveals defense-related genes and pathways against downy mildew in Vitis amurensis grapevine. Plant Physiol Biochem 95: 1–14

Liu C, Dong X, Xu Y, Dong Q, Wang Y, Gai Y, Ji X (2021) Transcriptome and DNA Methylome Reveal Insights Into Phytoplasma Infection Responses in Mulberry (Morus multicaulis Perr.). Front Plant Sci 12: 1–21

Liu J, Zhi P, Wang X, Fan Q, Chang C (2019) Wheat WD40-repeat protein TaHOS15 functions in a histone deacetylase complex to fine-tune defense responses to Blumeria graminis f.sp. tritici. J Exp Bot 70: 255–268

Liu L, Liu CY, Wang H, Yu SY, Guan TS, Huang YF, Li RC (2020a) The abscisic acid receptor gene VvPYL4 positively regulates grapevine resistance to Plasmopara viticola. Plant Cell Tissue Organ Cult 142: 483–492

Liu L, Zhang B, Wang H, Yu SY, Guan TS, Huang YF, Liu CY (2020b) Candidate resistance genes selection and transcriptome analysis for the early responses to Plasmopara viticola infection in grape cultivars. J Plant Pathol 102: 857–869

Liu Y, Teng C, Xia R, Meyers BC (2020c) PhasiRNAs in Plants: Their Biogenesis, genic sources, and roles in stress responses, development, and reproduction. Plant Cell 32: 3059–3080

Ma T, Chen S, Liu J, Fu P, Wu W, Song S, Gao Y, Ye W, Lu J (2021) Plasmopara viticola effector PvRXLR111 stabilizes VvWRKY40 to promote virulence. Mol Plant Pathol 22:

231–242

Magris G, Jurman I, Fornasiero A, Paparelli E, Schwope R, Marroni F, Di Gaspero G, Morgante M (2021) The genomes of 204 Vitis vinifera accessions reveal the origin of European wine grapes. Nat Commun 12: 1–12

Malacarne G, Vrhovsek U, Zulini L, Cestaro A, Stefanini M, Mattivi F, Delledonne M, Velasco R, Moser C (2011) Resistance to Plasmopara viticola in a grapevine segregating population is associated with stilbenoid accumulation and with specific host transcriptional responses. BMC Plant Biol 11: 1–13

Margaritopoulou T, Kizis D, Kotopoulis D, Papadakis IE, Anagnostopoulos C, Baira E, Termentzi A, Vichou AE, Leifert C, Markellou E (2022) Enriched HeK4me3 marks at Pm-0 resistance-related genes prime courgette against Podosphaera xanthii. Plant Physiol 188: 576–592

Margaryan K, Melyan G, Röckel F, Töpfer R, Maul E (2021) Genetic diversity of armenian grapevine (Vitis vinifera L.) germplasm: Molecular characterization and parentage analysis. Biology (Basel) 10: 1–22

Mauch-Mani B, Baccelli I, Luna E, Flors V (2017) Defense Priming: An Adaptive Part of Induced Resistance. Annu Rev Plant Biol 68: 485–512

Micheloni C (2017) Diseases and pests in viticulture

Morales-Cruz A, Aguirre-Liguori JA, Zhou Y, Minio A, Riaz S, Walker AM, Cantu D, Gaut BS (2021) Introgression among North American wild grapes (Vitis) fuels biotic and abiotic adaptation. Genome Biol 22: 1–27

Myles S, Boyko AR, Owens CL, Brown PJ, Grassi F, Aradhya MK, Prins B, Reynolds A, Chia JM, Ware D, Bustamante CD, Buckler ES (2011) Genetic structure and domestication history of the grape. Proc Natl Acad Sci U S A 108: 3530–3535

Nascimento R, Maia M, Ferreira AEN, Silva AB, Freire AP, Cordeiro C, Silva MS, Figueiredo A (2019) Early stage metabolic events associated with the establishment of Vitis vinifera – Plasmopara viticola compatible interaction. Plant Physiol Biochem 137: 1–13

Neto J, Silva M, Pandolfi V, Crovella S, Benko-Iseppon A, Kido E (2016) Epigenetic Signals on Plant Adaptation: a Biotic Stress Perspective. Curr Protein Pept Sci 18: 352–367

Niederhuth CE, Bewick AJ, Ji L, Alabady MS, Kim K Do, Li Q, Rohr NA, Rambani A, Burke JM, Udall JA, Egesi C, Schmutz J, Grimwood J, Jackson SA, Springer NM, Schmitz RJ (2016) Widespread natural variation of DNA methylation within angiosperms. Genome Biol 17: 1–19

Ospelt C (2022) A brief history of epigenetics. Immunol Lett 249: 1–4

De Palma M, Salzano M, Villano C, Aversano R, Lorito M, Ruocco M, Docimo T, Piccinelli AL, D'Agostino N, Tucci M (2019) Transcriptome reprogramming, epigenetic modifications and alternative splicing orchestrate the tomato root response to the beneficial fungus Trichoderma harzianum. Hortic Res 6: 1–15

Panigrahi GK, Sahoo A, Satapathy KB (2021) Insights to plant immunity: Defense signaling to epigenetics. Physiol Mol Plant Pathol 113: 1–7

Parker AH, Wilkinson SW, Ton J (2022) Epigenetics: a catalyst of plant immunity against pathogens. New Phytol 233: 66–83

Peng J, Wang X, Wang H, Li X, Zhang Q, Wang M, Yan J (2024) Advances in understanding grapevine downy mildew: From pathogen infection to disease management. Mol Plant Pathol 25: 1–21

Perazzolli M, Moretto M, Fontana P, Ferrarini A, Velasco R, Moser C, Delledonne M, Pertot I (2012) Downy mildew resistance induced by Trichoderma harzianum T39 in susceptible grapevines partially mimics transcriptional changes of resistant genotypes. BMC Genomics 13: 1–19

Pereira G, Pereira J, Santos RB, Figueiredo A (2022) Uncovering the role of DNA methyltransferases in grapevine – Plasmopara viticola interaction: From genome-wide characterization to global methylation patterns. Gene 837: 1–9

Polesani M, Bortesi L, Ferrarini A, Zamboni A, Fasoli M, Zadra C, Lovato A, Pezzotti M, Delledonne M, Polverari A (2010) General and species-specific transcriptional responses to downy mildew infection in a susceptible (Vitis vinifera) and a resistant (V. Riparia) grapevine species. BMC Genomics 11: 1–16

Prakash V, Devendran R, Chakraborty S (2017) Overview of plant RNA dependent RNA polymerases in antiviral defense and gene silencing. Indian J Plant Physiol 22: 493–505

Raju SKK, Ritter EJ, Niederhuth CE (2019) Establishment, maintenance, and biological roles of non-CG methylation in plants. Essays Biochem 63: 743–755

Ramirez-Prado JS, Abulfaraj AA, Rayapuram N, Benhamed M, Hirt H (2018a) Plant Immunity: From Signaling to Epigenetic Control of Defense. Trends Plant Sci 23: 833–844

Ramirez-Prado JS, Piquerez SJM, Bendahmane A, Hirt H, Raynaud C, Benhamed M (2018b) Modify the histone to win the battle: Chromatin dynamics in plant–pathogen interactions. Front Plant Sci 9: 1–18

Ramos-Cruz D, Troyee AN, Becker C (2021) Epigenetics in plant organismic interactions. Curr Opin Plant Biol 61: 1–10

Rossarolla MD, Tomazetti TC, Welter LJ, Santos HP, Stefanini M, Trapp O, Guerra MP, Nodari RO (2021) The interplay between hormone signaling and defense gene expression in grapevine genotypes carrying genetic resistance against Plasmopara viticola. Vitis - J Grapevine Res 60: 195–206

Saeed F, Chaudhry UK, Bakhsh A, Raza A, Saeed Y, Bohra A, Varshney RK (2022) Moving Beyond DNA Sequence to Improve Plant Stress Responses. Front Genet 13: 1–15

Saldívar-González FI, Gómez-García A, Chávez-Ponce De León DE, Sánchez-Cruz N, Ruiz-Rios J, Pilón-Jiménez BA, Medina-Franco JL (2018) Inhibitors of DNA methyltransferases from natural sources: A computational perspective. Front Pharmacol 9: 1–10

Samantara K, Shiv A, de Sousa LL, Sandhu KS, Priyadarshini P, Mohapatra SR (2021) A comprehensive review on epigenetic mechanisms and application of epigenetic modifications for crop improvement. Environ Exp Bot 188: 1–21

Sanan-Mishra N, Abdul Kader Jailani A, Mandal B, Mukherjee SK (2021) Secondary siRNAs in Plants: Biosynthesis, Various Functions, and Applications in Virology. Front Plant Sci 12: 1–32

Santos RB, Figueiredo A (2021) Two sides of the same story in grapevine-pathogen interactions. J Exp Bot 72: 3367

Seo JK, Wu J, Lii Y, Li Y, Jin H (2013) Contribution of small RNA pathway components in plant immunity. Mol Plant-Microbe Interact 26: 617–625

Shaikh AA, Chachar S, Chachar M, Ahmed N, Guan C, Zhang P (2022) Recent Advances in DNA Methylation and Their Potential Breeding Applications in Plants. Horticulturae 8: 1–28

Shang JY, He XJ (2022) Chromatin-remodeling complexes: Conserved and plant-specific subunits in Arabidopsis. J Integr Plant Biol 64: 499–515

Shilpa, Thakur RK, Prasad P, Bhardwaj SC, Gangwar OP, Kumar S (2022) Epigenetics of wheat–rust interaction: an update. Planta 255: 1–17

Shin H, Choi WL, Lim JY, Huh JH (2022) Epigenome editing: targeted manipulation of epigenetic modifications in plants. Genes and Genomics 44: 307–315

Sivan A, Rahimi O, Lavi B, Salmon-Divon M, Weiss E, Drori E, Hübner S (2021) Genomic evidence supports an independent history of Levantine and Eurasian grapevines. Plants People Planet 3: 414–427

Song ZT, Liu JX, Han JJ (2021) Chromatin remodeling factors regulate environmental stress responses in plants. J Integr Plant Biol 63: 438–450

Sudan J, Raina M, Singh R (2018) Plant epigenetic mechanisms: role in abiotic stress and their generational heritability. 3 Biotech 8: 1–12

Tang J, Gu X, Liu J, He Z (2021) Roles of small RNAs in crop disease resistance. Stress Biol 1: 1–15

Tao Z, Yan F, Hahn M, Ma Z (2023) Regulatory roles of epigenetic modifications in plant-phytopathogen interactions. Crop Heal 1: 1–20

Taskesenlioglu MY, Ercisli S, Kupe M, Ercisli N (2022) History of Grape in Anatolia and Historical Sustainable Grape Production in Erzincan Agroecological Conditions in Turkey. Sustain 14: 1–16

This P, Lacombe T, Thomas MR (2006) Historical origins and genetic diversity of wine grapes. Trends Genet 22: 511–519

Tirnaz S, Merce C, Bayer PE, Severn-Ellis AA, Edwards D, Batley J (2020) Effect of leptosphaeria maculans infection on promoter DNA methylation of defence genes in brassica napus. Agronomy 10: 1–12

Tirnaz S, Miyaji N, Takuno S, Bayer PE, Shimizu M, Akter MA, Edwards D, Batley J, Fujimoto R (2022) Whole-Genome DNA Methylation Analysis in Brassica rapa subsp. perviridis in Response to Albugo candida Infection. Front Plant Sci 13: 1–15

Tompkins JD (2022) Discovering DNA Methylation, the History and Future of the Writing on DNA. J Hist Biol 55: 865–887

Topart C, Werner E, Arimondo PB (2020) Wandering along the epigenetic timeline. Clin Epigenetics 12: 1–13

Ueda M, Seki M (2020) Histone modifications form epigenetic regulatory networks to regulate abiotic stress response1[OPEN]. Plant Physiol 182: 15–26

Vezzulli S, Vecchione A, Stefanini M, Zulini L (2018) Downy mildew resistance evaluation in 28 grapevine hybrids promising for breeding programs in Trentino region (Italy). Eur J Plant Pathol 150: 485–495

Waheed S, Anwar M, Saleem MA, Wu J, Tayyab M, Hu Z (2021) The critical role of small rnas in regulating plant innate immunity. Biomolecules 11: 1–21

Walley JW, Rowe HC, Xiao Y, Chehab EW, Kliebenstein DJ, Wagner D, Dehesh K (2008) The chromatin remodeler SPLAYED regulates specific stress signaling pathways. PLoS Pathog 4: 1–8

Wang B, Yang X, Wang Y, Xie Y, Zhou X (2018) Tomato Yellow Leaf Curl Virus V2 Interacts with Host Histone Deacetylase 6 To Suppress Methylation-Mediated Transcriptional Gene Silencing in Plants. J Viology 92: 1–16

Wang J, Liu C, Chen Y, Zhao Y, Ma Z (2021) Protein acetylation and deacetylation in plant-pathogen interactions. Environ Microbiol 23: 4841–4855

Wang L, Chen H, Li J, Shu H, Zhang X, Wang Y, Tyler BM, Dong S (2020) Effector gene silencing mediated by histone methylation underpins host adaptation in an oomycete plant pathogen. Nucleic Acids Res 48: 1790–1799

Wang Y, Hu Q, Wu Z, Wang H, Han S, Jin Y, Zhou J, Zhang Z, Jiang J, Shen Y, Shi H, Yang W (2017) HISTONE DEACETYLASE 6 represses pathogen defence responses in Arabidopsis thaliana. Plant Cell Environ 40: 2972–2986

Wendte JM, Zhang Y, Ji L, Shi X, Hazarika RR, Shahryary Y, Johannes F, Schmitz RJ (2019) Epimutations are associated with CHROMOMETHYLASE 3-induced de novo DNA methylation. Elife 8: 1–27

Wilkinson SW, Mageroslashy MH, Lopez Sanchez A, Smith LM, Furci L, Cotton TEA, Krokene P, Ton J (2019) Surviving in a Hostile World: Plant Strategies to Resist Pests and Diseases. Annu Rev Phytopathol 57: 505–529

Xiao D, Zhou K, Yang X, Yang Y, Ma Y, Wang Y (2021) Crosstalk of DNA Methylation

Triggered by Pathogen in Poplars With Different Resistances. Front Microbiol 12: 1–15

Yan L, Fan G, Li X (2019) Genome-wide analysis of three histone marks and gene expression in Paulownia fortunei with phytoplasma infection. BMC Genomics 20: 1–14

Yang L, Chen X, Wang Z, Sun Q, Hong A, Zhang A, Zhong X, Hua J (2020) HOS15 and HDA9 negatively regulate immunity through histone deacetylation of intracellular immune receptor NLR genes in Arabidopsis. New Phytol 226: 507–522

Yao Q, Song CX, He C, Kumaran D, Dunn JJ (2012) Heterologous expression and purification of Arabidopsis thaliana VIM1 protein: In vitro evidence for its inability to recognize hydroxymethylcytosine, a rare base in Arabidopsis DNA. Protein Expr Purif 83: 104–111

Yin L, An Y, Qu J, Li X, Zhang Y, Dry I, Wu H, Lu J (2017) Genome sequence of Plasmopara viticola and insight into the pathogenic mechanism. Sci Rep 7: 1–12

Yin X, Fu Q, Shang B, Wang Y, Liu R, Chen T, Xiang G, Dou M, Liu G, Xu Y (2022) An RxLR effector from Plasmopara viticola suppresses plant immunity in grapevine by targeting and stabilizing VpBPA1. Plant J 112: 104–114

Yu A, Lepère G, Jay F, Wang J, Bapaume L, Wang Y, Abraham AL, Penterman J, Fischer RL, Voinnet O, Navarro L (2013) Dynamics and biological relevance of DNA demethylation in Arabidopsis antibacterial defense. Proc Natl Acad Sci U S A 110: 2389–2394

Yu Y, Jiao L, Fu S, Yin L, Zhang Y, Lu J (2015) Callose Synthase Family Genes Involved in the Grapevine Defense Response to Downy Mildew Disease. Phytopathology 106: 56–64

Zarreen F, Chakraborty S (2020) Epigenetic regulation of geminivirus pathogenesis: A case of relentless recalibration of defence responses in plants. J Exp Bot 71: 6890–6906

Zdunić G, Preece JE, Aradhya M, Velasco D, Koehmstedt A, Dangl GS (2013) Genetic diversity and differentiation within and between cultivated (Vitis vinifera L. ssp. sativa) and wild (Vitis vinifera L. ssp. sylvestris) grapes. Vitis - J Grapevine Res 52: 29–32

Zeng W, Huang H, Lin X, Zhu C, Kosami K ichi, Huang C, Zhang H, Duan CG, Zhu JK, Miki D (2021) Roles of DEMETER in regulating DNA methylation in vegetative tissues and pathogen resistance. J Integr Plant Biol 63: 691–706

Zhang H, Lang Z, Zhu JK (2018) Dynamics and function of DNA methylation in plants. Nat Rev Mol Cell Biol 19: 489–506

Zhi P, Chang C (2021) Exploiting Epigenetic Variations for Crop Disease Resistance Improvement. Front Plant Sci 12: 1–13

Zhi P, Kong L, Liu J, Zhang X, Wang X, Li H, Sun M, Li Y, Chang C (2020) Histone deacetylase TaHDT701 functions in TaHDA6-TaHOS15 complex to regulate wheat defense responses to blumeria graminis f.sp. tritici. Int J Mol Sci 21: 1–17

Zhou Y, Massonnet M, Sanjak JS, Cantu D, Gaut BS (2017) Evolutionary genomics of grape (Vitis vinifera ssp. vinifera) domestication. Proc Natl Acad Sci U S A 114: 11715–11720

Zhu C, Liu T, Chang YN, Duan CG (2019) Small RNA functions as a trafficking effector in plant immunity. Int J Mol Sci 20:

Zou B, Sun Q, Zhang W, Ding Y, Yang DL, Shi Z, Hua J (2017) The arabidopsis chromatin-remodeling factor CHR5 regulates plant immune responses and nucleosome occupancy. Plant Cell Physiol 58: 2202–2216

Zvereva AS, Pooggin MM (2012) Silencing and innate immunity in plant defense against viral and non-viral pathogens. Viruses 4: 2578–2597

Chapter II - Analysis of grapevine susceptibility to *Plasmopara viticola*

II.1. Introduction

Viticulture is one of the most important agricultural activities worldwide. In 2020, 7.3 million hectares were estimated to be cultivated as vineyards (OIV 2021). The cultivated *Vitis vinifera* L. is highly susceptible to downy mildew, caused by the obligatory oomycete *Plasmopara viticola* (Berk. & Curtis) Berl. & De Toni, which significantly influences the plant lifespan, fruit quality and production quantity (Boso et al. 2014; Armijo et al. 2016; Buonassisi et al. 2017; Brilli et al. 2018; Eisenmann et al. 2019). Grapevine, when infected with *P. viticola* present damage in various organs but mainly on leaves and berries which decreases the quantity/quality of the products (Toffolatti et al. 2012; Banani et al. 2014; Buonassisi et al. 2017; Vezzulli et al. 2018). Spore propagation and plant infection depend on optimal environmental conditions (temperature and humidity), as well as the fungi's dual reproductive life cycle (sexual and asexual), which allows for more frequent outbreaks (Toffolatti et al. 2012; Buonassisi et al. 2017; Micheloni 2017; Fröbel and Zyprian 2019; Marfil et al. 2019).

High amounts of pesticides are used during the growing season to control this disease, harbouring both environmental and health impacts. To promote a more sustainable approach to control this disease, efforts have been conducted to expand the knowledge of this interaction and breeding new cultivars resistant to *P. viticola* have become a promising approach (Tirnaz and Batley 2019). The *P. viticola* impact on the Eurasian grapevines (the subspecies used for agricultural activities) leads to very frail outcome to the plant indicating its susceptibility meanwhile the American and Asian species has shown a resistant capability to this pathogen (Bove and Rossi 2020). The machinery that identified has resistance traits can occur as a cascade of events from reactive oxygen species, polyphenolic compounds production/accumulation, cell wall modification, enhancement of defense genes and enzymes, programmed cell death, necrosis and hypersensitive response (HR) (Malacarne et al. 2011; Perazzolli et al. 2012; Yu et al. 2012; Nascimento et al. 2019; Bove and Rossi 2020; Liu et al. 2020).

Breeding programs have been developed to introduce traits associated with biotic stress resistance while maintaining the good grape quality of *V. vinifera* (Li et al. 2015; Toffolatti et al. 2018). The way the breeding programs are able to improve crop resistance is through introgressive hybridization also known in viticulture as interspecific hybrids for a genetic variation improvement and acquisition of favourable genetic traits (Foria et al. 2022).

Several different genes have been transferred within the plant breeding programs for *V. vinifera* that can confer total or partial resistance to pathogens known as chromosomal locus resistant to *P. viticola* (RPV) such as RPV3–1, RPV3–2, RPV3–3, RPV10, RPV12 and RPV1/Run1 from a total of 33 RPVs already mapped (Bove and Rossi 2020; Foria et al. 2022; Possamai and Wiedemann-Merdinoglu 2022). These quantitative trait loci (QTL) have been identified in the several different studies focused on species of *Vitis* with different resistances/tolerances (Possamai and Wiedemann-Merdinoglu 2022). Breeding programs has tried to respect the demands of agro-economic activity search through improvement of different phenotypic characteristics (such as seeds and berry size) thus using *V. vinifera* with a Eurasian background crossed with other species resistant cultivars (American and Asia) which therefore gather one or multiple RPVs (Bove and Rossi 2020; Atak and Şen 2021). Several successful examples already being commercialized as Regent, Solaris and Calardis Blanc in regard of biotic resistance (Zini et al. 2019; Bove and Rossi 2020). Nevertheless, the resistance to implement introgression lines in the normal agricultural activities comes from preconceived ideas as well as historical agrochemical usage therefore the development of new disease resistance crossing lines, improvement of the quality status for mass production and reduction of fungicide application is still an ongoing process (Toffolatti et al. 2012; Vezzulli et al. 2018; Foria et al. 2022; Töpfer and Trapp 2022).

New different breeding programs focus on grapevine populations with only Eurasian background to obtain the desired and improved characteristics as well as fight diseases. In recent years, downy mildew disease affected a high percentage of vineyards in the Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di ricerca Viticoltura ed Enologia (CREA-VE) at Turi (Apulia Region, Italy). To better assess the tolerance/susceptibility level of table grape varieties/crossings to *P. viticola*, several assays in different years and on different organs have been performed by the CREA-VE researchers. The research focused on visual analysis which showed possible differences of susceptibility in several varieties as well as in the crossing populations (Marsico et al. 2018). In the literature generally *V. vinifera* is described as highly susceptible to *P. viticola* (Bove and Rossi 2020; Foria et al. 2022). Nevertheless, several authors have observed that the European *V. vinifera* cultivars can have different levels of susceptibility (Boso and Kassemeyer 2008; Boso et al. 2014; Bove and Rossi 2020; Foria et al. 2022). The breeding programme existant in CREA-VE created a cross population with parenthood Red Globe (female) x Regal seedless (male). With previous evaluations, the *P. viticola* infection in different grapevine cultivars and crossing were

an indication of possible different levels of susceptibility after plant-pathogen interaction. Therefore, the aim of our work is to identify grapevine crossing populations with opposite susceptibility to *P. viticola*.

II.2. Material and Methods

The *V. vinifera* accessions and crossing hybrids tolerance to *P. viticola* was previously evaluated through controlled inoculations of grape bunches and leaf disks (Table S1 and S2). The data collected from this evaluation and statistical analysis performed was the starting point for the work developed in this chapter (Table S3, Figure S1, Figure S2) with the intent to further analyse infected leaves *versus* mock inoculated with downy mildew disease.

II.2.1. In planta assay for the evaluation of V. vinifera susceptibility levels to P. viticola infection

The data referred (Table S3) allowed for the selection of the most contrasting genotypes. Wood cuttings from both selected cultivars (Italia, Red Globe and Crimson Seedless) and new genotypes (N20/020, N23/018, N20/012 and N20/029) were obtained from the experimental vineyard of the CREA-VE, located in Rutigliano (Apulia region, Italy). Grapevine cuttings were harvested, cut in similar sizes (with 2 buds per cutting) and partially immersed in a 30°C water bath within a 4°C chamber for 2 months. This system allows the radicular system to grow without the rapid development of the rest of the plant. Then, the cuttings were transferred to square plastic pots with a mixture of seedling substrate and perlite (3:1 v/v) and placed in a greenhouse for 2 months with natural light conditions at a temperature between 18°C and 25°C.

The *P. viticola* sporangia were collected from symptomatic grapevine leaves by brushing. A sporangia suspension ($1.8x10^4$ sporangia/ml) was sprayed on the abaxial side of the leaves (Figueiredo et al. 2017a) of the different grapevine genotypes. As a control of the *in vivo* assay, plants were sprayed with water (mock inoculation). After inoculation, plants were covered with plastic bags to create a moist chamber with high humidity and kept in the dark for 8h. Then, plants were maintained under greenhouse conditions. At 4, 6, 24 and 48 hours post infection

(hpi), the third to fifth fully expanded leaves were collected, frozen in liquid nitrogen and stored at -80°C (Figure II. 1A). For each cultivar and time point (inoculated and mock inoculated), 3 biological replicates were collected consisting of 3 leaves from 3 different plants.

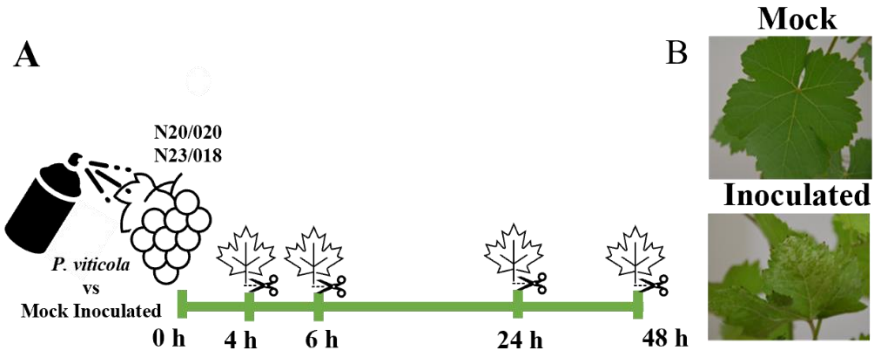


Figure II. 1 - Artificial inoculation of grapevine potted plants with *P. viticola* (*in planta* assay). A – Representation of the experimental protocol performed with collection of leaves of grapevine crossings at 4, 6, 24 and 48 hours after inoculation (hpi) with *P. viticola* and mock (control) conditions; B – Representative images of grapevine leaves with typical oil-spots lesions observed on leaves inoculated with the pathogen compared to healthy mock inoculated plants (controls)

The OIV descriptor 452-1 (OIV 2009) was used with some modifications (Table II.1) to evaluate infection. After infection assessment in the different varieties/crossings, Disease Incidence (DI) and Disease Severity (DS) were calculated on the different experimental assays. The percentage of DI was obtained following the formula (Ahmed 2018):

$$DI(\%) = \frac{n}{N} \times 100$$

The formula corresponding to the number of infected leaves (n) per total number of leaves (N) while DS was obtained by complying with the formula of Townsend-Heuberger (Lo Scalzo et al. 2012)

$$DS(\%) = \frac{\sum^{i} 1\ (n_i\ x\ v_i)}{NxV} \times 100$$

The infected leaves in one class (n_i), the class (v_i), the number of classes (i), the total number of leaves (N) and the highest class (V) are the main components of this formula (Lo Scalzo et al. 2012).

Table II. 1 - Classification of *P. viticola* infection signs on leaves, shoots and plant size after infection. When in the presence of sporangiophores on the abaxial surface of the leaf, the classification attributed was upgraded to the next class.

Class	oil spots lesion (%)	shoot infection (%)	Plant Size
0	0	0	cuttings without leaves
1	5-20	Only Apex affected	Stem with small number of leaves
2	25-40	25	Medium stem with medium number of leaves
3	45-60	50	Big Stem with allot of leaves
4	65-80	100	-
5	85-100	Shoot dead	-

II.2.2. Statistical analysis of the results collected from cultivars and crossing hybrid population inoculated with *P. viticola*

Data was evaluated to assess its distribution (Shapiro-Wilk's test) and homogeneity of variances (Levene's test). As data did not follow a normal distribution, a non-parametric statistic approach was used. In particular, permutational multivariate analysis of variance (PERMANOVA test (Anderson 2017), one-way ANOVA on ranks (Kruskal-Wallis H test (Kruskal and Wallis 1952) and Conover's test of multiple comparisons using rank sums as post hoc test were used to assess the statistical differences between experimental conditions. All the statistical analyses, as well as a descriptive analysis of the data were performed in R studio (version 3.5.0), from eigenvalue, the percentage of variance and the Principal Component Analysis (PCA).

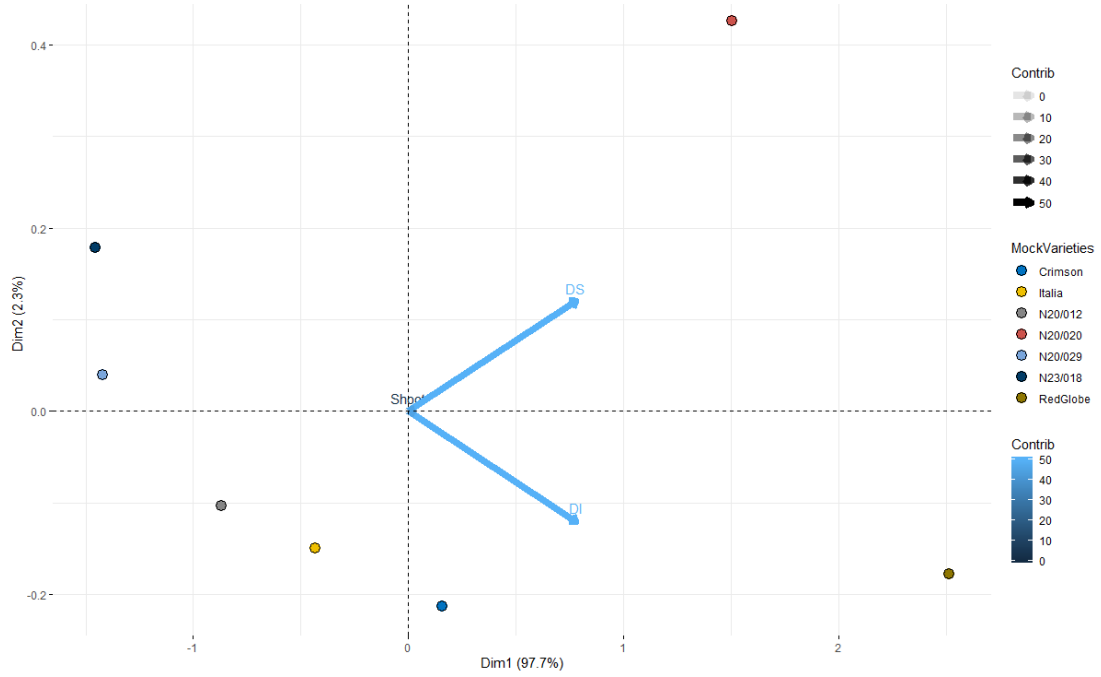
II.3. Results

II.3.1. V. vinifera table grape crossing populations can portray susceptible or tolerant characteristics to Downy mildew

The *in planta* assay phenotypic information allowed us to observe that in the inoculated plants there was a positive correlation ($p<0.05$) between all the variables analysed (DI, DS and Shoot infection). This correlation was verified through the analysis of the eigenvalue, the percentage of variance and the PCA (Figure II. 2). Also, in Figure II. 2B the PCA analysis shows a

categorization in two clusters of the inoculated plants. One comprehends N20/012, Crimson Seedless, Red Globe, N20/020 and Italia (more susceptible to *P. viticola*) while the other group is formed by N20/029 and N23/018 (less susceptible to *P. viticola*).

A



B

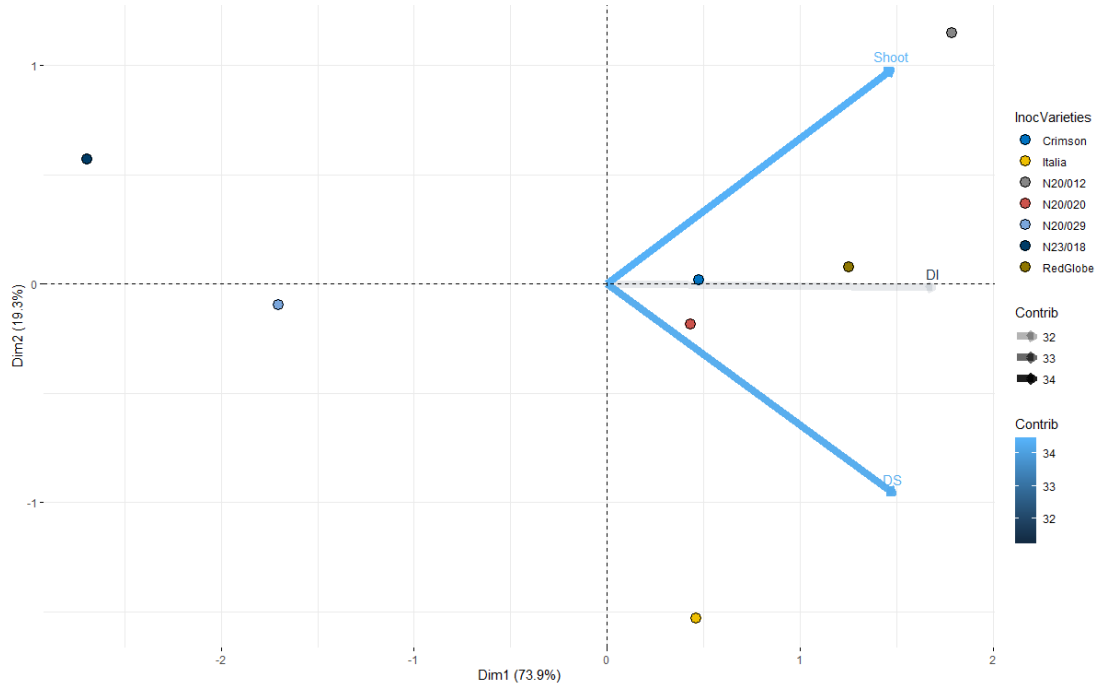


Figure II. 2 - Principal component analysis biplot of the phenotypic data from the *Vitis vinifera* cultivars and the progenies in the *in vivo* assay. The arrows positioning indicates the contribution of the variables (Disease incidence – DI, Disease severity – DS and Shoot) to the PCA components. A – Data from mock inoculated plants; B –Inoculated plants data.

With the statistical analysis it was possible to verify that in the overall data (mock and inoculated samples) there was neither a normal distribution nor a homogeneous variance. The PERMANOVA test showed that between conditions (mock and inoculated) there was statistically significant difference ($p<0.05$). With this information we bottlenecked our data to analyse only the inoculated phenotypic data. Regarding this, the statistical analysis shows that though Mardia's test and Levene's test the distribution maintained abnormal while the homogeneity of variance showed that only the shoot variable was statistically significant ($p<0.05$). With the PERMANOVA test, it was possible to verify a statistically significant difference ($p<0.05$) between varieties/crossings, when infected with *P. viticola*, but not between biological replicates.

To observe the statistical difference of each dependent variable (DI, DS and Shoot infection), the Kruskal-Wallis test and the Conover's test were performed. As shown in Table II.2, there was a significant difference of the several varieties/crossings inoculated in the variables analysed. The cultivar Red Globe and progeny N23/018 exhibited opposite responses in the different parameters (Table II.2 and Table S3).

Table II. 2 - Percentage of Disease Incidence (DI) and Severity (DS) of *P. viticola* infection in table grape varieties and crossings. The values with different letters are statistically different according to non-parametric Conover's test.

	Table Grape	***In planta* assay (Leaves)**				***In planta* assay (Shoot)**	
		DI (%)		**DS (%)**		**DI (%)**	
Varieties	**Regal Seedless**	-		-		-	
	Italia	47.5	*ab*	27.1	*ab*	0.3	*ab*
	Crimson Seedless	61.9	*ab*	15.6	*ab*	0.6	*ab*
	Red Globe	56.8	*b*	21.6	*b*	1.2	*ab*
Crossings	**N20/012**	58.3	*ab*	19.4	*ab*	1.9	*a*
	N20/020	51.5	*ab*	19.4	*ab*	0.7	*ab*
	N20/029	35.0	*ab*	10.7	*ab*	0.0	*b*
	N23/018	22.1	*a*	5.3	*a*	0.1	*b*

These data (Table II.2, Figure II. 3), together with the results from previous tests performed on these genotypes (Figures S1 and S2, Table S3) suggests that Red Globe and N20/020 are more susceptible to *P. viticola*, while N23/018 seems to be more tolerant to this oomycete.

Figure II. 3 - Effect of *P. viticola* inoculation on the development of the progeny and the parent Red Globe (♀) at 13 days after inoculation.

Among the crossing, N20/020 and N23/018 present, respectively, higher and lower susceptibility levels to *P. viticola* when compared to the other genotypes for all the performed assays, thus they were selected for the following molecular analyses.

II.4. Discussion

Domestication of *Vitis vinifera* species worldwide was intendant to improved cultivars for an economical point of view with a low genetic variability leading to increased problems with biotic and abiotic stresses (Csótó et al. 2023). Several breeding programs are using the introgression of resistance traits into grapevine cultivars with economic interest (Boso and Kassemeyer 2008; Boso et al. 2014; Marsico et al. 2018). Although, the success of the introgression hybrids, for wine purposes, these new specimens were not able to achieve the quality required (Töpfer and Trapp 2022). The main requirements to be able to approve hybrids for agricultural purposes go from climate change impact, environment resilient and quality (Töpfer and Trapp 2022).

In our study, the cultivar selection was based on the importance of these genotypes in the Italian table grape production and the levels of susceptibility measured in previous studies. The high variability in the susceptibility level observed among different cultivars and crossings and the presence of genotypes characterized by low susceptibility to *P. viticola* indicate possible defense traits in *Vitis vinifera* interesting to explore and eventually to transfer within breeding programs. The *in plant* assay, although performed with different experimental conditions from

the previous studies, revealed that some varieties/progenies presented similar susceptibility traits. The multivariate data set revealed two clusters in the PCA analysis which provides information about the varieties/crossings that are putatively more susceptible or tolerant to *P. viticola*.

In regards of the *P. viticola* used in the plant assays, it consists of a population of the pathogen instead of a single strain. The reason behind this choice was to mimic, as much as possible, the dynamics of the interaction in the field and to minimize the constrains that a specific pathogen strain could have on the susceptibility/tolerance response point of view (Bove and Rossi 2020)

The most consistent and interesting data when comparing with all assays performed, comes from the crossings N20/020 and N23/018 (putatively susceptible and tolerant, respectively). In the literature, it is known that the cross population with a cross background of an American or Asian *Vitis* variety leads to the transfer of pyrimed RPV, as observed by Bove and Rossi (Bove and Rossi 2020). However, in the case of these cross-hybrids with a parenthood pedigree of *Vitis vinifera sub. sativa* (Red Globe and Regal Seedless), this difference of susceptibility can be a consequence of transcriptomic, metabolomic or epigenetic influence/modification therefore reacting differently the pathogen invasion (Figueiredo et al. 2017b; Laureano et al. 2018; Pereira et al. 2022).

II.5. References

Ahmed MFA (2018) Evaluation of some biocontrol agents to control Thompson seedless grapevine powdery mildew disease. Egypt J Biol Pest Control 28: 1–7

Anderson MJ (2017) Permutational Multivariate Analysis of Variance (PERMANOVA). Wiley StatsRef Stat Ref Online 1–15

Armijo G, Schlechter R, Agurto M, Muñoz D, Nuñez C, Arce-Johnson P (2016) Grapevine Pathogenic Microorganisms: Understanding Infection Strategies and Host Response Scenarios. Front Plant Sci 7: 1–18

Atak A, Şen A (2021) A grape breeding programme using different Vitis species. Plant Breed 140: 1136–1149

Banani H, Roatti B, Ezzahi B, Giovannini O, Gessler G, Pertot I, Perazzolli M (2014) Characterization of resistance mechanisms activated by Trichoderma harzianum T39 and

benzothiadiazole to downy mildew in different grapevine cultivars. Plant Pathol 63: 334–343

Boso S, Alonso-Villaverde V, Gago P, Santiago JL, Martínez MC (2014) Susceptibility to downy mildew (Plasmopara viticola) of different Vitis varieties. Crop Prot 63: 26–35

Boso S, Kassemeyer HH (2008) Different susceptibility of European grapevine cultivars for downy mildew. Vitis - J Grapevine Res 47: 39–49

Bove F, Rossi V (2020) Components of partial resistance to Plasmopara viticola enable complete phenotypic characterization of grapevine varieties. Sci Rep 10: 1–12

Brilli M, Asquini E, Moser M, Bianchedi PL, Perazzolli M, Si-Ammour A (2018) A multi-omics study of the grapevine-downy mildew (Plasmopara viticola) pathosystem unveils a complex protein coding and noncoding-based arms race during infection. Sci Rep 8: 1–12

Buonassisi D, Colombo M, Migliaro D, Dolzani C, Peressotti E, Mizzotti C, Velasco R, Masiero S, Perazzolli M, Vezzulli S (2017) Breeding for grapevine downy mildew resistance: a review of "omics" approaches. Euphytica 1–21

Csótó A, Nagy A, Laurinyecz N, Nagy ZA, Csaba N, Németh EK, Csikász-Krizsics A, Rakonczás N, Fontaine F, Fekete E, Flipphi M, Karaffa L, Sándor E (2023) Hybrid Vitis Cultivars with American or Asian Ancestries Show. 1–16

Eisenmann B, Czemmel S, Ziegler T, Buchholz G, Kortekamp A, Trapp O, Rausch T, Dry I, Bogs J (2019) Rpv3–1 mediated resistance to grapevine downy mildew is associated with specific host transcriptional responses and the accumulation of stilbenes. BMC Plant Biol 19: 1–17

Figueiredo A, Martins J, Sebastiana M, Guerreiro A, Silva A, Matos AR, Monteiro F, Pais MS, Roepstorff P, Coelho AV (2017a) Specific adjustments in grapevine leaf proteome discriminating resistant and susceptible grapevine genotypes to Plasmopara viticola. J Proteomics 152: 48–57

Figueiredo J, Maia M, Sousa Silva M, Figueiredo A (2017b) Grapevine Subtilase Family: Update on New Sequences and Nomenclature Proposal. Front Plant Sci 8: 1–3

Foria S, Magris G, Jurman I, Schwope R, De Candido M, De Luca E, Ivanišević D, Morgante M, Di Gaspero G (2022) Extent of wild-to-crop interspecific introgression in grapevine (Vitis vinifera) as a consequence of resistance breeding and implications for the crop

species definition. Hortic Res 9: 1–16

Fröbel S, Zyprian E (2019) Colonization of Different Grapevine Tissues by Plasmopara viticola—A Histological Study. Front Plant Sci 10: 1–13

Kruskal WH, Wallis WA (1952) Use of ranks in one-criterion variance analysis. J Am Stat Assoc 47: 583–621

Laureano G, Figueiredo J, Cavaco AR, Duarte B, Caçador I, Malhó R, Sousa Silva M, Matos AR, Figueiredo A (2018) The interplay between membrane lipids and phospholipase A family members in grapevine resistance against Plasmopara viticola. Sci Rep 8: 1–15

Li X, Wu J, Yin L, Zhang Y, Qu J, Lu J (2015) Comparative transcriptome analysis reveals defense-related genes and pathways against downy mildew in Vitis amurensis grapevine. Plant Physiol Biochem 95: 1–14

Liu L, Zhang B, Wang H, Yu SY, Guan TS, Huang YF, Liu CY (2020) Candidate resistance genes selection and transcriptome analysis for the early responses to Plasmopara viticola infection in grape cultivars. J Plant Pathol 102: 857–869

Malacarne G, Vrhovsek U, Zulini L, Cestaro A, Stefanini M, Mattivi F, Delledonne M, Velasco R, Moser C (2011) Resistance to Plasmopara viticola in a grapevine segregating population is associated with stilbenoid accumulation and with specific host transcriptional responses. BMC Plant Biol 11: 1–13

Marfil C, Ibañez V, Alonso R, Varela A, Bottini R, Masuelli R, Fontana A, Berli F (2019) Changes in grapevine DNA methylation and polyphenols content induced by solar ultraviolet-B radiation, water deficit and abscisic acid spray treatments. Plant Physiol Biochem 135: 287–294

Marsico AD, Perniola R, Bergamini C (2018) Nuovi genotipi di V. vinifera poco sensibili a Peronospora ottenuti dal Crea. Riv di Fruttic e di Ortofloric 82: 26–30

Micheloni C (2017) Diseases and pests in viticulture

Nascimento R, Maia M, Ferreira AEN, Silva AB, Freire AP, Cordeiro C, Silva MS, Figueiredo A (2019) Early stage metabolic events associated with the establishment of Vitis vinifera – Plasmopara viticola compatible interaction. Plant Physiol Biochem 137: 1–13

OIV (2009) 2ND Edition of the OIV Descriptor List for Grape Varieties and Vitis species.

doi.org/10.1192/bjp.111.479.1009-a

OIV (2021) State of the world vitivinicultural sector in 2020. Int Organ Vine Wine 1–19

Perazzolli M, Moretto M, Fontana P, Ferrarini A, Velasco R, Moser C, Delledonne M, Pertot I (2012) Downy mildew resistance induced by Trichoderma harzianum T39 in susceptible grapevines partially mimics transcriptional changes of resistant genotypes. BMC Genomics 13: 1–19

Pereira G, Pereira J, Santos RB, Figueiredo A (2022) Uncovering the role of DNA methyltransferases in grapevine – Plasmopara viticola interaction: From genome-wide characterization to global methylation patterns. Gene 837: 1–9

Possamai T, Wiedemann-Merdinoglu S (2022) Phenotyping for QTL identification: A case study of resistance to Plasmopara viticola and Erysiphe necator in grapevine. Front Plant Sci 13: 1–22

Lo Scalzo R, Fibiani M, Pietromarchi P, Mandalà C, La Torre A (2012) Effects of different fungicide treatments on grape, must and wine quality. Commun Agric Appl Biol Sci 77: 151–161

Tirnaz S, Batley J (2019) DNA Methylation: Toward Crop Disease Resistance Improvement. Trends Plant Sci 24: 1137–1150

Toffolatti SL, De Lorenzis G, Costa A, Maddalena G, Passera A, Bonza MC, Pindo M, Stefani E, Cestaro A, Casati P, Failla O, Bianco PA, Maghradze D, Quaglino F (2018) Unique resistance traits against downy mildew from the center of origin of grapevine (Vitis vinifera). Sci Rep 8: 1–11

Toffolatti SL, Venturini G, Maffi D, Vercesi A (2012) Phenotypic and histochemical traits of the interaction between Plasmopara viticola and resistant or susceptible grapevine varieties. BMC Plant Biol 12: 1–16

Töpfer R, Trapp O (2022) A cool climate perspective on grapevine breeding: climate change and sustainability are driving forces for changing varieties in a traditional market. Theor Appl Genet 135: 3947–3960

Vezzulli S, Vecchione A, Stefanini M, Zulini L (2018) Downy mildew resistance evaluation in 28 grapevine hybrids promising for breeding programs in Trentino region (Italy). Eur J Plant Pathol 150: 485–495

Yu Y, Zhang Y, Yin L, Lu J (2012) The mode of host resistance to Plasmopara viticola infection of grapevines. Phytopathology 102: 1094–1101

Zini E, Dolzani C, Stefanini M, Gratl V, Bettinelli P, Nicolini D, Betta G, Dorigatti C, Velasco R, Letschka T, Vezzulli S (2019) R-Loci Arrangement Versus Downy and Powdery Mildew Resistance Level: A Vitis Hybrid Survey. Int J Mol Sci 20: 1–29

Chapter III - Transcriptomic and methylation analysis of susceptible and tolerant grape genotypes following *Plasmopara viticola* infection

III.1. Introduction

The grapevine defense system relies on intricate communication between host and pathogen, leading to broad physiological modulation and the activation of molecular layers of defense. Previous studies have shown that a broad modulation of transcripts, proteins, lipids, and metabolites occurs in the first hours of interaction between grapevine and *P. viticola* (Buonassisi et al. 2017; Figueiredo et al. 2017, 2018; Laureano et al. 2018; Fröbel and Zyprian 2019; Maia et al. 2019; Nascimento et al. 2019; Liu et al. 2020b; Santos et al. 2020). Upon being challenged with biotic or abiotic stresses, plants can create a "memory" of the response obtained. This response is known as defense priming and it can also be mediated by different epigenetic mechanisms (Espinas et al. 2016; Lämke and Bäurle 2017; Ramirez-Prado et al. 2018a; Laurell et al. 2021). Epigenetic modulation is known to regulate gene expression that leads to phenotypic plasticity aiming at plant adaptation and survival to external stimuli (Gallusci et al. 2017; Xie et al. 2017; Yan et al. 2019; Ramos-Cruz et al. 2021). Epigenetic modifications may affect plant defence at a long-term level contributing to a transgenerational inheritable defence strategy (Yang et al. 2011; Espinas et al. 2016; Lämke and Bäurle 2017; Xie et al. 2017; Ramirez-Prado et al. 2018c; Laurell et al. 2021).

In the context of plant-pathogen interactions, epigenetic modulation has been shown to impact the outcome of the host defense. Several mechanisms leading to epigenetic modifications have been described so far, including DNA methylation, chromatin rearrangement, histone modifications and the establishment of gene silencing through RNA interference (RNAi) (Espinas et al. 2016; Barozai and Aziz 2018; Hoang et al. 2018; Ramirez-Prado et al. 2018c; Yan et al. 2019; Ramos-Cruz et al. 2021). Several studies on DNA methylation have been performed on different types of host-pathogens interaction (Huang and Jin 2022). As an adaptive strategy to biotic stresses, plant DNA methylation may lead to a broad transcriptome reprogramming since the pressure on the different positions of the cytosine methylation patterns on different genomic regions could influence the plant defence gene response and furthermore obtain distinct responses (Dowen et al. 2012; Deleris et al. 2016; Espinas et al. 2016; Sánchez et al. 2016; Köhler and Springer 2017; Brocklehurst et al. 2018; Ali et al. 2020; Rambani et al. 2020; Ramos-Cruz et al. 2021; Zhi and Chang 2021). Sánchez and authors reported that Arabidopsis mutants at the epigenetic mechanism level (Nuclear DNA-dependent RNA polymerase V - *nrpe1* - from the RdDM pathway and Repressor of Silencing 1 - *ros1*- from the DNA demethylation pathway) presented a resistance and susceptible phenotype,

respectively, when infected with *Hyaloperonospora arabidopsidis* and a possible corelation between DNA methylation Salicylic Acid (SA)-dependent defence against the pathogen (Sánchez et al. 2016).

Chromatin organisation is considered another important epigenetic mechanism (Berr et al. 2012; Ramirez-Prado et al. 2018c; Alonso et al. 2019; Panigrahi et al. 2021) and its role in plant-pathogen interactions was also highlighted recently. Several chromatin remodelling complexes have been analysed to play a role in the immune defense system against biotic stresses (Ramirez-Prado et al. 2018c; Alonso et al. 2019; Panigrahi et al. 2021). SWItch/Sucrose Non-Fermentable (SWI/SNF) complex and/or SWI2/SNF2-Related 1 Chromatin Remodeling Complex (SWR1) subunits are some of the chromatin remodelers that have important functions in the defense system as well as a regulatory impact on SA and Jasmonic Acid/Ethylene (JA/ET) pathways (March-Díaz et al. 2008; Berriri et al. 2016; Ramirez-Prado et al. 2018c; Panigrahi et al. 2021). Also, histone modification enzymes have been identified as influencers of the defence related networks, such as JA/ET, Abscisic Acid (ABA) and/or SA-dependent pathways which therefore could contribute to a resistance or susceptible phenotypic behaviour after the biotic stresses (De-La-Peña et al. 2012; Ding and Wang 2015; Ramirez-Prado et al. 2018a; Chen et al. 2020; Kim 2021).

Another important epigenetic mechanism is the RNA-directed DNA methylation (RdDM) which acts parallelly with other epigenetic mechanisms for plant development and defense response (López et al. 2011; Zhu et al. 2016; Ali et al. 2020). It is known that RdDM is involved in the DNA methylation pathway and associated with the RNAi mechanism as a plant defense mechanism, specifically reported in plant-viruses interactions (Erdmann and Picard 2020). López and colleagues have observed that mutant Arabidopsis, for several different components of the RdDM pathway (especially RNA Pol V), when infected with bacteria or fungi presented a resistance and susceptible phenotype, respectively and opposite regulation of the SA and JA pathways (López et al. 2011). Weiberg and colleagues identified, in *Botrytis cinerea* Pers.: Fr., small RNAs (sRNA) that silenced the plants RNAi machinery, specifically Argonaute 1, to undermine and weaken the host's immune defense system (Weiberg et al. 2013). Interestingly, the same research group reported that in RNAi transgenic *B. cinerea-Dicer-like* 1/2 (*DCL 1/2*) gene in Arabidopsis and tomato plants (without host DCL change) presented a lower disease manifestation and fungus *DCL* gene expression. It indicated that the hosts could counterattack against pathogens through the same RNAi strategy, which might be a possible novel agronomic approach through RNA-directed fungicides (Wang et al. 2016).

Epigenetic machinery has also been analysed as playing a role in the plant pathogen's development, pathogenesis and metabolism (Zhu et al. 2016; He et al. 2020a). Interestingly, Chen and colleagues reported that DNA methylation marks on *Phytophthora* oomycetes (positioned at the N6-methyladenine (6mA)) were influenced by their lifecycle and important virulent elements (effectors and transposable elements (TE)), suggesting that the activation of these patterns could lead to pathogen adaptation to plant defense (Chen et al. 2018; Rojas-Rojas and Vega-Arreguín 2021). Therefore, the DNA methylation mechanism influences plants and pathogens from an adaptive response perspective by expanding their phenotypical and genomic range for either organism's survival.

In grapevine, little is known about epigenetic regulation in the context of its interaction with pathogens, namely with *P. viticola*. To further understand the epigenetic regulation mechanisms underneath grapevine-*P. viticola* interaction, a 5-mC DNA methylation analysis was done during the first hours of contact between grapevine crossing hybrids and *P. viticola*. We have further looked into epigenetic and defense-related transcripts modulation during this interaction in order to characterise the main mechanisms behind grapevine response to the downy mildew pathogen.

III.2. Material and Method

III.2.1. DNA methylation evaluation

To evaluate the effect of pathogen on grapevine DNA methylation, global 5-Metyhlcytosine assay was performed.

III.2.1.1. Global 5-methylcytosine (5-mC) methylation assay

Leaf samples of the genotypes N20/020 (S - susceptible) and N23/018 (T - tolerant), collected at 6 and 24 hours after *P. viticola* or water inoculation (see Chapter II), were used for 5-mC

analysis. These two genotypes were selected for molecular analysis since all previously performed evaluations showed a constant phenotypic response to downy mildew infection.

III.2.1.1.1. DNA extraction

Genomic DNA isolation was performed using NucleoSpin Plant II (Macherey-Nagel) according to the manufacturer's instructions. Genomic DNA quality was evaluated at 260/280 nm and 260/230 nm using a spectrophotometer (NanoDrop-1000, Thermo Scientific). Prior to the global DNA methylation evaluation and further microarray analysis, each individual was analysed by conventional Polymerase Chain Reaction (PCR) (Table III.1) with primers (Table III.2) specifically design to identify *P. viticola* with a PCR product sizes approximately 591 base pairs (bp), as described by Kong and colleagues (Kong et al. 2016) . For each sample, the biological replicates showing presence (inoculated samples) or absence (mock control) of the *P. viticola*-specific amplicon were used for the following analyses.

Table III. 1 - Intensity scale observed in the Polymerase chain reaction (PCR) products.

Scale	Band Intensity
0	No Band
1	Faint band
2	Visible band
3	Defined Band
4	High intensity Band
5	Higher intensity band

Table III. 2 - Sequence of primers design on the *P. viticola cox2* gene (5'-3')

Pv-cox2_Forward	CAAGATCCAGCAACTCCAGTTATGGA
Pv-cox2_Reverse	ACATTGTCCATAAAAAACACCTTCTC

III.2.1.1.2. ELISA ASSAY

The 5-mC DNA ELISA Kit (Zymo Research, California) was used to measure the percentage (%) of 5-methylcytosine (5-mC) present in the genomic DNA, according to the manufacturer's instruction. The biological replicates were pooled for each sample. An Anti-5-mC monoclonal antibody, that is both sensitive and specific for 5-mC, was used. A horseradish peroxidase (HRP) conjugate was used as a secondary antibody. The absorbance was read at 405 nm, approximately 20 minutes after the colour development. Each sample was assessed in triplicate. According to the manufacturer's instructions, the percent 5-mC in a DNA sample has been quantified from a standard curve generated with *Escherichia coli* DNA methylated with a CpG methylase. Considering that the density of CpG dinucleotides varies between species, in order to quantitate the percentage of 5-mC, the calculated % 5-mC was multiplied by the fold difference in CpG density between *E. coli* and the sample species. Therefore, the % CpG methylation of each sample was corrected by the CpG/genome length ratio of *V. vinifera* (4.4749).

To determine the % of global methylation, the % CpG methylation was multiplied by the ratio of *V. vinifera* total cytosines/genome length (0.1483). The percentage of global 5-mC methylation for each sample was calculated as fold change of inoculated versus mock (control). Statistical analysis was also performed to compare the fold change between N20/020 and N23/018 at each time-point, using Welch's t-test from GraphPad Prism software version 8.2.1. 1 (Pereira et al. 2022)

The contribution of *P. viticola* DNA methylation was not taken into account for the global methylation calculations, as in Pereira and colleagues work (Pereira et al. 2022). It is reported that in oomycetes the methylation of adenines (6mA) is the more frequent DNA methylation modification (Chen et al. 2018; Atighi et al. 2020; Pereira et al. 2022).

III.2.2. Transcriptomics by microarray assay

Leaf samples of the genotypes N20/020 (S - susceptible) and N23/018 (T - tolerant) were collected at 6 and 24 hours after inoculation with *P. viticola* or water (see Chapter II) and used for high throughput gene expression analysis by microarray technique.

III.2.2.1. Microarrays

Total RNA extraction was performed from plant leaves using the Agilent Plant RNA Isolation Mini kit (Agilent Technologies, USA) according to the manufacturer's protocol. The total RNA purity and concentration were analysed by the measurement of the RNA at A260/280nm in a spectrophotometer (Nanodrop, Spain), 2100 Bioanalyzer (Agilent Technologies, USA) and Qubit 4 Fluorometer (Invitrogen, USA). Samples of biological replicates were pooled as previously described (Chapter III, paragraph III.2.1.2.2. ELISA ASSAY). A total of 1500 ng per pooled sample were used for the cDNA synthesis. Both cDNA synthesis, labelling and hybridization procedures were made in accordance with the manufacturer's instructions (Agilent Technologies Protocol version 6.9.1).

A custom *V. vinifera* Agilent array with 44 000 probes (Catacchio et al. 2019) was used. The Agilent Feature Extraction software version 12.0 (Agilent Technologies, USA) was used for the data extraction.

III.2.2.1.1. Statistical Analysis

To improve the reproducibly of the microarray gene expression, a resampling *in silico* approach applied to the microarray gProcessed Signals was performed on the outliers samples, based on the workflow of the Sincell R work package, as described by Julià and colleagues (Juliá et al. 2015). To obtain the computational replicates, the average of the 10 *in silico* replicates for each technical replicate were produced. To test the robustness of this strategy Pearson Correlation was performed (Table III.3).

For the data analysis, GeneSpring MultiOmics Analysis version 14.9 (Agilent Technologies, USA) was used. Data was normalised through a percentile shift of 75%, baseline median of all samples and different types of filters (expression, flag, data file and error - coefficient of variance <20%).

For the statistical analysis, Rank Product (RP) method was used to determine the total number of differentially expressed genes (DEGs) (Breitling et al. 2004), through a comparison between the RP of 1000 balanced permutations, the RP value of each gene, and the comparison of

inoculation *versus* mock conditions. The DEGs cut-off determination was based on the False Discovery Rate (FDR) < 0.17.

III.2.2.1.2. Differentially Expressed Genes (DEGs) characterization

In order to view the pathways that belong to the obtained DEGs, *V. vinifera* annotation was queried, as described by Catacchio and colleagues (Catacchio et al. 2019). To complement the DEG's annotation, V3 version of *V. vinifera* gene annotation (https://urgi.versailles.inra.fr/Species/Vitis/Annotations), as well as, NCBI Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi) were used.

The Cytoscape platform v3.7.2 and ClueGo plug-in v2.5.5 (Shannon et al. 2003; Bindea et al. 2009) were used for the gene network analysis, by using the V1 *V. vinifera* gene annotation. The biological processes, cellular components, molecular functions and KEGG pathways significantly modulated between inoculated and control samples, between tolerant and susceptible genotypes and between time points were considered. GO term fusion was applied. The Gene Ontology (GO) hierarchy was restricted between GO term levels 0 to 20. Kappa Score grouping was applied, as well as the Kappa Score Threshold of 0.4. To enrich the GO analysis, the AMIGO2 v2.5.13 platform (http://amigo.geneontology.org/amigo) was used. An overview of DEGs intersection between different experimental conditions was possible by the usage of Venn Diagram tools (http://bioinformatics.psb.ugent.be/webtools/Venn/). This multiple annotation method allowed to improve the identification of the epigenetic-related DEGs (ER-DEGs) in targeted pathways for further analysis. Subsequently, the data was reported through hierarchical clustering (HC) and heatmap of the defense-related (DR-) and ER- gene expression profile (gene microarray normalized intensities) through the Multiple Experiment Viewer (MeV) (Howe et al. 2010), applying the average linkage clustering and Pearson Correlation as distance metric to the HC.

III.2.3. Quantitative real-time PCR validation

Six ER-DEGs were selected, and their expression was validated by quantitative real-time PCR (qPCR).

In order to design the primers, mRNA sequences were attained at the Genoscope Grape Genome Browser (https://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/) by the genes identifiers (GSVIT) (Catacchio et al. 2019). With the Primer3web version 4.1.0 (https://primer3.ut.ee/), primers were designed taking in account primer size (18-23 base pairs (bp)), melting temperature (60-62ºC), GC content (30-70%) and amplicon size (90-150 bp). Primers nearest to 3' UTR and showing lowest self and 3'-end complementarity were selected for the Real time analyses. For the verification of primer specificity, NCBI primer blast (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) was used, where the amplicons were cross matched with *V. vinifera* species and the presence/absence of splicing forms was verified. For a more accurate confirmation of the primers chosen (Table III.3), primers were aligned to the target sequences by BioEdit software 7.2.5 (Hall 1999). This analysis was performed in the presence of splicing forms, since primers able to amplify one or more sequences were designed in accordance with the ability of microarray probes to catch one or more splicing forms.

Table III. 3 - Primers used for qPCR analyses.

Target Sequence	Gene ID	Primer Sequence (5'-3')	Length (bp)	Tm (ºC)
Atpase splayed (SYD)	VIT_205s0020g02000	FW: CCTCCTTCCCCTTCTGTTGC RV: TGAGCCTTCTGACTTTGCCC	92	60
Lysm domain gpi-anchored protein 1 precursor (LYM1)	VIT_218s0001g03080	FW: TCCTAGTCCTTCGGTGGTGC RV: AGGGAAACTGGCTAAGGGGT	118	61
High expression of osmotically response genes 15 (HOS15)	VIT_218s0001g09610	FW: ACCCTTGATTGGAATGGAGATGG RV: AAACAACATACCAACCCTGCTCC	90	60
Meiotic recombination protein spo11-1 (SPO11-1)	VIT_219s0015g00280	FW: AGAGTTCCGGGTCATGGTCT RV: TCCTCACTTGGCATTTTCTCGT	124	60
Protein dicer-like 2 (DCL2)	VIT_204s0023g00920	FW: CGAGCATAAGAATAACACGCCCA RV: CCAAGCGAAGTGAAGCGAAC	116	61
Gamma histone variant H2AX (GAMMA-H2AX)	VIT_207s0104g00960	FW: AAACTTCTGGGAGGCGTGAC RV: GATGCGGACCCAATCTCTCC	110	60

Complementary DNA was synthesised from 1 ug of total RNA, using SuperScript IV Reverse Transcriptase kit (Invitrogen, Thermo Fisher Scientific) and anchored oligo(dT)16, according to the manufacturer's instructions. Expression analysis was performed with an ABI Prism 7900HT instrument (Applied Biosystems, Thermo Fisher Scientific) according to the manufacturer's protocol and using the Platinum SYBR Green qPCR SuperMix-UDG with ROX

(Invitrogen, Thermo Fisher Scientific) and gene-specific primers (Table III.3). All reactions were performed in triplicate. After each assay, a dissociation kinetics analysis was performed to verify the specificity of the amplification products. Relative amounts of all mRNAs were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001), where ΔCt = Ct (target gene) – Ct (reference gene). The housekeeping gene actin was used as an endogenous reference for normalisation.

III.3. Results

III.3.1. DNA methylation patterns of grapevine leaves after interaction with P. viticola

III.3.1.1. 5-mC DNA methylation differs between N20/020 and N23/018 genotypes

To check the presence of *P. viticola* in the leaf samples collected from N20/020 and N23/018 at 6 hpi and 24 hpi. At 8 dpi oil spots were present, and a PCR analysis was done to confirm the presence of the pathogen (Figure III. 1). To pursue further molecular analysis, only samples mock inoculated without bands were pooled, while for the inoculated samples the opposite was performed, all by genotype and time point. To carry out the epigenomics and transcriptomics analysis technical replicates were applied.

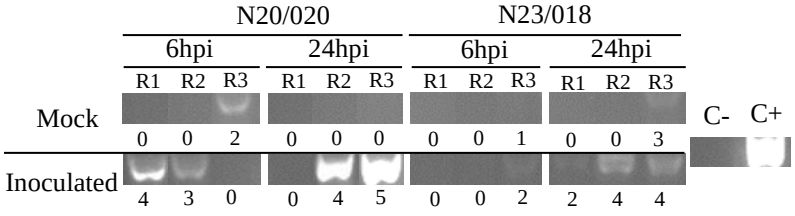


Figure III. 1 - Electrophoresis of a conventional PCR performed on susceptible (N20/020) and tolerant (N23/018) table grape varieties at 6 and 24 hours post inoculation with *P. viticola*-specific primers. Analysis of mock (water) and inoculated (pathogen suspension) biological replicates (R1-R3) with specific primers designed from *P. viticola cox2* gene (591bp) are presented on a 2.5% agarose gel. The C- (negative Control) and the C+ (positive Control) obtained from a plant with symptoms of *P. viticola* were used. 0 to 5 is the band intensity evaluation, as shown in Table III.1.

To assess if the global DNA methylation pattern reflects the contrasting susceptibility towards *P. viticola* of the two crossings, an evaluation of the percentage of the 5-methylcytosine (5-mC) was performed at 6 and 24hpi. The global methylation pattern differs when comparing the susceptible (N20/020) and tolerant (N23/018) genotypes. The fold change reflects the comparison between the % 5-mC levels in inoculated and mock inoculated samples (Figure III. 2). At both 6 and 24 hpi the % 5-mC levels are higher in both genotypes (positive fold change) (Figure III. 2). However, the % 5-mC alteration in the tolerant genotype was lower than in the susceptible genotype (Figure III. 2). The N20/020 presents higher modulation of the % 5-mC levels at 6 hpi. At 24 hpi modulation of the % 5-mC is similar in both genotypes (Figure III. 2).

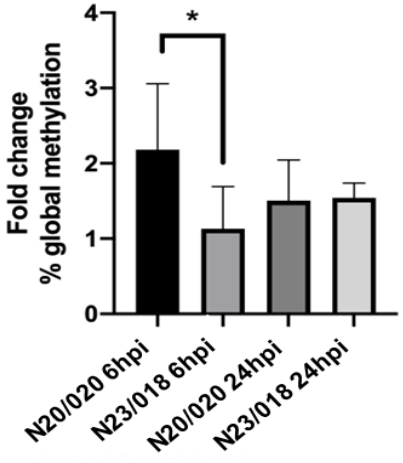


Figure III. 2 - Fold change of the percentage of global 5-methylcytosine (5-mC) when comparing inoculated and mock condition at 6 and 24 hours post infection (hpi). Asterisk (*) represents the significant difference ($p \leq 0.05$) between the susceptible (N20/020) and tolerant (N23/018) genotypes analysed.

III.3.2. Transcriptome modulation at the first hours of interactions with P. viticola

Microarray assay was performed with RNA isolated from leaves of susceptible (N20/020) and tolerant (N23/018) genotypes, collected at 6 and 24 hpi upon inoculation with *P. viticola*. Mock inoculated samples were also analysed. A cDNA microarray approach was followed and a custom *V. vinifera* microarray with 44 000 probes was used (Catacchio et al. 2019). With the need to improve the reproducibility of our data, resampling was performed through an *in silico* approach. A Pearson Correlation coefficient analysis shows a good relationship between all replicates analysed (Table III.4).

Table III. 4 - Pearson Correlation analysis between all three replicates (R) used for statistical analysis (S – Susceptible genotype N20/20, T – Tolerant genotype N23/018; M – Mock; I – Inoculated)

	S6I	S6M	T6I	T6M	T24M
R1 vs R2	0,991873	0,986164	0,954454	0,973070	0,957430
R1 vs R3	0,996075	0,996452	0,980500	0,987271	0,986968
R2 vs R3	0,997753	0,996239	0,991029	0,994047	0,989982

III.3.2.1. Differentially expressed genes (DEGs) associated with different grapevine defence strategies

After data filtering, normalization and statistical analysis with the rank product, a correlation of the DEGs with False Discovery Rate (FDR) < 0.17 was performed by principal component analysis (PCA) (Figure III. 3). The analysis shows that most of samples are distributed in two main groups based on the genotype (S and T), indicating that wide differences in both constitutive and induced gene expression exist between the two genotypes analysed. Interestingly, N20/020 at 24 hpi (S24I) and N23/018 (T) at 6 hpi do not group with the others indicating strong differences in their gene expression pattern, compared to the other samples.

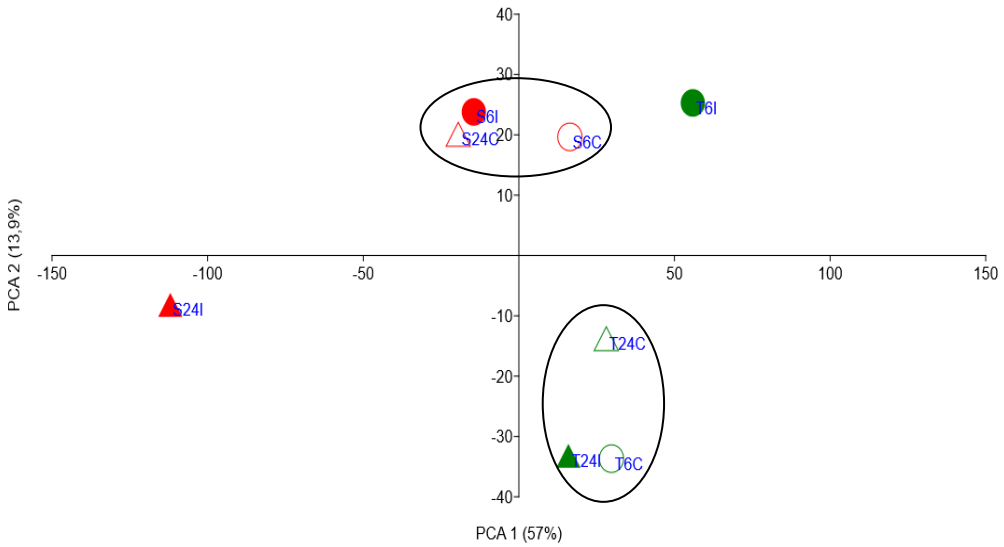


Figure III. 3 - Principal component analysis of the differentially expressed genes obtained from FDR < 17% cut-off.

Overall, 3108 genes were shown to be modulated (FDR < 0.17), 1995 in the susceptible genotype and 1549 in the tolerant genotype (Figure III. 4A). In N20/020, the number of DEGs is consistent between the time-points analysed (1068 at 6 hpi and 1130 at 24hpi), in N23/018

transcriptome modulation is higher at 6 hpi, with the majority of the DEGs being down-regulated (Table III. 5).

Table III. 5 - Overview of the differentially expressed genes (DEGs) after *P. viticola* infection of the susceptible (S - N20/020) and tolerant (T - N23/018) genotypes. Up- or down- regulation of genes was observed through the comparison of inoculated versus mock conditions.

Conditions	**Total DEGs**	**Up-Regulated**	**Down-Regulated**
S6hpi	1068	611	457
S24hpi	1130	839	291
T6hpi	1151	371	782
T24hpi	504	239	265

When comparing both genotypes, at 6 and 24 hpi in both time points, the majority of the DEGs are specific to each interaction. At 6 hpi only 176 DEGs are commonly modulated and at 24 hpi, 105 DEGs are commonly modulated (Figure III. 4A). Within these commonly modulated genes at 6 hpi, there are 37 defence-related DEGs (DR-DEGs). Meanwhile, at 24 hpi, 21 DR-DEGs were identified. The DR-DEGs are characterised by GO terms related to defense and stress response, and hormone biosynthesis and signalling.

To increase the knowledge of the functional networks affecting grapevine response to *P. viticola,* between N20/020 and N23/018, a gene network analysis was conducted on Cytoscape plug-in ClueGo (Figure III. 4B). A total of 33 clusters were significantly enriched ($p<0.01$), with the N20/020 genotype presenting a significantly higher percentage of DEGs involved in nitrilase activity, iron ion binding activity and transmembrane transporter activity when compared with the tolerant genotype. The N23/018 genotype showed a significant modulation of genes involved in chitinase activity (Figure III. 4B).

The network analysis of DEGs at each time point, showed that different biological pathways and molecular functions are affected during the time course (Figure III. 4C and Figure III. 4D). For instance, in the susceptible variety at 6 hpi, a higher number of DEGs are associated with iron ion binding processes and photosynthesis processes (Figure III. 4C). Several processes connecting to manganese ion binding, enzyme inhibitor activity, carbohydrate derivative catabolic process, sugar transmembrane transporter activity, carboxylic acid transmembrane transporter activity and nitrilase activity are also enriched also in the susceptible genotype (Figure III. 4C). Regarding the tolerant genotype, polysaccharide metabolic processes,

anchored components of membrane, chitinase activity, water channel activity, hydrolase activity, hydrolyzing O-glycosyl compounds are significantly modulated at 6 hpi (Figure III. 4D).

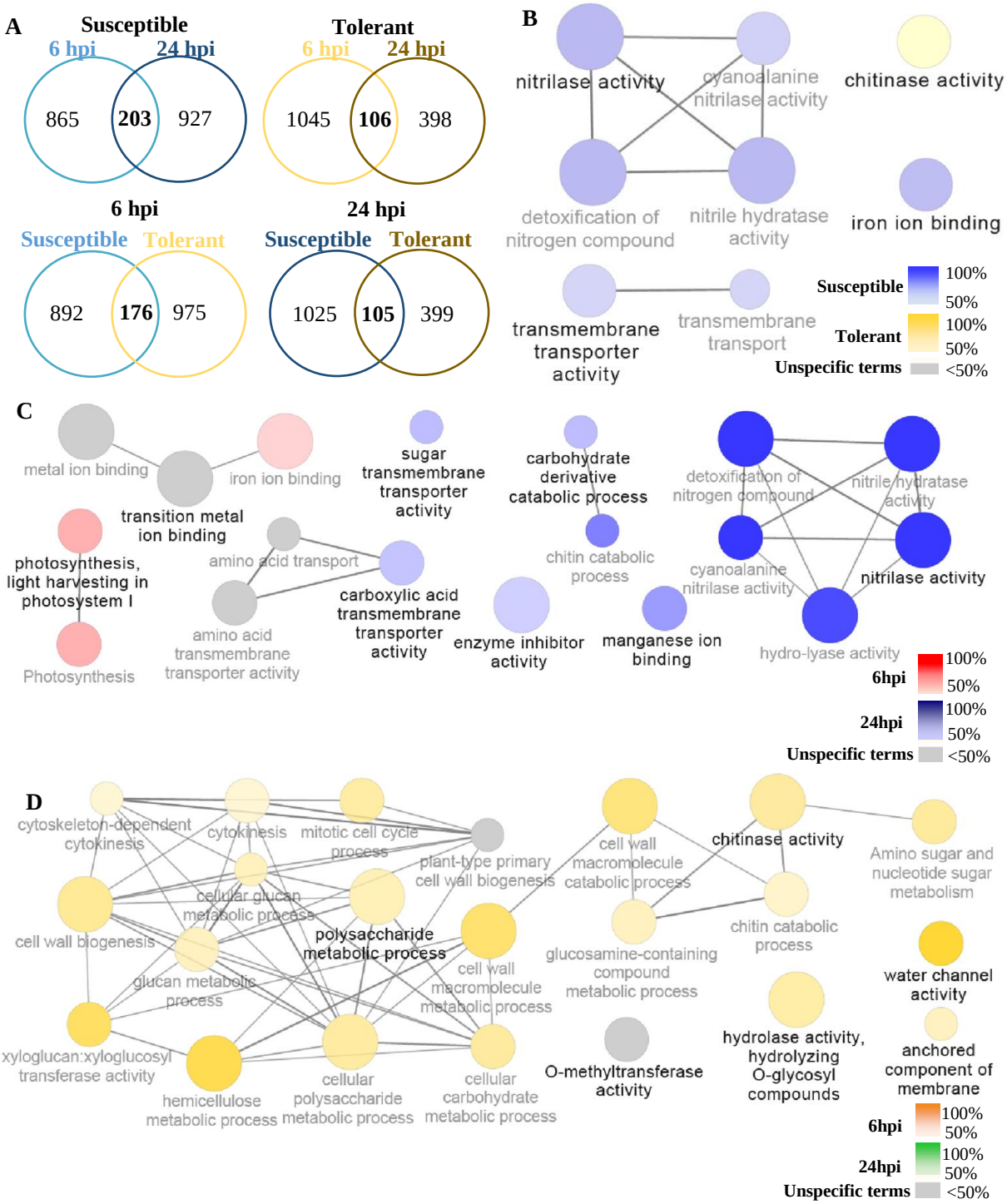


Figure III. 4 - Identification of grapevine genes differentially expressed following *Plasmopara viticola* infection. Venn diagrams and network analysis of differentially expressed genes (DEGs) obtained from the comparison between inoculated and control (mock) samples. (A) Venn diagrams show differentially expressed genes at different hours post inoculation (hpi) in susceptible and tolerant genotypes. (B-D) Cluster distribution network of DEGs showing the GO terms significantly represented in N20/020 (susceptible) versus N23/018 (tolerant) genotypes (B) and those significantly represented in the comparison between 6 and 24 hpi in the susceptible (C) and the tolerant (D) cultivars. Gene network analysis was performed by Cytoscape plug-in ClueGo. Only significant ($p<0.01$) terms belonging to GO biological process, GO cellular components, GO molecular

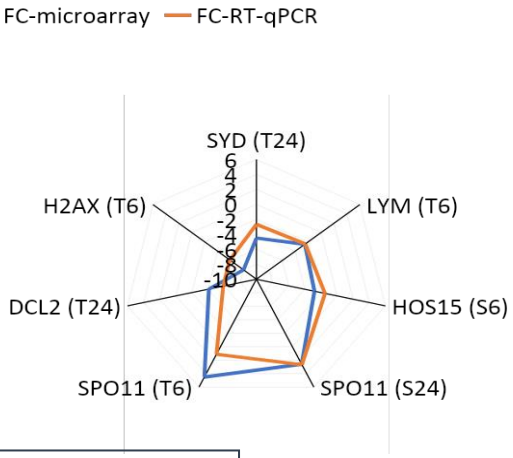
Function and Kegg ontologies are shown. The colour gradient shows the gene proportion of each cluster with at least five genes on a GO interval from four to eight levels. Equal proportions of the two clusters are represented in grey. The node size is proportional to the term significance.

Six DEGs were analysed by qPCR in order to validate microarray data (Figure III. 5). The analyses confirmed the expression trend observed.

A

Gene	Gene ID	Fold change microarray	Fold change RT-qPCR
SYD (T24)	VIT_205s0020g02000	-4,5	-2,66
LYM (T6)	VIT_218s0001g03080	-2,5	-2,44
HOS15 (S6)	VIT_218s0001g09610	-2,8	-1,49
SPO11 (S24)	VIT_219s0015g00280	2,6	2,68
SPO11 (T6)	VIT_219s0015g00280	4,5	1,13
DCL2 (T24)	VIT_204s0023g00920	-4,1	-5,88
H2AX (T6)	VIT_207s0104g00960	-8,0	-5,88

Pearson correlation = 0,876749751



B

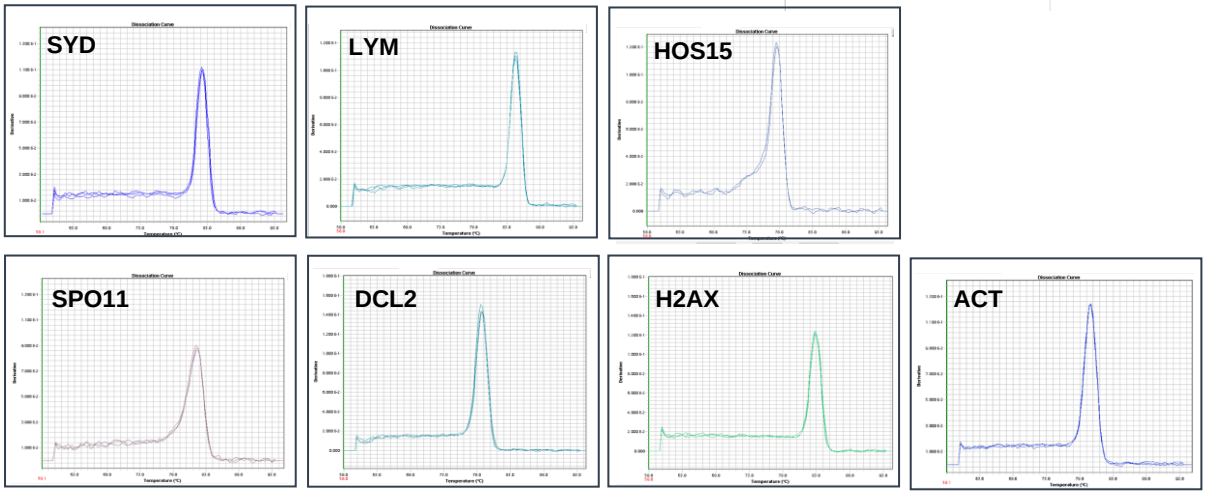


Figure III. 5 - qPCR analysis. (A) Comparison of gene expression between microarray and qPCR, calculated as fold change of *P. viticola* inoculated samples (leaves) *vs* control (mock). (B) Dissociation curves of qPCR analyses. SYD: Chromatin structure-remodeling complex protein SPLAYED; LYM: LysM domain-containing GPI-anchored protein 1; HOS15: F-box-like/WD repeat-containing protein TBL1X; SPO11: Meiotic recombination protein SPO11-1; DCL2: Dicer-like homolog 2; H2AX: Histone H2AX; ACT: Actin (housekeeping gene).

III.3.2.1.1. Epigenetic related transcripts modulated upon grapevine-pathogen recognition

Eighty-nine genes differentially expressed in the inoculated samples, compared to mock, were identified as being associated with epigenetic regulation (ER-DEGs), corresponding to 2% (susceptible) and 4% (tolerant) of the total DEGs identified (Figure III. 6). Among them, 25 genes were also classified as defense-related (Table III. 6).

Table III. 6 – Genes common to the ER-DEGs and DR-DEGs data sets.

Gene ID V1	S6	S24	T6	T24	Gene Name	Product Name	Gene Bank
VIT_19s0015g00280		2,6	4,7		*ATSPO11-1*	PREDICTED: *V. vinifera* meiotic recombination protein SPO11-1	XM_010646261.2
VIT_09s0002g01350				3,6	*GRF5*	PREDICTED: *V. vinifera* growth-regulating factor 1	XM_010656138.2
VIT_00s0532g00020			-2,1		*ATK1*	PREDICTED: *V. vinifera* kinesin-like protein KIN-14C	XM_002265264.3
VIT_07s0129g00210		13,2	-2,7		*BT4*	BTB/POZ and TAZ domain-containing protein 4	XM_003632444.2
VIT_05s0020g02000				-4,5	*SYD*	PREDICTED: *V. vinifera* chromatin structure-remodeling complex protein SYD	XM_010651496.2
VIT_05s0077g00940			-2,4		*PHYB*	phytochrome B	XM_002278227.2
VIT_04s0023g00920				-4,1	*DCL2*	PREDICTED: *V. vinifera* endoribonuclease Dicer homolog 2	XM_019219502.1
VIT_14s0006g00640	2			-4,0	*RGA1*	PREDICTED: *V. Vinifera* DELLA protein GAI	XM_002266231.4
VIT_08s0040g01440	1,8				*PGK1*	PREDICTED: *V. vinifera* phosphoglycerate kinase	XM_019221715.1
VIT_13s0067g01660		5,3			*SYN1*	sister chromatid cohesion 1 protein 1	XM_002273343.3
VIT_06s0004g05920			-2,5		*PCNA2*	PREDICTED: *V. vinifera* proliferating cell nuclear antigen-like	XM_002281054.3
VIT_03s0038g00300			-5,9		*MCM4*	DNA replication licensing factor MCM4	XM_002274498.2
VIT_01s0150g00390			-10,3		*MCM2*	DNA replication licensing factor MCM2	XM_002273639.2
VIT_18s0001g03080			-2,5		*LYM1*	lysM domain-containing GPI-anchored protein 1	XM_002285812.2
VIT_00s0227g00130				-4,1	*VIT_200s0227g00130*	PREDICTED: *V. vinifera* adenine DNA glycosylase	XM_002264991.4
VIT_04s0008g02570			5,1		*RPA2*	PREDICTED: *V. vinifera* replication protein A 32 kDa subunit A	XM_002282760.3
VIT_10s0003g03080			-3,4		*MCM3*	DNA replication licensing factor MCM3 homolog 2	XM_010657342.1
VIT_06s0061g01270				-5,3	*DME*	PPREDICTED: *V. vinifera* transcriptional activator DEMETER	XM_019220413.1
VIT_16s0013g00310	-2,6				*SUVH5*	PREDICTED: *V. vinifera* histone-lysine N-methyltransferase, H3 lysine-9 specific SUVH5	XM_002277738.3
VIT_07s0005g01430			-3		*PRL*	PREDICTED: *V. vinifera* DNA replication licensing factor MCM7	XM_002276293.4
VIT_17s0000g09790	2,1				*BT1*	BTB/POZ and TAZ domain-containing protein 1-like	XM_002278156.3
VIT_11s0016g00340			-2,4		*RAD51*	DNA repair protein RAD51 homolog	XM_002273767.2
VIT_06s0061g01610	-2,5				*TIL1*	DNA polymerase epsilon catalytic subunit A	XM_002269884.3
VIT_16s0022g01820			-2,1		*PLE*	65-kDa microtubule-associated protein 3	XM_002267548.2
VIT_11s0016g05490			-3		*ARP6*	PREDICTED: *V. vinifera* actin-related protein 6	XM_010658136.2

The susceptible genotype, at 6 hours after inoculation, presented 17 modulated transcripts with GO annotation related to epigenetic machinery, while, at 24 hpi the number decreased slightly (14) (Figure III. 6). In contrast, in the tolerant genotype, a higher number of differentially expressed genes (54 ER-DEGs) were observed at an early time point (6 hpi) (Figure III. 6).

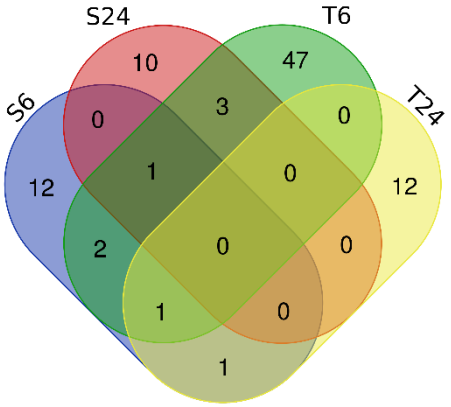


Figure III. 6 - Venn diagram showing the number of epigenetic related differentially expressed genes (ER-DEGs) at 6 and 24 hours post inoculation (hpi) in susceptible (S – N20/020) and tolerant (T – N23/018) genotypes. Genes significantly modulated in the inoculated versus mock were considered as differentially expressed for each sample.

The main GO terms associated with ER mechanisms after *P. viticola* inoculation were methylation and demethylation and chromatin remodelling (Figure III. 7).

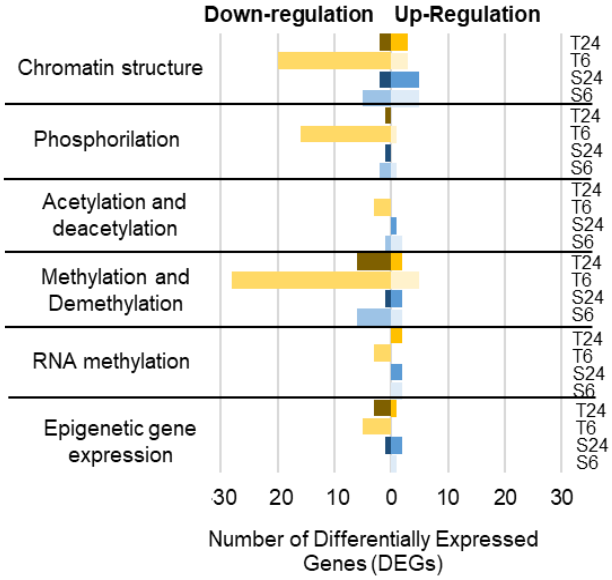


Figure III. 7 Histogram of the ER-DEGs grouped by the most representative GO terms related to epigenetic machinery. Up- and Down-regulation of inoculated *versus* mock is attributed by the Rank Product statistical method. T24: tolerant at 24 hpi; T6: tolerant at 6 hpi; S24: susceptible at 24 hpi; S6: susceptible at 6 hpi. The following processes were considered for each GO subcategory. Chromatin structure: organization, modification, regulation, involvement and histone modification; Phosphorilation: histone biological process and post-translational modifications; Acetylation and deacetylation: histone post-translational modifications, pathways, transferase complexes and activity; Methylation and Demethylation: DNA and Histone pathways, maintenance, regulation, enzyme activity and modifications; RNA methylation: pathways, complexes and enzyme activity; Epigenetic gene expression: regulation.

Considering the functional networks more represented in each genotype at different time points, genes related to histone modifications were significantly modulated on the susceptible genotype (Figure III. 8A) whereas, DNA methylation machinery, nuclear chromosome, chromatin microtubule cytoskeleton and DNA metabolic processes were significantly affected in the tolerant genotype (Figure III. 8A). Nonetheless, histone kinase activity was significantly modulated at 6 hpi on the S genotypes (Figure III. 8B). Meanwhile, DNA methylation category showed significant number of genes modulated during pathogen inoculation at 24 hpi on the T genotype (Figure III. 8C).

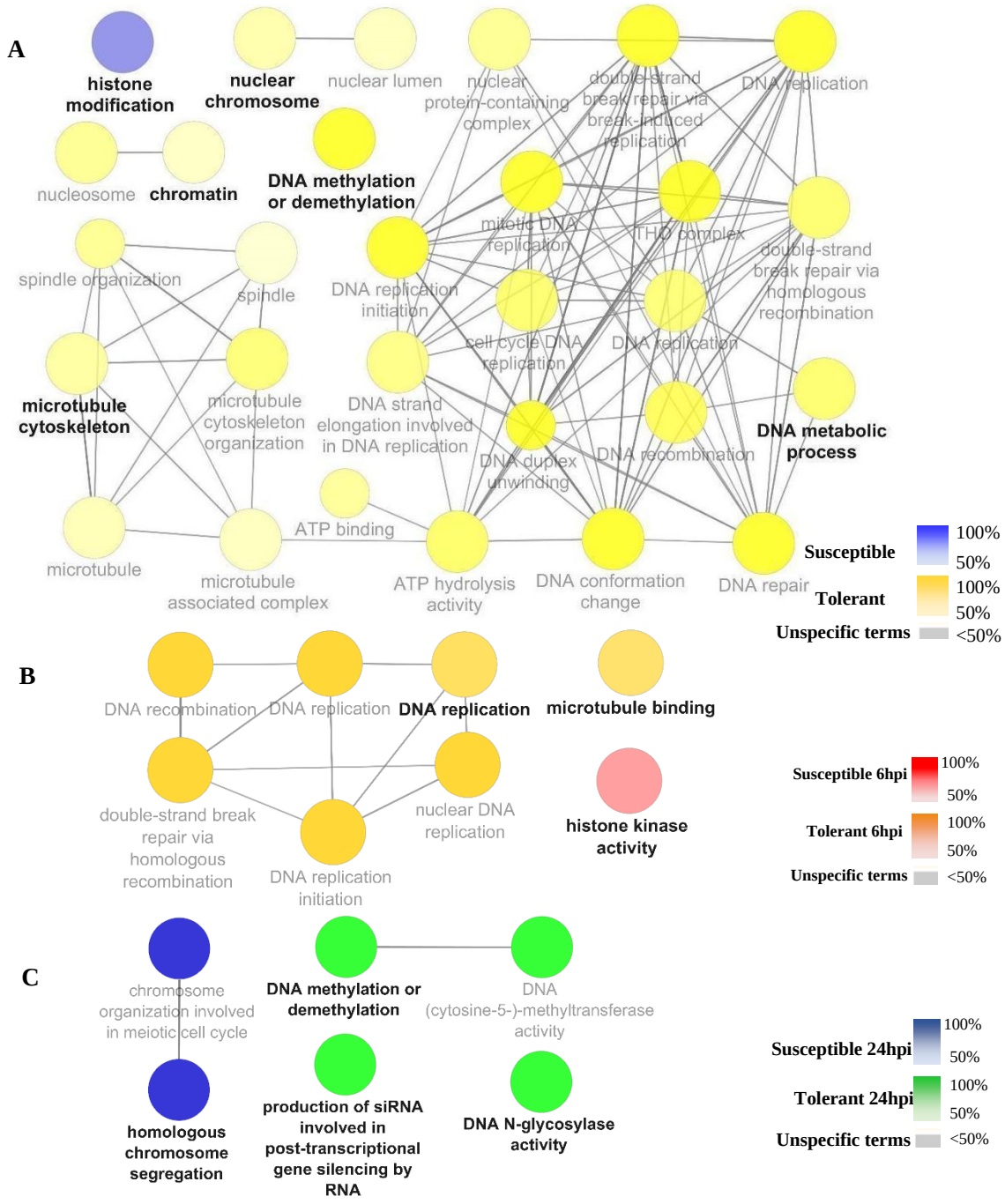


Figure III. 8 - Network analysis of ER-DEGs showing the most significant pathways affected in grapevines after *P. viticola* infection. Gene network analysis was performed by Cytoscape plug-in ClueGo. Only significant ($p<0.01$) terms belonging to

GO biological process, GO cellular components, GO molecular Function and Kegg ontologies were shown. Networks of the ER-DEGs at the overall Susceptible versus Tolerant (A), at 6hpi (B) and at 24hpi (C). Gene proportion of each cluster was presented by colour gradient containing at least four genes per term belonging to GO interval from six to eight levels (A) and from four to eight (B, C). Equal proportions of the two clusters are represented in grey. The node size is proportional to the term significance.

DNA methylation related genes such as *DNA Methyltransferase 1* (*MET1*), *Chromomethylase 1* (*CMT1*) and *Chromomethylase3* (*CMT3*) were all significantly down-regulated on the tolerant genotype (Table III.7). Genes involved in DNA replication, damage response and repair mechanisms are significantly modulated in this work (Table III.7). Chromatin remodelers play an important role since they crosstalk with several different defense and developmental networks**.** Genes related to chromatin structure (as *ATPase SPLAYED - SYD, High Expression of Osmotically Responsive Genes 15 - HOS15, Histone H2A 12 - HTA12, HTA12.1, Histone H2A 8 - HTA8 and linker Histone 1 - H1*), histone methylation (e.g. *SU(var)3-9 homologue 5 - SUVH5*) and acetylation (*Histone acetyltransferase of the GNAT family 2 - HAG2.1, HAG2.2*) were differentially expressed after pathogen infection. Moreover, differences in their regulation were found between the two genotypes analysed. Epigenetic mechanisms besides chromatin, histone and DNA modifications, are also influenced by RNA such as small RNAs. In this study, genes encoding small RNAs such as *Arginine/Serine-Rich Splicing Factor 40/41* (*RSP40/RS41*) and *DCL2* were differentially expressed after pathogen infection and also showed a different regulation in each genotype analysed (Table III.7). Finally, genes involved in the DNA damage repair machinery (as *Retinoblastoma-Related 1 - RBR1, Gamma-Histone variant H2AX - GAMMA-H2A.X, RAD51*) were differentially expressed after pathogen inoculation.

Table III. 7 - Epigenetic related DEGs after *P. viticola* infection in both N20/020 (S) and N23/018 (T) at 6 and 24hpi.

Gene ID	S6	S24	T6	T24	Gene Name	Product	Gene Bank
DNA Damage/Repair Response							
VIT_04s0008g02780		2,48			*RBR1*	PREDICTED*: V. vinifera* retinoblastoma-related protein-like (RBR)	XM_010650145.2
VIT_07s0104g00960			-8,07		*γ-H2AX*	histone H2AX	XM_002271470.3
VIT_11s0016g00340			-2,40		*RAD51*	DNA repair protein RAD51 homolog	XM_002273767.2
DNA Methylation/Demethylation							
VIT_08s0007g06800			-2,39		*CMT1*	PREDICTED*: V. vinifera* putative DNA (cytosine-5)-methyltransferase CMT1	XM_002275896.3
VIT_06s0004g01080				-3,62	*CMT3*	DNA (cytosine-5)-methyltransferase CMT3	XM_010653042.1
VIT_12s0035g01770				-4,37	*MET1*	PREDICTED: *V. vinifera* DNA (cytosine-5)-methyltransferase 1B-like	XM_019223723.1
VIT_06s0061g01270				-5,27	*DME*	PREDICTED: *V. vinifera* transcriptional activator DEMETER	XM_019220413.1
Chromatin remodeling							
VIT_05s0020g02000				-4,47	*SYD*	PREDICTED*: V. vinifera* chromatin structure-remodeling complex protein SYD	XM_010651496.2
VIT_11s0016g05490			-3,01		*ARP6*	PREDICTED: *V. vinifera* actin-related protein 6	XM_010658136.2
Histone modification							
VIT_11s0016g02620			-3,71		*HAG2.1*	histone acetyltransferase type B catalytic subunit	XM_002282895.2
VIT_13s0047g00150	2,03				*HAG2.2*	PREDICTED: *V. vinifera* histone acetyltransferase type B catalytic subunit	XM_002282895.3
VIT_00s0179g00340			-2,33		*HTA8*	PREDICTED: *V. vinifera* histone H2A variant 1	XM_002281230.2
VIT_16s0013g00310	-2,59				*SUVH5*	PREDICTED: *V. vinifera* histone-lysine N-methyltransferase, H3 lysine-9 specific SUVH5	XM_002277738.3
VIT_18s0001g09610	-2,84				*HOS15*	PREDICTED*: V. vinifera* F-box-like/WD repeat-containing protein TBL1Y	XR_002032367.1
VIT_06s0004g04270			-2,10		*HTA12*	histone H2A	XM_002284269.3
VIT_14s0060g02360		-10,82			*HTA12.1*	histone H2A.1	XM_002283935.3
VIT_07s0005g01060			-2,68		*H1*	histone H1	XM_002269443.3
Small RNA biogenesis and RNA regulation							
VIT_04s0023g00920				-4,10	*DCL2*	PREDICTED: *V. vinifera* endoribonuclease Dicer homolog 2	XM_019219502.1
VIT_15s0048g01870		2,66			*RSP40*	serine/arginine-rich splicing factor RS41-like	XM_002273715.3
VIT_11s0016g03220				2,58	*RDR5*	probable RNA-dependent RNA polymerase 5	XM_010657967.1

The hierarchical clustering of the ER-DEGs was reported in Figure III. 9. It was possible to observe the formation of three main clusters. The first and most notable cluster includes the main genes of epigenetic machinery such as *MET1*, *CMT3*, *DME*, *SYD* and *DCL2*.

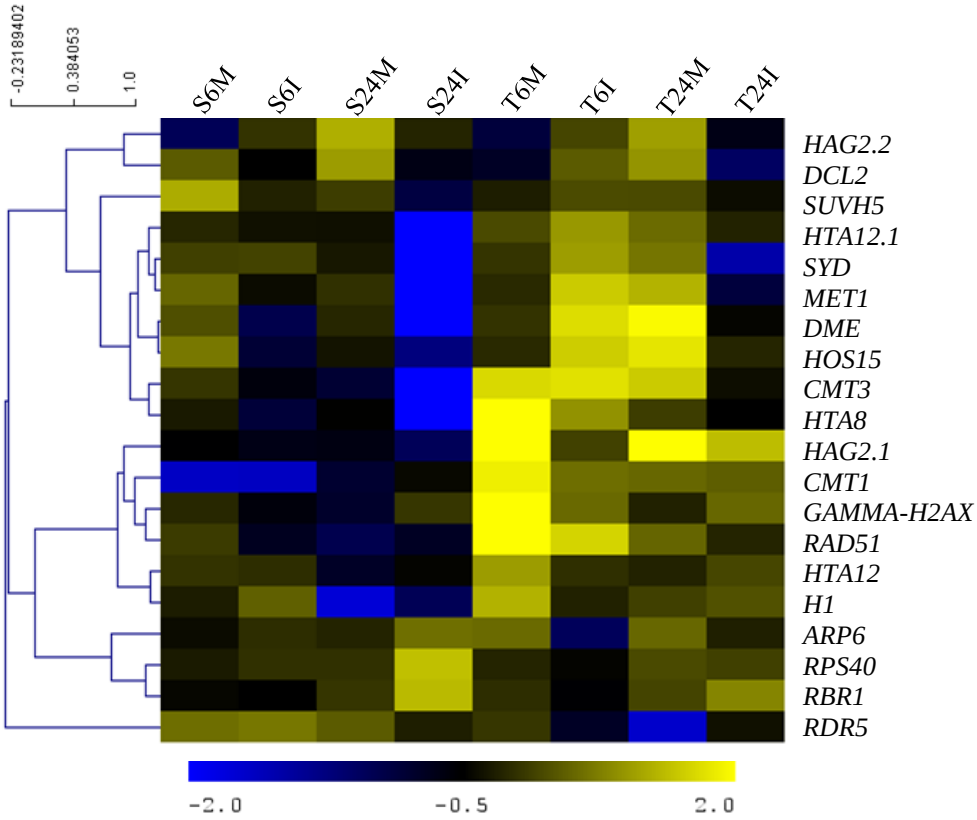


Figure III. 9 - Hierarchical clustering of selected epigenetic related genes differentially expressed in grapevine leaves after *Plasmopara viticola* infection. Microarray expression data of the putatively susceptible (S) and tolerant (T) genotypes at 6 and 24 hours post inoculation with different treatments, mock (M) and inoculated (I), were used for clustering analysis. A selection of genes significantly differentially expressed in the inoculated versus mock conditions is reported. For each gene, the Log_2 normalised expression value was used. The different colours indicate different expression values, as reported in the colour bar. Lowest and highest expression values are in blue and yellow, respectively. Gene IDs of the selected genes are those reported in Table III.7.

III.3.2.1.2. Pathogen impacts the grapevine defense related gene network

It is well established that *P. viticola* inoculation leads to a broad modulation of grapevine transcriptome (Polesani et al. 2010; Figueiredo et al. 2012). In our study, a total of 578 genes were identified as being associated with grapevine defense (DR-DEGs), corresponding to 18.4% and 19.6% of the total DEGs for susceptible and tolerant genotypes respectively (Figure III. 10).

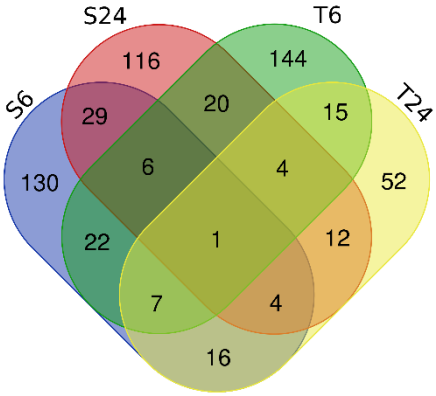


Figure III. 10 - Venn diagram showing the distribution of defense related differentially expressed genes (DR-DEGs) at 6 and 24 hours post inoculation (hpi) in susceptible (S - N20/020) and tolerant (T - N23/018) genotypes. Genes significantly modulated in the inoculated *versus* mock were considered as differentially expressed for each sample.

In N20/020, genes related to defense and DR signalling pathways were mainly up-regulated at both time points. In this genotype, the number of signalling related genes was higher after 24 hours of the pathogen inoculation (Figure III. 11). Meanwhile, in the tolerant genotype a predominant down-regulation of the defense genes was observed at 6 hpi, while signalling related genes were mainly down-regulated at both time points (Figure III. 11).

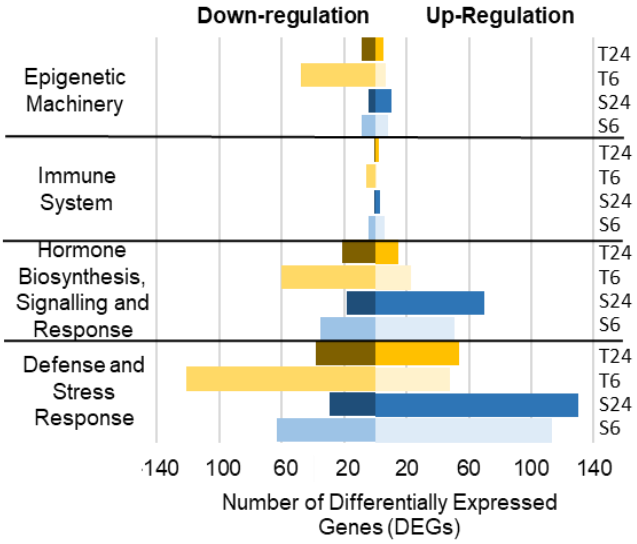


Figure III. 11 - Histogram of the DR-DEGs grouped by the most representative GO terms related to plant defense responses. Up- and Down-regulation of inoculated *versus* mock is attributed by the Rank Product statistical method. T24: tolerant at 24 hpi; T6: tolerant at 6 hpi, S24: susceptible at 24 hpi; S6: susceptible at 6 hpi.

To assess if the level of plant susceptibility/tolerance to this pathogen is related to different defense mechanisms, a gene network analysis of DR-DEGs was conducted (Figure III. 12). Although numerous GO terms were equally represented in the two genotypes (grey clusters), a number of biological processes and molecular functions were more represented in S or T

genotypes. For instance, genes involved DNA replication and chitinase activity were more represented in T DEGs (Figure III. 12A). Nonetheless, observing the clusters by timeframe (6 and 24 hpi), the T variety presented at 6 hpi a significant clustered subset of genes on DNA replication cascade (Figure III. 12B).

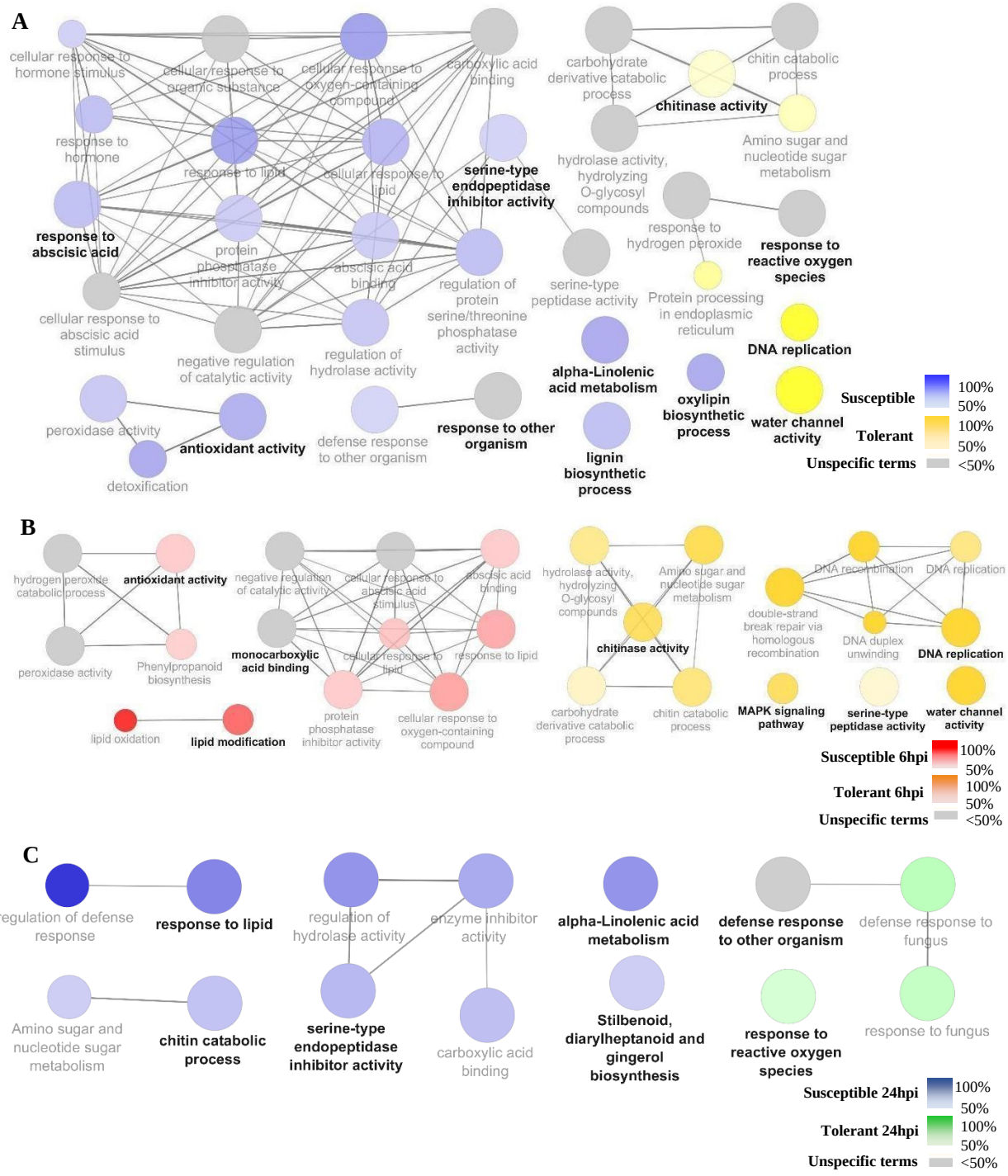


Figure III. 12 - Network of DR-DEGs showing most represented defense pathways. Networks of the DR-DEGs at the overall Susceptible *versus* Tolerant (A), at 6hpi (B) and at 24hpi (C). Gene network analysis was performed by Cytoscape plug-in ClueGo. Only significant *($p<0.01$)* terms belonging to GO biological process, GO cellular components, GO molecular function and Kegg ontologies were shown. Gene proportion of each cluster is presented by colour gradient containing at least five genes on a GO interval from four to eight levels. Equal proportions of the two clusters are represented in grey. The node size is proportional to the term significance.

Genes related to signalling pathways (JA, SA, ABA and ET) were found to be differentially modulated in our study after pathogen colonisation. The susceptible genotype at 24 hpi presents more signalling related genes with high fold change (as *protein tify 9 – JAZ10, lipoxygenase 2 – Lox3, BTB/POZ and TAZ domain-containing protein 4 – BT4, alpha-dioxygenase 1 – DOX1_1* and *ethylene-responsive transcription factor 1B – ERF1*) than 6 hpi. Meanwhile the tolerant variety has more DEGs related to signalling pathways at 6 hpi (as *abscisic acid receptor pyl4 – PYL4* and *probable N-acetyltransferase HLS1-like - VIT_205s0077g01020*) (Table III.8).

Metabolism plays a role in different levels of defense responses. In this work, we found that genes involved in both primary and secondary metabolism were modulated in grapevine-*P. viticola* interaction. On the susceptible genotype, most of them were up-regulated in the at 24 hpi such as *isoflavone 2-hydroxylase* (*CYP81E8*) and *reticuline oxidase-like* (*VIT_210s0003g05550*) (Table III.8), suggesting that the infection has a less impact on the metabolic related genes of the tolerant genotype.

Both genotypes showed significant differential expression of genes encoding for important classes of genes involved in plant defense against pathogens, such as pattern recognition receptors (PRR-like), pathogenesis-related (PR) proteins and disease resistance proteins (Table III. 8, Figure III. 13). The susceptible variety showed that ribonuclease-like *PR10 (MLP423)* and *lipid transfer 14* (*VIT_213s0019g02690 – DIR1*) genes were differentially modulated. Meanwhile, the tolerant genotype presented several different pathogenesis-related protein genes significantly down-regulated early after pathogen colonisation such as *OSM34 genes*. These data show that the PR proteins were mostly downregulated in the two genotypes studied. Nevertheless, there was a higher modulation of genes encoding PR proteins on the tolerant variety (Table III. 8).

Effector triggered immunity with R genes also seems to play an important role when comparing the susceptible and tolerant varieties in the presence of *P. viticola.* The highest up-regulated genes observed are present at 6 hpi on the tolerant variety such as *putative disease resistance RPP13-like protein 1* (*VIT_213s0084g00510* and *VIT_213s0064g01780*), *disease resistance protein RFL1-like* (*VIT_212s0035g01280*) and *RPS5*. Meanwhile the susceptible genotype showed a downregulation expression of R genes (Table III. 8).

Finally, within genes related with the immune defense network, it's possible to observe a modulation of a wide variety of DEGs. Interestingly on the susceptible genotypes one of the most curious genes is *MYB108* which is upregulated throughout the first hours of pathogen colonisation (Table III. 8).

Table III. 8 - Selection of defence related DEGs (DR-DEGs) with higher fold change regulation after *P. viticola* infection grouped through the pathway/gene they are related to.

Gene ID V1	S6	S24	T6	T24	Gene Name	Product	Gene Bank
Pathogen-Associated Molecular Pattern (PAMP) triggered immunity							
VIT_15s0024g01270	13,80				*VIT_215s0024g01270*	receptor-like protein kinase HSL1	XM_010662858.1
VIT_00s0429g00010		15,60			*CRK26*	PREDICTED: *V. vinifera* cysteine-rich receptor-like protein kinase 29	XM_010649338.2
VIT_03s0038g03600		-12,98			*VIT_203s0038g03600*	serine/threonine-protein kinase CDL1-like	XM_002279438.2
VIT_18s0041g00040	3,06	-12,69			*WAK2*	wall-associated receptor kinase 2-like	XM_010667126.1
VIT_18s0001g03740			7,59		*VIT_218s0001g03740*	PREDICTED: *V. vinifera* non-functional pseudokinase ZED1	XM_019217903.1
VIT_11s0016g02970			-5,21		*MKK6*	mitogen-activated protein kinase kinase 6	XM_002283455.3
Jasmonic Acid pathway							
VIT_01s0146g00480		18,76			*JAZ10*	protein tify 9	XM_002262714.2
VIT_00s0265g00170		25,45		-4,52	*LOX3*	lipoxygenase 2	
Salicylic Acid pathway							
VIT_07s0129g00210		13,22	-2,73		*BT4*	BTB/POZ and TAZ domain-containing protein 4	XM_003632444.2
VIT_12s0028g04040		-11,64			*VIT_212s0028g04040*	PREDICTED: *V. vinifera* protein ACCELERATED CELL DEATH 6-like	XM_019223359.1
VIT_14s0066g01670		10,09			*DOX1_1*	alpha-dioxygenase 1	XM_002279848.3
VIT_14s0066g01690		-12,93			*DOX1_2*	alpha-dioxygenase 1-like	XM_010662647.2
Abscisic Acid pathway							
VIT_04s0008g03950	-2,66	-18,41		2,07	*RD22*	dehydration-responsive protein rd22	
VIT_08s0058g00470			6,08		*PYL4*	abscisic acid receptor pyl4	XM_002264122.4
Ethylene pathway							
VIT_05s0049g00510		14,45	-3,16		*ERF1*	ethylene-responsive transcription factor 1B	XM_010651768.2
VIT_19s0014g02240			-6,67		*ERF4*	ethylene-responsive transcription factor 4	XP_002285146.1
VIT_05s0077g01020			6,10		*VIT_205s0077g01020*	probable N-acetyltransferase HLS1-like	XM_002272501.2
Metabolism related Proteins							
VIT_07s0129g00800		13,02			*CYP81E8*	isoflavone 2 -hydroxylase	XM_002283756.4
VIT_12s0059g02150	-2,57	11,76	-3,33		*ACO3*	aconitate hydratase cytoplasmic	XM_002279224.3
VIT_10s0003g05550		29,38			*VIT_210s0003g05550*	reticuline oxidase-like	XP_002269462.1
VIT_14s0066g01210		-30,10			*CA1*	PREDICTED: *V. vinifera* beta carbonic anhydrase 1, chloroplastic	XM_010662610.2
VIT_04s0008g01300		-19,02		-3,86	*GLDP1*	PREDICTED: *V. vinifera* glycine dehydrogenase (decarboxylating), mitochondrial	XM_010650056.1
VIT_04s0043g00620	-12,47	-10,43	-10,43	17,08	*VIT_204s0043g00620*	PREDICTED: *V. vinifera* GDSL esterase/lipase At5g33370-like	XM_010650646.2

Gene ID V1	S6	S24	T6	T24	Gene Name	Product	Gene Bank
Pathogenesis-Related (PR) Proteins							
VIT_05s0077g01690		17,35			*MLP423_1*	PREDICTED: *V. vinifera* pathogenesis-related protein 10.8 (PR10.8)	XM_002273779.4
VIT_05s0077g01670	-8,58	-10,23	-9,00		*VIT_205s0077g01670*	REDICTED: *V. vinifera* pathogenesis-related protein 10.7 (PR10.7)	XM_002273754.4
VIT_01s0026g00570	-7,84	-54,44		2,34	*MLP423_2*	MLP-like protein 423	XM_002267183.3
VIT_13s0019g02690	-2,60	-11,73			*VIT_213s0019g02690*	putative lipid-transfer protein DIR1	XM_002281518.3
VIT_05s0077g01650		-8,90	6,31		*MLP423_3*	PREDICTED: *V. vinifera* pathogenesis-related protein 10.5	XM_002273946.4
VIT_17s0000g02470			-9,29		*ATLP-1*	thaumatin-like protein	XM_003634158.2
VIT_02s0025g04310	3,40		-12,99	2,87	*OSM34_1*	*V. vinifera* thaumatin-like protein (Tl3)	AF532965.1
VIT_02s0025g04330	3,11		-7,76	2,53	*OSM34_2*	*V. vinifera* VVTL1 (TL1), mRNA	NM_001281132.2
VIT_02s0025g04330	5,14		-7,66	3,80	*OSM34_3*	*V. vinifera* VVTL1 (TL1), mRNA	NM_001281132.2
VIT_06s0004g04440			-5,59		*VIT_206s0004g04440*	osmotin-like protein	XP_002281193.1
VIT_08s0058g01210	-2,96		-6,20	2,16	*LP1*	non-specific lipid-transfer protein P5	XM_002270934.2
VIT_07s0130g00030			-36,42	2,91	*PDF2*	defensin-like protein 1	XM_010654727.2
Effector Triggered Immunity (ETI) - R Genes							
VIT_13s0139g00210		-20,15			*VIT_213s0139g00210*	putative disease resistance RPP13-like protein 1	XR_787311.1
VIT_09s0002g05840		-16,21			*RPS5_1*	probabble disease resistance protein rps5-like	XP_002281195.1
VIT_15s0045g01020		-13,50			*RPP8*	putative disease resistance protein At1g50180	XM_010662983.1
VIT_13s0084g00510			6,92		*VIT_213s0084g00510*	PREDICTED: *V. vinifera* putative disease resistance RPP13-like protein 1	XM_019224396.1
VIT_12s0035g01280	-5,23		6,07		*VIT_212s0035g01280*	PREDICTED: *V. vinifera* disease resistance protein RFL1-like	XM_019223277.1
VIT_19s0027g01780			6,13		*RPS5_2*	probable disease resistance protein At1g12280	XM_010646742.1
VIT_13s0064g01780			6,58		*VIT_213s0064g01780*	putative disease resistance RPP13-like protein 1	XP_010659292.1
VIT_19s0027g00660			6,51		*VIT_219s0027g00660*	putative disease resistance protein RGA4	XM_010646688.1
VIT_19s0027g01580			5,81	-6,77	*VIT_219s0027g01580*	PREDICTED: *V. vinifera* probable disease resistance protein At1g12280-like	XM_019217064.1
Imune response network							
VIT_05s0077g00500	3,72	8,74			*MYB108*	*V. vinifera* R2R3 transcription factor MYB108-like protein 1	NM_001281062.1
VIT_03s0038g03410		-15,43			*NAC036*	PREDICTED: *V. vinifera* NAC domain-containing protein 35	XM_002281780.4
VIT_16s0039g01710			5,85	-4,58	*MYB9*	transcription factor MYB39	XM_003633958.2
VIT_08s0007g00410			-6,75		*AS1*	PREDICTED: *V. vinifera* transcription factor AS1	XM_002266391.3

Gene ID V1	S6	S24	T6	T24	Gene Name	Product	Gene Bank
Imune response network (continuation)							
VIT_06s0061g01270				-5,27	*DME*	PREDICTED: *V. vinifera* transcriptional activator DEMETER	XM_019220413.1
VIT_05s0062g00700		30,82			*IAGLU_1*	PREDICTED: *V. vinifera* crocetin glucosyltransferase, chloroplastic	XM_002263265.4
VIT_05s0062g00710	2,19	27,56			*IAGLU_2*	PREDICTED: *V. vinifera* crocetin glucosyltransferase, chloroplastic-like	XM_010652096.2
VIT_09s0002g04180		7,80			*VIT_209s0002g04180*	protein PLANT CADMIUM RESISTANCE 8	XM_002279124.3
VIT_11s0016g04750		10,94	-2,89		*AKT1*	potassium channel AKT1-like	XM_002281751.2
VIT_11s0016g00560		-12,77			*ATPRX*	peroxiredoxin Q	NM_001281040.1
VIT_10s0523g00010	-8,82	-12,81			*VIT_210s0523g00010*	heat shock protein 70 -interacting	
VIT_09s0054g00350		-12,72			*VIT_209s0054g00350*	atp gtp binding protein	
VIT_11s0037g00850		-17,24			*VIT_211s0037g00850*	PREDICTED: *V. vinifera* protein TSS	XM_003633119.2
VIT_04s0008g01530			6,52		*HSP17II*	17.3 kDa class II heat shock protein-like	XP_003631809.1
VIT_08s0007g00430			9,81		*VIT_208s0007g00430*	elicitor-responsive protein 1-like	XM_002270572.2
VIT_00s0371g00010	-2,60		6,49		*CAD9*	PREDICTED: *V. vinifera* probable mannitol dehydrogenase	XM_002271543.4
VIT_19s0090g01480			5,92		*NHX3*	sodium hydrogen exchanger 4-like	XM_002276777.2
VIT_11s0016g05850			6,60		*VAMP711*	vesicle-associated membrane	
VIT_19s0090g00400	-2,44		-5,61		*VIT_219s0090g00400*	NEP1-interacting protein-like 1	XM_010646150.1
VIT_00s0388g00070			5,17	-5,37	*PGP9*	PREDICTED: *V. vinifera* ABC transporter B family member 9	XM_019222449.1
VIT_18s0001g13200			-3,72	-9,07	*CKX5*	cytokinin dehydrogenase 5	XM_002280761.2
Genome Machinery							
VIT_13s0064g01260		-15,44			*VIT_213s0064g01260*	DNA-damage-repair/toleration protein DRT100-like precursor	NM_001281258.1
VIT_00s0274g00010			7,48		*MSH3*	PREDICTED: *V. vinifera* DNA mismatch repair protein MSH3-like	XM_019218309.1
VIT_01s0150g00390			-10,25		*MCM2*	DNA replication licensing factor MCM2	XM_002273639.2
VIT_03s0038g00300			-5,94		*MCM4*	DNA replication licensing factor MCM4	XM_002274498.2
VIT_06s0061g00600				-5,68	*VIT_206s0061g00600*	PREDICTED: *V. vinifera* protein NUCLEAR FUSION DEFECTIVE 4	XM_002273067.4

Figure III. 13 shows an overall microarray expression profile/hierarchical clustering of the DR-DEGs with the formation of four main clusters. Cluster one indicates a proximity between genes of PAMP triggered immunity, signalling pathways (JA, SA and ET), PR proteins, metabolism related proteins and genes involved in immune network (Figure III. 13). Meanwhile in cluster two, the smallest cluster of all, shows an interaction between genes related with PAMP tiggered

immunity and thaumatin-like proteins (*OSM34*) (Figure III. 13). Cluster three displays a clear relationship between R genes, genes related the immune network and some influence of genes involved with the signalling pathways (SA, ABA and ET) (Figure III. 13). Then the four cluster demonstrates the behaviour of genes encoding PR proteins and their engagement with genome machinery, immune response and some influence with signalling pathways (Figure III. 13).

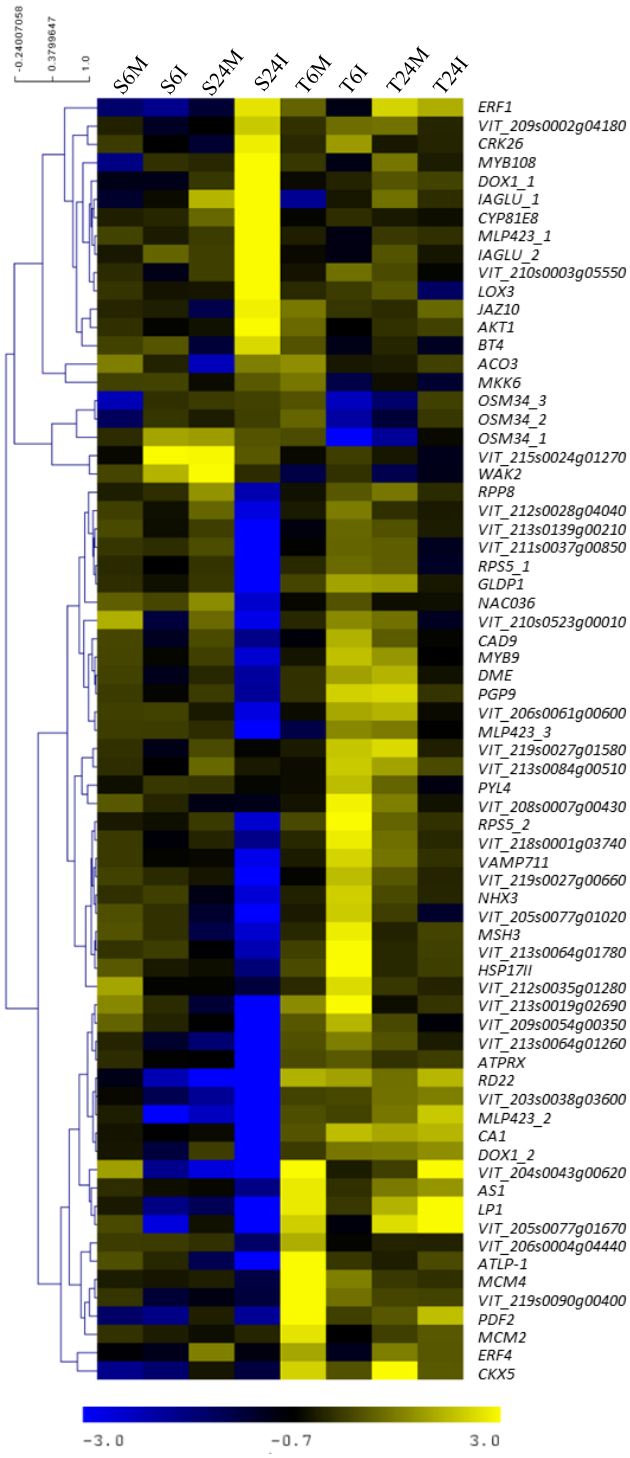


Figure III. 13 - Hierarchical clustering of defense related genes (DR-DEGs) differentially expressed after *P. viticola* infection. Microarray expression data of the putatively susceptible (S) and tolerant (T) genotypes at 6 and 24 hours post inoculation (hpi)

with different treatments, mock (M) and inoculated (I) are reported on a heat map outline. Gene IDs of the selected genes are reported in Table III.8. A selection of defense gene is reported.

III.4. Discussion

In this study, two grapevine crossing populations (S - N20/020 and T - N23/018), selected from chapter II, where molecularly analysed after *P. viticola* inoculation to observe the different molecular responses. To further understand if epigenetic regulation is one of the key factors influencing the higher tolerance/susceptibility of the crossing hybrids, we have conducted a 5-mC % evaluation in the first hours (6 and 24 hpi) after pathogen contact.

We have also performed through microarray technique a characterisation of transcriptome modulation on S and T genotypes performed at 6 and 24 hpi, as pathogen recognition and development were described to occur in a short period of time after inoculation (Unger et al. 2007). After statistical and comparative analysis of the differentially expressed genes, both genotypes presented different response periods to the *P. viticola* infection. N20/020 genotype showed a more delayed response to *P. viticola* and N23/018 a quicker response resembling the timing and response of a resistant species to *P. viticola* inoculation (Figueiredo et al. 2012). Also, the low overlapping DEGs between S and T indicates a genotypic-specific response to pathogen stimulus. This is in accordance with that observed in grapevine response to other kinds of stresses (Catacchio et al. 2019). We have also performed a characterisation of transcriptome modulation focusing on both defence-related (DR-DEGs) and epigenetics related transcripts (ER-DEGs).

III.4.1. Modulation of defense pathways after pathogen infection

The recognition of pathogen molecules on the host cells is done through pattern recognition receptors (PRR) localized in plasma membranes that further initiate the first steps of immunity (Ramirez-Prado et al. 2018a; Iqbal et al. 2021). By recognising pathogen-associated molecular pattern (PAMP) molecules, they activate down-stream signalling pathways responsible for the activation of plant defense mechanisms, a process called PAMP-triggered immunity (PTI) (Ramirez-Prado et al. 2018b; Parker et al. 2022). Differences in the modulation of PRR-like

genes as well as genes involved in the down-stream signalling pathway, like mitogen-activated protein kinase, were observed between susceptible and tolerant genotypes, in accordance with their different phenotypic response. The mitogen-activated protein kinase (MAPK) signalling cascade is known to play a pivotal role in plant immunity after recognition of biotic stress through the involvement with phytohormone signalling cascades and regulation of transcriptional activity (Tsuda et al. 2013; Chen et al. 2021). The MAPK cascade was somewhat present in our study, the MKK6 gene was downregulated on the tolerant variety as early as 6phi presenting an opposite outcome from the literature (Lazar et al. 2014; Wang et al. 2017). Several studies have observed the role of MKK6 in different plants such as cotton (Wang et al. 2017) or potato (Lazar et al. 2014) play a role in the plant immunity against different types of pathogens from *Fusarium oxysporum* to Potato virus Y, respectively (Lazar et al. 2014; Wang et al. 2017). These studies reported that this MKK6 is involved with different phytohormone signalling pathways (SA and JA cascades) and their defense related genes expression, ROS cascade generation and hypersensitive response to pathogens which overall indicates that MKK6 plays an important role on plant-pathogen resistance (Lazar et al. 2014; Wang et al. 2017).

Our results indicated that genes related to several different pathways were modulated in the presence of the oomycete. However, differences between susceptible and tolerant varieties were also observed. JA, ABA and ET pathways are important phytohormones with cascade networks to help regulate the defense system of *V. vinifera* (Chong et al. 2008; Figueiredo et al. 2018). Genes encoding the signalling phytohormones that are involved in plant defense were overrepresented in our datasets in accordance with their pivotal role in the modulation of plant defense responses (Chong et al. 2008; Figueiredo et al. 2018; Laureano et al. 2018). Signalling pathways such as ABA are considered host species dependent (Lievens et al. 2017). Nevertheless, studies reporting plant infection with either nematodes or oomycetes show ABA signalling pathway playing a role in their plant-pathogen interaction (Liu et al. 2020b; Modesto et al. 2021).In our results PYL4 gene, a known ABA pathway gene (Liu et al. 2020a), presented an up-regulation expression on the tolerant variety at an early stage of infections (6 hpi) by *P. viticola*. In the literature, ABA and JA pathways cooperate in the presence of infections specially through PYL4 which also contributes for the regulation effect on defence response genes (Liu et al. 2020a; Modesto et al. 2021). PLY4 could be considered an ABA pathway activator and/or inducer of genes related to both signalling cascades facing nematode colonization (Modesto et al. 2021). Similar to our results, Liu and colleagues have observed the

presence of PYL4 gene in *V. vinifera* after pathogen colonization presenting with up-regulated expression on a resistant cultivar (Liu et al. 2020a). Therefore, our and other studies have shown that PYL4 could play a role in *V. vinifera* infected with *P. viticola* (Liu et al. 2020b, 2020a)

On the other hand, SA and JA/ET have a key role in the activation of plant defense response dependent on the pathogens feeding relationship with the host (He et al. 2020a). Regulatory genes involved in signalling pathways (SA, JA and ET) cascades such as BT4 are known to be involved with the increased resistance against *Botritis cineria* (Hao et al. 2013) and *Pseudomonas syringae* (Zheng et al. 2019). Also, the engagement between BT4 and the phytohormone signalling cascades while plant-pathogen interaction impacts the downstream defense related genes (Hao et al. 2013; Zheng et al. 2019). In our study BT4 is upregulated on the susceptible variety at 24 hours after *P. viticola* colonization while on the tolerant variety a down-regulation expression early after infection (6h) was observed. Interestingly, Zheng and colleagues reported a relationship between BT4, ERF, SA and ET signalling pathways while the Arabidopsis defense against *P. syringae* (Zheng et al. 2019). Although, SA and JA behave antagonistically depending on the pathogen trophic relationship (biotrophic/hemi biotrophic (Hao et al. 2013) and necrotrophic (Hao et al. 2013), respectively) some genes can participate in both cascades which is the case of BT4 in plant immunity (Zheng et al. 2019).

It is reported that ET phytohormone signalling pathways components such as the transcription factors ERF can cooperatively help BT4, other signalling paths and defense genes important for plant immunity (Zheng et al. 2019). Our results show that ERF1 gene is, similarly to BT4 gene, positively differentially expressed on the susceptible genotype at 24 hpi and oppositely regulate at 6 hpi on the tolerant variety. Literature has shown that plant resistance capabilities against necrotrophic pathogens is correlated with ET signalling pathway specifically ERF transcription factors such as ERF1 (Lorenzo et al. 2003; Darino et al. 2021). ERF1 is known to be a key player involved with JA and ET signalling pathways. It is a gene that is reported to be stimulated downstream of these pathways, and concomitantly regulates defense related genes (such as PDF1.2 and PR3) in plant immunity (Lorenzo et al. 2003; Li et al. 2015, 2021; Huang et al. 2016; Darino et al. 2021). A study done by Li and colleagues analysing *Vitis amurensis* reported that the significantly expression of ERF1 was evident on the resistant genotype to downy mildew disease at later in the pathogen colonization period (12 and 24 hours) (Li et al. 2015). ERF1 was also induced in maize after infection with *Ustilago maydis* (Darino et al. 2021). Likewise, in Arabidopsis infected by necrotrophic pathogens, the expression of ERF1 was up-regulated (Berrocal-Lobo et al. 2002). However, it's been reported that the crosstalk

between different pathogens and plants, the ET pathway could have different action modes, from a synergistic to antagonistic behaviour with other signalling pathways interactions (Berrocal-Lobo et al. 2002). Also, epigenetic machinery was shown to play a role on the expression of ERF1 such as SYD (Walley et al. 2008) and HDA19 (Wight et al. 2009; Berr et al. 2012) while plant-pathogens interaction. These molecular interaction have been reported to be related with defense strategies after *B. cineria* (Walley et al. 2008; Berr et al. 2012), *P. syringae* (Wight et al. 2009), *Alternaria brassicicola* infection (Berr et al. 2012).

The JA signalling pathway can be repressed through different proteins such as the JAZ family (Zhu et al. 2016). In our study, JAZ10 gene is up regulated on the susceptible genotype at 24 hpi. In *A. thaliana* studies with grey mold disease, reports of the presence JAZ10 activity as well as its interconnection with JA responses, gibberellins pathway and genes related with light stress responses (such as phyB) (Cerrudo et al. 2012, 2017; Iqbal et al. 2021). Also, Demianski and colleagues reported through transcriptionally analysing *Pseudomonas syringae* interaction with *A. thaliana* that there is an activation of JAZ10 gene although mutants' analyses showcase JAZ10 playing a role on disease susceptibility (Demianski et al. 2012). Meanwhile Tify9, known to be synonymous of JAZ10, has been studied in *V. vinifera-Erysiphe necator* interaction (Yu et al. 2019). Yu and colleagues observed the *V. vinifera* Tify9 gene behaviour after the pathogen attack which was activated in the grapevine leaves (Yu et al. 2019). Furthermore, the profile of this *V. vinifera* gene (analysed on overexpressed *V. vinifera* tify9 Arabidopsis mutant) indicates that important defense genes related to JA and SA pathways were expressed, specially PR1 (SA marker gene) (Yu et al. 2019).

Within the JA pathway also lipoxygenases from the oxylipin family have been reported to be an intricate part of the plant defense (Gao et al. 2009, 2017). Studies have observed that maize-pathogen interactions could be affected by ZmLox3 which contribute to plant defense response dependently on the pathogen species, and a possible relationship with the pathogen attack strategy for its survival (Gao et al. 2009, 2017; Battilani et al. 2018; Ramu et al. 2020). In our study Lox3 gene presented at 24 hpi an activated expression on the susceptible genotype and repressed on the tolerant genotype. In studies done of *V. vinifera* defense related genes against several different pathogens, a correlation between Lox3 and genes encoding thaumatin-like proteins (*V. quinquangularis* TLP29) (Yan et al. 2017) or genes encoding Toll-interleukin-1(TIR)-NBS-LRR (*V. amurensis* RGA1) (Tian et al. 2020) show the intricate link between JA and different plant defense strategies that can help to obtain a resistance or susceptible phenotypic in *Vitis* species (Yan et al. 2017; Tian et al. 2020).

Another important oxylipin is DOX1 since it has been shown in *A. thaliana* affected with *P. syringae* or *B. cineria* could contribute to plant defense and signalling pathway against the pathogens (Shah 2003; Vicente et al. 2012; Salas-Marina et al. 2015). In our study the DOX1 gene was only found on the susceptible genotype as being upregulated at 24 hpi. In tomato plants colonized by *B. cineria* an activation of DOX1 gene was shown at 24 hpi (Crespo-Salvador et al. 2018).

The probable N-acetyltransferase HLS1-like is a known ethylene related plant development gene with crosstalk between several other different signalling pathways (Dolgikh et al. 2019; Zhao et al. 2021). In our study, this gene was up-regulated on the tolerant genotype at 6 hpi which, as well as shown in the literature, could be part of the plan strategy to survive biotic stresses. Studies in different environmental stresses (from light (Lyu et al. 2019)), viruses (beet necrotic yellow vein virus and Beet soil-borne mosaic virus (Gil et al. 2020)), insects (Egyptian cotton worm (Stotz et al. 2001)) and pathogens (*Xanthomonas campestris pv campestris* (Buell 2002), *B. cineria* or *P. seryngae* (Liao et al. 2016)) as shown that HLS1 is associated with susceptibility and/or resistance to the different stresses. This phenotypic variability could occur through HLS1 regulatory behaviour on genes related with signalling pathways such as ET, auxin, gibberellin or ABA that further encodes an array of defense related proteins (Gil et al. 2020; Zhang et al. 2020; Liao et al. 2022). In a study performed by Liao and colleagues, it was observed a link between HLS1 gene with histone acetylation and ABA signalling throughout several different targets (H3Ac, MED18, ABI5 and WRKY33) whenever plant-pathogen interaction (Liao et al. 2016).

The metabolic system of plants can play a role has a plant defense strategy (Almeida et al. 2014; Liang et al. 2015) has shown in our work which either isoflavone 2-hydroxylase and reticuline oxidase-like on the susceptible genotype were up-regulated in the susceptible genotype at 24 hpi. Other studies have shown that these secondary metabolites such as Isoflavone 2-hydroxylase that was observed on cucumber and grass pea infected with pests and pathogens, respectively, identifying the importance of isoflavonoids pathway to plants resistance to a variety of diseases in plants strategy (Almeida et al. 2014; Liang et al. 2015). Also, reticuline oxidase-like has been observed to be present on the study of different plants (grass pea, grapevine and coffee) inoculated with different pathogens also indicating its importance in restricting pathogen colonization (Almeida et al. 2014; Guerra-Guimarães et al. 2015; Toth et al. 2016).

From the genes belonging to pathogenesis-related (PR) proteins, plant defense related genes such as MLP423 or OSM34 in our study present a diverse differential modulation in both genotypes which could indicate a role on *Vitis* species in the presence of a *P. viticola*. Studies has shown that MLP423 biologic behaviour is not established although it's known to be homologous and correlated to the PR family specifically PR10, with a RNase activity, therefore playing a role in the plant immunity as well as the phytohormone signalling pathways at biotic stress-related situations (He et al. 2013, 2020b; Galindo-González and Deyholos 2016; Xu et al. 2019; Fujita and Inui 2021). Several different host such as cucumber (Xu et al. 2019), apple (He et al. 2020b), flax (Galindo-González and Deyholos 2016) infected with pathogens (powdery mildew (Xu et al. 2019), *Botryosphaeria berengeriana f. sp. piricola* (He et al. 2020a), *Alternaria alternata* (He et al. 2020b) *Fusarium oxysporum* f. sp. *lini* (Galindo-González and Deyholos 2016), respectively) were reported to present different MLP423 expression (Galindo-González and Deyholos 2016; Xu et al. 2019; He et al. 2020b; Fujita and Inui 2021). The OSM34 gene was shown to be induced in citrus plants after the bacteria *Candidatus Liberibacter asiaticus* infection (Albrecht and Bowman 2012). However, when comparing the susceptible and tolerant genotypes used in that study, to the control plants, the OSM34 expression was higher on the tolerant variety even in the absence of the pathogen indicating an independent behaviour of the gene from the pathogen colonization (Albrecht and Bowman 2012). Also, the analysis in *V. vinifera* with either infection by *E. necator* or after methyl salicylate treatment (MeSA), show that OSM34 was induced by both experimental approaches suggesting a correlation between this gene and the SA signalling pathways (Toth et al. 2016).

To counterattack pathogens effectors, an improved plant immune system nominated effector triggered immunity (ETI) develops resistance (R) genes that encodes identifier proteins (NB-LRR) against pathogen specific molecules (Jones and Dangl 2006; Cheng et al. 2018; Santos et al. 2020) *disease resistance recognition of Peronospora parasitica 13 (RPP13)-like protein 1* (Cheng et al. 2018; Eichmeier et al. 2019), *disease resistance protein RFL1-like* (Sari et al. 2019) or *RPS5* (Wang et al. 2013; Islam et al. 2015; Lee et al. 2016), thus evolving the defense to a stage of hypersensitivity resistance (HR) (Cheng et al. 2018). In our study RPP13-like 1 protein homologous gene is essentially up-regulated on the tolerant variety. This encoding gene has been acknowledged as part of the plant resistance against different pathogens through several plant analysis such as Arabidopsis and barley affected with downy mildew, powdery mildew, *Pseudomonas syringae* (Bhattarai et al. 2018; Cheng et al. 2018; Chandran et al. 2021)

although also a susceptible phenotype also has been observed in plant-pathogen interactions (Bhattarai et al. 2018). As an example, studies have observed the recognition task of RPP13 to the *Hyaloperonospora arabidopsidis* ATR13 gene, a pathogen avirulent effector protein, which therefore indicate a possible role in the plant defense immune system (Möller and Stukenbrock 2017; Serra et al. 2018; Herlihy et al. 2019). Also, Sari and colleagues observed that in the durum wheat genotypes with resistance phenotype presented a possible association between these physical characteristics, the SA-independent pathway, the expression of RPP13 and other defense related genes, such as *RFL1* (Sari et al. 2019). In our study, the disease resistance protein RFL1-like encoding gene presents essentially an up-regulation expression on the tolerant genotype indicating a role in the plant-pathogen interaction. Also, studies have observed a 74% RFL1 coding region similarity with RPS5 (Resistance *Pseudomonas syringae*-5) gene (Warren et al. 1998; Henk et al. 1999; Islam et al. 2015) an identified R gene in plant defense strategy, which in our work presented a highly downregulated expression on the susceptible genotype at 24 hpi while on the tolerant genotype an up-regulation expression in the early hour of infection. Equally, to other R genes, RPS5 has been studied as an intricate part of the ETI defense system in *Vitis* species it has been observed a role of RPS5 gene in plant defense system against *P. viticola* with lower expression on genotypes with higher sensibility to this pathogen which agrees with the results of our work (Wang et al. 2013). Analysis by Islam and colleagues in other pathogen species indicate that RPS5 variants has been differentially expressed in *V. flexuosa* indicating a role in defense (Islam et al. 2015)**.** Also, research on RPS5 and epigenetic marks indicate a possible influence of histone modifications on the transcriptional expression of this defense related gene and therefore also influence the plant defense strategy (Lee et al. 2016).

Other genes important for the plant defense were observed in this study such as the transcription factor MYB108 gene which is present in our work essentially up-regulated on the susceptible genotype on the overall colonization. Studies in grapevine, Arabidopsis, cotton, flax and chickpea have observed that this transcription factor of the family R2R3-MYB is an important player on biotic stresses of a variety of pathogens essentially through direct or indirect contribution to SA, JA and ABA signalling-mediated responses (Mengiste et al. 2003; Cheng et al. 2016; Galindo-González and Deyholos 2016; Toth et al. 2016; Pirrello et al. 2022). Research performed by Cheng and colleagues in regard of cotton roots infected by the fungus *Verticillium dahlia* indicate an expression profile of the MYB108 induced as early as 6 hpi (Cheng et al. 2016). Also, observing knock down and overexpressed plants of the MYB108

gene colonized by this pathogen presented a possible tolerance behaviour of the plant to the pathogen through a regulatory role of the defense system (Cheng et al. 2016). Similarly, to this research, chickpea roots infected with *Fusarium oxysporum* was reported by Gupta and colleagues, the same expression of MYB108 gene was reported (Gupta et al. 2017). Interestingly Neu and authors reported that susceptible rose leaves infected with different types of fungus presented an up-regulation expression of the MYB108 gene which they suggest that in agreement with the upregulated PR genes observed in their transcriptomic study they propose that the PTI process could be "awaken" after pathogen recognition nevertheless not sufficient to promote a resistance behaviour of the plant to the pathogens (Neu et al. 2019). Meanwhile, the study performed by Chandra and colleagues focusing on susceptible and resistant rose genotypes infected by powdery mildew observed the up-regulation of *MYB108* transcript on the resistance genotype with the contribution of other important transcription factors (TFs) such as other MYBs, WRKYs, ERFs, bhlhs and TFs contributing to pathways important for defense (Chandran et al. 2021). In regard of the literature observed it's possible to suggest that in grapevine infected with downy mildew the susceptible genotype of our study could be recognizing the pathogens interaction though its defense system is still not sufficient to counterattack or overpowered by the pathogen.

Transcriptional and post-transcriptional modifications play an important role in the regulation of the activation of grapevine defense networks (Figueiredo et al. 2008). For instance, we found that genes encoding for transcription factors and those involved in protein modifications were differentially expressed after pathogen infection. Moreover, a modulation in the expression of important classes of genes involved in plant immunity, such as PTI- and ETI-related genes, PR and disease resistance proteins were found after the pathogen inoculation, and differences between the two genotypes were observed in accordance with their different susceptibility level to *P. viticola*.

III.4.2. DNA methylation plays a role in the plant immune defense system

In the stress adaptation mechanism, epigenetic modulation may play different roles. DNA methylation regulates gene expression in plant defense responses. Based on our results we hypothesise that *V. vinifera-P. viticola* interaction influences epigenetic mechanisms such as the DNA methylation maintenance and associated components such as DCL2. We observed a

down-regulation of genes encoding the DNA methyltransferases in the tolerant genotype at 6 hpi (*CMT1*) and 24 hpi (*MET1* and *CMT3*). Reports on the lacking of either MET1 or CMT3 methyltransferase activity in genetic mutants of different plant species induce resistance after the interaction with an array of pathogens (viruses, fungus and bacteria) (Dowen et al. 2012; Sánchez et al. 2016; Chen et al. 2018; Geng et al. 2019; Kuźnicki et al. 2019; Cui et al. 2021; Pujara et al. 2021). Also, DNA methylation can affect either SA pathway genes or *pathogenesis responsive gene 1 (PR1*) expression (Dowen et al. 2012; Hewezi et al. 2018; Chan and Zimmerli 2019).

On the other hand, our results also provided evidence of the modulation of genes related to DNA demethylation in the grapevine-*P. viticola* interaction. *DME* was down-regulated in our study on the tolerant cultivar, although, it is considered a key player in biotic and abiotic stress protection (Schumann et al. 2019; Ashapkin et al. 2020; Kong et al. 2020; Vyse et al. 2020; Zeng et al. 2021). Zeng and colleagues reported that DME is important for the regulation of genes related to defense/resistance since *dme* mutant presents a disease susceptibility and a pathogenesis related protein 5 (PR5) repression in Arabidopsis-bacteria/fungus interaction with increase pathogen presence (Zeng et al. 2021). In our study PR5, also known as thaumatin-like proteins, are up-regulated at the same time as DME was down-regulated (24 hpi). Nevertheless, studies have shown that DNA demethylation status is regulated by DME which therefore influences gene expression through targeting defense genes structure (Schumann et al. 2019; Zeng et al. 2021). Also, it has been reported that DME could need help of other DNA demethylases for their regulatory activity (Schumann et al. 2019; Zeng et al. 2021).

To further understand if epigenetic regulation is one of the key factors influencing the higher tolerance/susceptibility of the crossing hybrids, we have conducted a global DNA methylation evaluation at the first hours after pathogen contact. The 5-methylcytosine (5-mC) DNA ELISA kit provided in this work a highly specific 5-mC antibodies for any DNA context (CG, CHG or CHH) allowing the quantification of global DNA methylation. This methodology was already used in several works published (Atighi et al. 2020; Rasmussen et al. 2021). In fact, a similar approach has been already used in grapevine to differentiate the % 5-mC of two grapevine genotypes, a Portuguese susceptible cultivar and a RPV3 background crossing hybrid (Pereira et al. 2022). In our study, the global methylation patterns (5-mC) differ between the susceptible and tolerant crossing hybrids. In accordance with that, global methylation focusing on 5-mC presented a lower increase in the tolerant genotype. Analysis of global DNA methylation pattern modification on several species after infection with different pathogens has been reported on

tomato roots, Poplar bark or Arabidopsis leaves (Leonetti and Molinari 2020; Xiao et al. 2021). Often, resistant species showed DNA hypomethylation patterns while susceptible ones presented a hypermethylated DNA after pathogens attack (Leonetti and Molinari 2020; Xiao et al. 2021). Also, studies have observed that altering DNA methylation levels (hypo- or hypermethylation) on genomic regions could influence gene and phenotypic responses to plant diseases (Geng et al. 2019; Leonetti and Molinari 2020). Our results are in agreement with recent work done by Pereira and authors which reported that the global cytosine analysis performed on the tolerant cultivar Regent revealed a decrease of global methylation levels in the early hours of infection by *P. viticola* in comparison to the Trincadeira, a susceptible genotype (Pereira et al. 2022).

III.4.3. Involvement of epigenetic mechanisms in grapevine defense response

In our study variation in the expression of chromatin remodelers subunits such as SYD (part of the SWI/SNF chromatin complexes) could lead to alteration of chromatin conformation. Studies in Arabidopsis thaliana have shown that SYD is recruited to the promoter of a set of JA and ET responsive defense genes, and it is essential for their expression (Walley et al. 2008; Berr et al. 2012; Ding and Wang 2015; Johnson et al. 2015; Ramirez-Prado et al. 2018c, 2018a; Zhi and Chang 2021; Kim 2021; Panigrahi et al. 2021; Ramos-Cruz et al. 2021; Song et al. 2021).

Besides chromatin and DNA methylation, histone modifications are also involved in the plant defense responses (Ramirez-Prado et al. 2018c). In our study, several genes related to histones modifications, encoding for structural or regulatory proteins, were differentially expressed after *P. viticola* infection (Table III. 8). An important gene encoding protein of the WD-40 repeat family (HOS15) was downregulated on both analysed genotypes at different time periods of infection. HOS15 plays an important role in the plant immunity against different stresses via histone acetylation/deacetylation pathways (Kumar et al. 2021). Li and colleagues observed that wheat infected with powdery mildew has a negative regulatory role on HOS15 in the defense system (Liu et al. 2019). The authors suggested that a mediation between HOS15-HDA6 and histone acetyltransferases could be occurring to regulate the transcription of defense related genes (Liu et al. 2019).

Additionally, in our study the gene expression of one of the encoding genes of histone variant H2A.Z (HTA8) is repressed in the susceptible genotype early after the infection which could be a preliminary indication of the H2A.Z complex relevance on the plant-pathogen interaction, as shown in the literature (Berriri et al. 2016; Cai et al. 2021). Cai and colleagues observed that an increased plant susceptibility is present in Arabidopsis H2A.Z mutants (*hta8 hta9 hta11*) after inoculated with *Slerotinia sclerotiorum*, a necrotrophic fungus (Cai et al. 2021). Also, in case of H2A.Z encoding genes inexistence, the others could play a compensatory role (Berriri et al. 2016). Moreover, studies have shown that H2A.Z could be a player in the resistance against different pathogens with influence in JA/ET pathways (Berriri et al. 2016; Cai et al. 2021; Pujara et al. 2021).

The deposition of H2A.Z histone variant on nucleosomes and histone modifications, is possible through the SWR1 complex and ER signalling pathway thus modulate gene's expression in plant immunity against biotic stress (Cai et al. 2021). Interestingly, in our work ARP6, subunit of SWR1 complex, was downregulated on the tolerant variety. Several studies have shown that Arabidopsis SWR1 subunits mutated displays different susceptibilities against pathogens such as ARP6 that presents a wild-type behaviour (Berriri et al. 2016; Cai et al. 2021). Still Cai and authors reported that with a gene-gene interaction analysis between double mutants arp6 and ERECTA- mitogen-activated protein kinase (ER-MPK) signalling genes, a susceptible phenotype in the presence of the *S. sclerotiorum* was obsereved (Cai et al. 2021).

Furthermore, the linker histone 1 (H1) was downregulated in the tolerant genotype at 6 hpi. Histone H1 is known to regulate chromatin condensation (Sheikh et al. 2023). Sheikh and colleagues observed the effect of the absence of H1 in Arabidopsis when inoculated *P. seryngae* and treated with flagellin 22 (flg22) for prime analysis (Sheikh et al. 2023). They eluded that the genes related to defense are influenced by H1 to respond immediately or not to the pathogen attack (Sheikh et al. 2023). Also, while analysing DNA methylation and histone modifications throughout the plant immune defense system, Arabidopsis H1 indicated a possible role in plant immunity since H1 impacts chromatin rearrangement through the influence of the epigenetic profile (Sheikh et al. 2023). Therefore, H1 seems to part of the defense and epigenetic regulation of the incompatible interaction between grapevine and *P. viticola*.

Beyond the more studied epigenetic traits, small RNA can influence plant defense responses and plant adaptation mechanisms. It is well known that Dicer-like proteins are essential components of the miRNA and siRNA biogenesis and that these molecules have a recognised role in plant defense mechanisms (Qin et al. 2017). In our study the repression of the Dicer-like

2 (DCL2) gene in the tolerant genotype could suggest a role in grapevine-oomycete interaction. Studies have shown that DCL2 and DCL4 are associated with virus infection immune defense with the purpose of attaining viral siRNA (vsiRNA) as a strategy against viruses (Prakash et al. 2017; Lee and Carroll 2018; Ashapkin et al. 2020; Erdmann and Picard 2020). In agreement with our finding Brilli et al. were able to observe modulation of different components of the RNA machinery while analysing *V. vinifera-P. viticola* interaction (Brilli et al. 2018). Also, Wang et al analysed *B. cineria* interaction with a wide variety of hosts where it was identified a bi-directional defense process between host and pathogen RNAi and DCL2 targeted genes (Wang et al. 2016). Another important small RNA biogenesis molecule evident in our study is the serine/arginine-rich splicing factor RS41-like (RSP40) that were up-regulated in the susceptible cultivar. Literature has shown that DCL1 plays a defense role in plant-*B. cinerea* interaction whereas Coolen and colleagues observed that the expression of DCL1 in RS40/41 mutant Arabidopsis after *B. cinerea* infection decreased (Weiberg et al. 2013; Coolen et al. 2019).

The Retinoblastoma Related 1 (RBR1), RAD51 and gamma-H2AX are corelated with to the epigenetic machinery as well as interact with defense mechanisms against biotic stresses (Wang et al. 2010, 2014; Bouyer et al. 2018; Camborde et al. 2019; Desvoyes and Gutierrez 2020). RBR1 has been revealed as a member of the DNA Damage repair machinery (DDR) together with RAD51 (Bouyer et al. 2018; Camborde et al. 2019). Furthermore, gamma-H2AX and RAD51 affect the DNA through direct DSB when interacting with other plant-necrotrophic pathogens (Song and Bent 2014). Overall, these data support the involvement of these mechanisms in the epigenetic regulation of the grapevine at the defense response against *P. viticola* and open the possibility of new functional regulations of stress responses in grapevine.

Evidence of the involvement of epigenetic modulation in plants resistance has been increasingly reported for a wide variety of biotic stresses (Ding and Wang 2015; Elhamamsy 2016; Espinas et al. 2016; Ramirez-Prado et al. 2018a; Kuźnicki et al. 2019; Kong et al. 2020; Cui et al. 2021; Zhi and Chang 2021). Therefore, in this work we focused on the DEGs involved in the plant epigenetic machinery and to its interplay with its defense system (Figure III. 14). The Figure III. 14 represents at a nuclear and cytoplasmic level the interaction of the epigenetic related DEGs and the correlation with signalling pathways after *P. viticola* inoculation in either grapevine populations studied (yellow – Tolerant and Blue – susceptible) indicating the possible impact of intermediary genes (grey) that could be playing also a role in these connections.

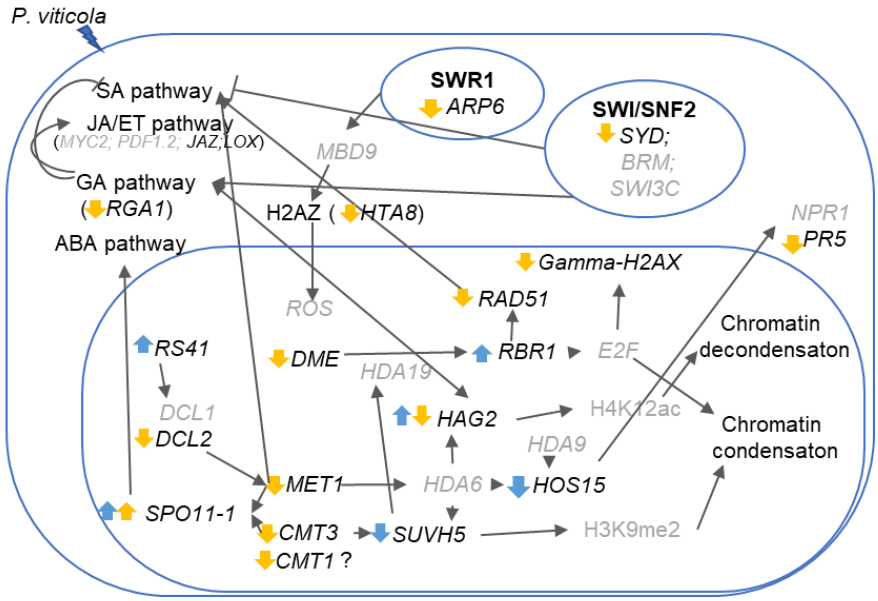


Figure III. 14 - Schematic representation of the epigenetic machinery interaction and modulation after *Plasmopara viticola* infection of grapevine leaf tissue. Main blue structure: cell wall; Iner blue structure: nucleus; two circular structures: chromatin remodelling complexes; Yellow arrows: genes differentially expressed in the tolerant genotype. Blue arrows: genes differentially expressed in the susceptible genotype. Up arrows indicate up-regulation, down arrows indicate down-regulation; Grey genes: intermediated genes reported to interact with genes.

III.5. References

Albrecht U, Bowman KD (2012) Transcriptional response of susceptible and tolerant citrus to infection with Candidatus Liberibacter asiaticus. Plant Sci 185–186: 118–130

Ali M, Javaid A, Naqvi SH, Batcho A, Kayani WK, Lal A, Sajid IA, Nwogwugwu JO (2020) Biotic stress triggered small RNA and RNAi defense response in plants. Mol Biol Rep 47: 5511–5522

Almeida NF, Leitão ST, Krezdorn N, Rotter B, Winter P, Rubiales D, Vaz Patto MC (2014) Allelic diversity in the transcriptomes of contrasting rust-infected genotypes of, a lasting resource for smart breeding. BMC Plant Biol 14: 1–15

Alonso C, Ramos-Cruz D, Becker C (2019) The role of plant epigenetics in biotic interactions. New Phytol 221: 731–737

Ashapkin V V., Kutueva LI, Aleksandrushkina NI, Vanyushin BF (2020) Epigenetic mechanisms of plant adaptation to biotic and abiotic stresses. Int J Mol Sci 21: 1–32

Atighi MR, Verstraeten B, De Meyer T, Kyndt T (2020) Genome-wide DNA hypomethylation shapes nematode pattern-triggered immunity in plants. New Phytol

227: 545–558

Barozai MYK, Aziz AN (2018) Recent plant growth and stress management related significant advancements in epigenetics. Ann Agrar Sci 16: 416–421

Battilani P, Lanubile A, Scala V, Reverberi M, Gregori R, Falavigna C, Dall'asta C, Park YS, Bennett J, Borrego EJ, Kolomiets M V. (2018) Oxylipins from both pathogen and host antagonize jasmonic acid-mediated defence via the 9-lipoxygenase pathway in Fusarium verticillioides infection of maize. Mol Plant Pathol 19: 2162–2176

Berr A, Ménard R, Heitz T, Shen WH (2012) Chromatin modification and remodelling: A regulatory landscape for the control of Arabidopsis defence responses upon pathogen attack. Cell Microbiol 14: 829–839

Berriri S, Gangappa SN, Kumar SV (2016) SWR1 Chromatin-Remodeling Complex Subunits and H2A.Z Have Non-overlapping Functions in Immunity and Gene Regulation in Arabidopsis. Mol Plant 9: 1051–1065

Berrocal-Lobo M, Molina A, Solano R (2002) Constitutive expression of Ethylene-Response-Factor1 in arabidopsis confers resistance to several necrotrophic fungi. Plant J 29: 23–32

Bhattarai K, Wang W, Cao Z, Deng Z (2018) Comparative analysis of impatiens leaf transcriptomes reveal candidate genes for resistance to downy mildew caused by plasmopara obducens. Int J Mol Sci 19: 1–17

Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, Fridman WH, Pagès F, Trajanoski Z, Galon J (2009) ClueGO: A Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. Bioinformatics 25: 1091–1093

Bouyer D, Heese M, Chen P, Harashima H, Roudier F, Grüttner C, Schnittger A (2018) Genome-wide identification of RETINOBLASTOMA RELATED 1 binding sites in Arabidopsis reveals novel DNA damage regulators. PLOS Genet 14: e1007797

Breitling R, Armengaud P, Amtmann A, Herzyk P (2004) Rank products: A simple, yet powerful, new method to detect differentially regulated genes in replicated microarray experiments. FEBS Lett 573: 83–92

Brilli M, Asquini E, Moser M, Bianchedi PL, Perazzolli M, Si-Ammour A (2018) A multi-omics study of the grapevine-downy mildew (Plasmopara viticola) pathosystem unveils a complex protein coding and noncoding-based arms race during infection. Sci Rep 8: 1–

12

Brocklehurst S, Watson M, Carr IM, Out S, Heidmann I, Meyer P (2018) Induction of epigenetic variation in Arabidopsis by over-expression of DNA METHYLTRANSFERASE1 (MET1). PLoS One 13: 1–22

Buell CR (2002) Interactions Between Xanthomonas Species and Arabidopsis thaliana. Arab B 1: 1–11

Buonassisi D, Colombo M, Migliaro D, Dolzani C, Peressotti E, Mizzotti C, Velasco R, Masiero S, Perazzolli M, Vezzulli S (2017) Breeding for grapevine downy mildew resistance: a review of "omics" approaches. Euphytica 1–21

Cai H, Huang Y, Chen F, Liu L, Chai M, Zhang M, Yan M, Aslam M, He Q, Qin Y (2021) ERECTA signaling regulates plant immune responses via chromatin-mediated promotion of WRKY33 binding to target genes. New Phytol 230: 737–756

Camborde L, Raynaud C, Dumas B, Gaulin E (2019) DNA-Damaging Effectors: New Players in the Effector Arena. Trends Plant Sci 24: 1094–1101

Catacchio CR, Alagna F, Perniola R, Bergamini C, Rotunno S, Calabrese FM, Crupi P, Antonacci D, Ventura M, Cardone MF (2019) Transcriptomic and genomic structural variation analyses on grape cultivars reveal new insights into the genotype-dependent responses to water stress. Sci Rep 9: 1–15

Cerrudo I, Caliri-Ortiz ME, Keller MM, Degano ME, Demkura P V., Ballaré CL (2017) Exploring growth-defence trade-offs in Arabidopsis: phytochrome B inactivation requires JAZ10 to suppress plant immunity but not to trigger shade-avoidance responses. Plant Cell Environ 40: 635–644

Cerrudo I, Keller MM, Cargnel MD, Demkura P V., de Wit M, Patitucci MS, Pierik R, Pieterse CMJ, Ballaré CL (2012) Low red/far-red ratios reduce arabidopsis resistance to Botrytis cinerea and jasmonate responses via a COI1-JAZ10-dependent, salicylic acid-independent mechanism. Plant Physiol 158: 2042–2052

Chan C, Zimmerli L (2019) The Histone Demethylase IBM1 Positively Regulates Arabidopsis Immunity by Control of Defense Gene Expression. Front Plant Sci 10: 1–10

Chandran NK, Sriram S, Prakash T, Budhwar R (2021) Transcriptome changes in resistant and susceptible rose in response to powdery mildew. J Phytopathol 169: 556–569

Chen H, Shu H, Wang L, Zhang F, Li X, Ochola SO, Mao F, Ma H, Ye W, Gu T, Jiang L, Wu Y, Wang Y, Kamoun S, Dong S (2018) Phytophthora methylomes are modulated by 6mA methyltransferases and associated with adaptive genome regions. Genome Biol 19: 1–16

Chen J, Clinton M, Qi G, Wang D, Liu F, Qing Fu Z (2020) Reprogramming and remodeling: Transcriptional and epigenetic regulation of salicylic acid-mediated plant defense. J Exp Bot 71: 5256–5268

Chen X, Wang W, Cai P, Wang Z, Li T, Du Y (2021) The role of the MAP kinase−kinase protein StMKK1 in potato immunity to different pathogens. Hortic Res 8: 1–9

Cheng HQ, Han LB, Yang CL, Wu XM, Zhong NQ, Wu JH, Wang FX, Wang HY, Xia GX (2016) The cotton MYB108 forms a positive feedback regulation loop with CML11 and participates in the defense response against Verticillium dahliae infection. J Exp Bot 67: 1935–1950

Cheng J, Fan H, Li L, Hu B, Liu H, Liu Z (2018) Genome-wide Identification and Expression Analyses of RPP13-like Genes in Barley. Biochip J 12: 102–113

Chong J, Le Henanff G, Bertsch C, Walter B (2008) Identification, expression analysis and characterization of defense and signaling genes in Vitis vinifera. Plant Physiol Biochem 46: 469–481

Coolen S, Van Pelt JA, Van Wees SCM, Pieterse CMJ (2019) Mining the natural genetic variation in Arabidopsis thaliana for adaptation to sequential abiotic and biotic stresses. Planta 249: 1087–1105

Crespo-Salvador Ó, Escamilla-Aguilar M, López-Cruz J, López-Rodas G, González-Bosch C (2018) Determination of histone epigenetic marks in Arabidopsis and tomato genes in the early response to Botrytis cinerea. Plant Cell Rep 37: 153–166

Cui N, Chen X, Shi Y, Chi M, Hu J, Lai K, Wang Z, Wang H (2021) Changes in the epigenome and transcriptome of rice in response to Magnaporthe oryzae infection. Crop J 9: 843–853

Darino M, Chia KS, Marques J, Aleksza D, Soto-Jiménez LM, Saado I, Uhse S, Borg M, Betz R, Bindics J, Zienkiewicz K, Feussner I, Petit-Houdenot Y, Djamei A (2021) Ustilago maydis effector Jsi1 interacts with Topless corepressor, hijacking plant jasmonate/ethylene signaling. New Phytol 229: 3393–3407

De-La-Peña C, Rangel-Cano A, Alvarez-Venegas R (2012) Regulation of disease-responsive genes mediated by epigenetic factors: Interaction of Arabidopsis-Pseudomonas. Mol Plant Pathol 13: 388–398

Deleris A, Halter T, Navarro L (2016) DNA Methylation and Demethylation in Plant Immunity. Annu Rev Phytopathol 54: 579–603

Demianski AJ, Chung KM, Kunkel BN (2012) Analysis of Arabidopsis JAZ gene expression during Pseudomonas syringae pathogenesis. Mol Plant Pathol 13: 46–57

Desvoyes B, Gutierrez C (2020) Roles of plant retinoblastoma protein: cell cycle and beyond. EMBO J 39: 1–18

Ding B, Wang GL (2015) Chromatin versus pathogens: The function of epigenetics in plant immunity. Front Plant Sci 6: 1–8

Dolgikh VA, Pukhovaya EM, Zemlyanskaya E V. (2019) Shaping Ethylene Response: The Role of EIN3/EIL1 Transcription Factors. Front Plant Sci 10: 1–9

Dowen RH, Pelizzola M, Schmitz RJ, Lister R, Dowen JM, Nery JR, Dixon JE, Ecker JR (2012) Widespread dynamic DNA methylation in response to biotic stress. Proc Natl Acad Sci U S A 109: E2183–E2191

Eichmeier A, Kiss T, Penazova E, Pecenka J, Berraf-tebbal A, Baranek M, Pokluda R, Cechova J, Gramaje D, Grzebelus D (2019) MicroRNAs in Vitis vinifera cv. Chardonnay Are Differentially Expressed in Response to Diaporthe Species. Genes (Basel) 10: 1–19

Elhamamsy AR (2016) DNA methylation dynamics in plants and mammals: overview of regulation and dysregulation. Cell Biochem Funct 34: 289–298

Erdmann RM, Picard CL (2020) RNA-directed DNA Methylation. PLOS Genet 16: 1–31

Espinas NA, Saze H, Saijo Y (2016) Epigenetic control of defense signaling and priming in plants. Front Plant Sci 7: 1–7

Figueiredo A, Figueiredo J, Maia M, Cavaco AR, Laureano G, Nascimento R, Silva C da, Matos AR, Silva MS (2018) Subtilisin-like proteases and lipid signalling events play an important role in grapevine resistance to downy mildew: a systems biology approach. Rev Ciências Agrárias 41: 61–66

Figueiredo A, Fortes AM, Ferreira S, Sebastiana M, Choi YH, Sousa L, Acioli-Santos B,

Pessoa F, Verpoorte R, Pais MS (2008) Transcriptional and metabolic profiling of grape (Vitis vinifera L.) leaves unravel possible innate resistance against pathogenic fungi. J Exp Bot 59: 3371–3381

Figueiredo A, Martins J, Sebastiana M, Guerreiro A, Silva A, Matos AR, Monteiro F, Pais MS, Roepstorff P, Coelho AV (2017) Specific adjustments in grapevine leaf proteome discriminating resistant and susceptible grapevine genotypes to Plasmopara viticola. J Proteomics 152: 48–57

Figueiredo A, Monteiro F, Fortes AM, Bonow-Rex M, Zyprian E, Sousa L, Pais MS (2012) Cultivar-specific kinetics of gene induction during downy mildew early infection in grapevine. Funct Integr Genomics 12: 379–386

Fröbel S, Zyprian E (2019) Colonization of Different Grapevine Tissues by Plasmopara viticola—A Histological Study. Front Plant Sci 10: 1–13

Fujita K, Inui H (2021) Review: Biological functions of major latex-like proteins in plants. Plant Sci 306: 1–16

Galindo-González L, Deyholos MK (2016) RNA-seq transcriptome response of flax (Linum usitatissimum L.) to the pathogenic fungus fusarium oxysporum f. sp. lini. Front Plant Sci 7: 1–22

Gallusci P, Dai Z, Génard M, Gauffretau A, Leblanc-Fournier N, Richard-Molard C, Vile D, Brunel-Muguet S (2017) Epigenetics for Plant Improvement: Current Knowledge and Modeling Avenues. Trends Plant Sci 22: 610–623

Gao M, Yin X, Yang W, Lam SM, Tong X, Liu J, Wang X, Li Q, Shui G, He Z (2017) GDSL lipases modulate immunity through lipid homeostasis in rice. PLoS Pathog 13: 1–24

Gao X, Brodhagen M, Isakeit T, Brown SH, Göbel C, Betran J, Feussner I, Keller NP, Kolomiets M V. (2009) Inactivation of the lipoxygenase ZmLOX3 increases susceptibility of maize to Aspergillus sp. Mol Plant Microbe Interact 22: 222–231

Geng S, Kong X, Song G, Jia M, Guan J, Wang F, Qin Z, Wu L, Lan X, Li A, Mao L (2019) DNA methylation dynamics during the interaction of wheat progenitor Aegilops tauschii with the obligate biotrophic fungus Blumeria graminis f. sp. tritici. New Phytol 221: 1023–1035

Gil JF, Wibberg D, Eini O, Savenkov EI, Varrelmann M, Liebe S (2020) Comparative Transcriptome Analysis Provides Molecular Insights into the Interaction of Beet necrotic

yellow vein virus and Beet soil-borne mosaic virus with Their Host Sugar Beet. Viruses 12: 1–21

Guerra-Guimarães L, Tenente R, Pinheiro C, Chaves I, Silva M do C, Cardoso FMH, Planchon S, Barros DR, Renaut J, Ricardo CP (2015) Proteomic analysis of apoplastic fluid of Coffea arabica leaves highlights novel biomarkers for resistance against Hemileia vastatrix. Front Plant Sci 6: 1–16

Gupta S, Bhar A, Chatterjee M, Ghosh A, Das S (2017) Transcriptomic dissection reveals wide spread differential expression in chickpea during early time points of Fusarium oxysporum f. sp. ciceri Race 1 attack. PLoS One 12: 1–37

Hall AT (1999) BioEdit: a user-friendly biological sequence alignmt editor and analysis program for Windows 95/96/NT. Oxford Univ Press 41: 95–98

Hao CC, Jia J, Chen Z, Xing JH, Weng QY, Wang FR, Dong JG, Han JM (2013) Functional analysis of BT4 of Arabidopsis thaliana in resistance against Botrytis cinerea. Australas Plant Pathol 42: 393–401

He C, Zhang Z, Li B, Tian S (2020a) The pattern and function of DNA methylation in fungal plant pathogens. Microorganisms 8: 1–14

He M, Xu Y, Cao J, Zhu Z, Jiao Y, Wang Y, Guan X, Yang Y, Xu W, Fu Z (2013) Subcellular localization and functional analyses of a PR10 protein gene from Vitis pseudoreticulata in response to Plasmopara viticola infection. Protoplasma 250: 129–140

He S, Yuan G, Bian S, Han X, Liu K, Cong P, Zhang C (2020b) Major Latex Protein MdMLP423 Negatively Regulates Defense against Fungal Infections in Apple. Int J Mol Sci 21: 1–20

Henk AD, Warren RF, Innes RW (1999) A new Ac-like transposon of arabidopsis is associated with a deletion of the RPS5 disease resistance gene. Genetics 151: 1581–1589

Herlihy J, Ludwig NR, van den Ackerveken G, McDowell JM (2019) Oomycetes Used in Arabidopsis Research. Arab B 2019: 1–26

Hewezi T, Pantalone V, Bennett M, Neal Stewart C, Burch-Smith TM (2018) Phytopathogen-induced changes to plant methylomes. Plant Cell Rep 37: 17–23

Hoang TV, Vo KTX, Hong WJ, Jung KH, Jeon JS (2018) Defense Response to Pathogens Through Epigenetic Regulation in Rice. J Plant Biol 61: 1–10

Howe E, Holton K, Nair S, Schlauch D, Sinha R, Quackenbush J (2010) MeV: MultiExperiment viewer

Huang CY, Jin H (2022) Coordinated Epigenetic Regulation in Plants: A Potent Managerial Tool to Conquer Biotic Stress. Front Plant Sci 12: 1–11

Huang PY, Catinot J, Zimmerli L (2016) Ethylene response factors in Arabidopsis immunity. J Exp Bot 67: 1231–1241

Iqbal Z, Iqbal MS, Hashem A, Abd_Allah EF, Ansari MI (2021) Plant Defense Responses to Biotic Stress and Its Interplay With Fluctuating Dark/Light Conditions. Front Plant Sci 12: 1–22

Islam Z, Ahn SY, Yun HK (2015) Analysis of structure and differential expression of Pseudomonas syringae 5-like (RPS5-like) genes in pathogen-infected vitis flexuosa. Turkish J Biol 39: 775–789

Johnson KCM, Xia S, Feng X, Li X (2015) The Chromatin Remodeler SPLAYED Negatively Regulates SNC1-Mediated Immunity. Plant Cell Physiol 56: 1616–1623

Jones JDG, Dangl JL (2006) The plant immune system. Nature 444: 323–329

Juliá M, Telenti A, Rausell A (2015) Sincell: An R/Bioconductor package for statistical assessment of cell-state hierarchies from single-cell RNA-seq. Bioinformatics 31: 3380–3382

Kim JH (2021) Multifaceted chromatin structure and transcription changes in plant stress response. Int J Mol Sci 22: 1–25

Köhler C, Springer N (2017) Plant epigenomics—deciphering the mechanisms of epigenetic inheritance and plasticity in plants. Genome Biol 18: 1–3

Kong L, Liu Y, Wang X, Chang C (2020) Insight into the role of epigenetic processes in abiotic and biotic stress response in wheat and barley. Int J Mol Sci 21: 1–15

Kong X, Qin W, Huang X, Kong F, Schoen CD, Feng J, Wang Z, Zhang H (2016) Development and application of loop-mediated isothermal amplification (LAMP) for detection of Plasmopara viticola. Sci Rep 6: 1–9

Kumar V, Thakur JK, Prasad M (2021) Histone acetylation dynamics regulating plant development and stress responses. Cell Mol Life Sci 78: 4467–4486

Kuźnicki D, Meller B, Arasimowicz-Jelonek M, Braszewska-Zalewska A, Drozda A,

Floryszak-Wieczorek J (2019) BABA-induced DNA methylome adjustment to intergenerational defense priming in potato to Phytophthora infestans. Front Plant Sci 10: 1–16

Lämke J, Bäurle I (2017) Epigenetic and chromatin-based mechanisms in environmental stress adaptation and stress memory in plants. Genome Biol 18: 1–11

Laureano G, Figueiredo J, Cavaco AR, Duarte B, Caçador I, Malhó R, Sousa Silva M, Matos AR, Figueiredo A (2018) The interplay between membrane lipids and phospholipase A family members in grapevine resistance against Plasmopara viticola. Sci Rep 8: 1–15

Laurell C, Berglund T, Ohlsson AB (2021) Transcriptome analysis shows nicotinamide seed treatment alters expression of genes involved in defense and epigenetic processes in roots of seedlings of Picea abies. J For Res doi.org/10.1007/s11676-021-01410-z

Lazar A, Coll A, Dobnik D, Baebler Š, Bedina-Zavec A, Žel J, Gruden K (2014) Involvement of potato (Solanum tuberosum L.) MKK6 in response to potato virus Y. PLoS One 9: 1–15

Lee CH, Carroll BJ (2018) Evolution and Diversification of Small RNA Pathways in Flowering Plants. Plant Cell Physiol 59: 2169–2187

Lee S, Fu F, Xu S, Lee SY, Yun D-J, Mengiste T (2016) Global regulation of plant immunity by histone lysine methyl transferases. Plant Cell 28: 1640–1661

Leonetti P, Molinari S (2020) Epigenetic and metabolic changes in root-knot nematode-plant interactions. Int J Mol Sci 21: 1–17

Li B, Wang R, Wang S, Zhang J, Chang L (2021) Diversified Regulation of Cytokinin Levels and Signaling During Botrytis cinerea Infection in Arabidopsis. Front Plant Sci 12: 1–14

Li X, Wu J, Yin L, Zhang Y, Qu J, Lu J (2015) Comparative transcriptome analysis reveals defense-related genes and pathways against downy mildew in Vitis amurensis grapevine. Plant Physiol Biochem 95: 1–14

Liang D, Liu M, Hu Q, He M, Qi X, Xu Q, Zhou F, Chen X (2015) Identification of differentially expressed genes related to aphid resistance in cucumber (Cucumis sativus L.). Sci Rep 5: 1–10

Liao CJ, Hailemariam S, Sharon A, Mengiste T (2022) Pathogenic strategies and immune mechanisms to necrotrophs: Differences and similarities to biotrophs and hemibiotrophs.

Curr Opin Plant Biol 69: 1–15

Liao CJ, Lai Z, Lee S, Yun DJ, Mengiste T (2016) Arabidopsis HOOKLESS1 regulates responses to pathogens and abscisic acid through interaction with MED18 and acetylation of WRKY33 and ABI5 chromatin. Plant Cell 28: 1662–1681

Lievens L, Pollier J, Goossens A, Beyaert R, Staal J (2017) Abscisic acid as pathogen effector and immune regulator. Front Plant Sci 8: 1–15

Liu J, Zhi P, Wang X, Fan Q, Chang C (2019) Wheat WD40-repeat protein TaHOS15 functions in a histone deacetylase complex to fine-tune defense responses to Blumeria graminis f.sp. tritici. J Exp Bot 70: 255–268

Liu L, Liu CY, Wang H, Yu SY, Guan TS, Huang YF, Li RC (2020a) The abscisic acid receptor gene VvPYL4 positively regulates grapevine resistance to Plasmopara viticola. Plant Cell Tissue Organ Cult 142: 483–492

Liu L, Zhang B, Wang H, Yu SY, Guan TS, Huang YF, Liu CY (2020b) Candidate resistance genes selection and transcriptome analysis for the early responses to Plasmopara viticola infection in grape cultivars. J Plant Pathol 102: 857–869

Livak KJ, Schmittgen TD (2001) Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. Methods 25: 402–408

López A, Ramírez V, García-Andrade J, Flors V, Vera P (2011) The RNA silencing enzyme RNA polymerase V is required for plant immunity. PLoS Genet 7: 1–10

Lorenzo O, Piqueras R, Sánchez-Serrano JJ, Solano R (2003) ETHYLENE RESPONSE FACTOR1 integrates signals from ethylene and jasmonate pathways in plant defense. Plant Cell 15: 165–178

Lyu M, Shi H, Li Y, Kuang K, Yang Z, Li J, Chen D, Li Y, Kou X, Zhong S (2019) Oligomerization and Photo-Deoligomerization of HOOKLESS1 Controls Plant Differential Cell Growth. Dev Cell 51: 78-88.e3

Maia M, Maccelli A, Nascimento R, Ferreira AEN, Crestoni ME, Cordeiro C, Figueiredo A, Silva MS (2019) Early detection of Plasmopara viticola-infected leaves through FT-ICR-MS metabolic profiling. Acta Hortic 1248: 575–580

March-Díaz R, García-Domínguez M, Lozano-Juste J, León J, Florencio FJ, Reyes JC (2008) Histone H2A.Z and homologues of components of the SWR1 complex are required to

control immunity in Arabidopsis. Plant J 53: 475–487

Mengiste T, Chen X, Salmeron J, Dietrich R (2003) The Botrytis Susceptible1 Gene Encodes an R2R3MYB Transcription Factor Protein That Is Required for Biotic and Abiotic Stress Responses in Arabidopsis. Plant Cell 15: 2551–2565

Modesto I, Sterck L, Arbona V, Gómez-Cadenas A, Carrasquinho I, Van de Peer Y, Miguel CM (2021) Insights Into the Mechanisms Implicated in Pinus pinaster Resistance to Pinewood Nematode. Front Plant Sci 12: 1–18

Möller M, Stukenbrock EH (2017) Evolution and genome architecture in fungal plant pathogens. Nat Rev Microbiol 15: 756–771

Nascimento R, Maia M, Ferreira AEN, Silva AB, Freire AP, Cordeiro C, Silva MS, Figueiredo A (2019) Early stage metabolic events associated with the establishment of Vitis vinifera – Plasmopara viticola compatible interaction. Plant Physiol Biochem 137: 1–13

Neu E, Domes HS, Menz I, Kaufmann H, Linde M, Debener T (2019) Interaction of roses with a biotrophic and a hemibiotrophic leaf pathogen leads to differences in defense transcriptome activation. Plant Mol Biol 99: 299–316

Panigrahi GK, Sahoo A, Satapathy KB (2021) Insights to plant immunity: Defense signaling to epigenetics. Physiol Mol Plant Pathol 113: 1–7

Parker AH, Wilkinson SW, Ton J (2022) Epigenetics: a catalyst of plant immunity against pathogens. New Phytol 233: 66–83

Pereira G, Pereira J, Santos RB, Figueiredo A (2022) Uncovering the role of DNA methyltransferases in grapevine – Plasmopara viticola interaction: From genome-wide characterization to global methylation patterns. Gene 837: 1–9

Pirrello C, Malacarne G, Moretto M, Lenzi L, Perazzolli M, Zeilmaker T, Van den Ackerveken G, Pilati S, Moser C, Giacomelli L (2022) Grapevine DMR6-1 Is a Candidate Gene for Susceptibility to Downy mildew. Biomolecules 12: 1–21

Prakash V, Devendran R, Chakraborty S (2017) Overview of plant RNA dependent RNA polymerases in antiviral defense and gene silencing. Indian J Plant Physiol 22: 493–505

Pujara DS, Kim S-I, Nam JC, Mayorga J, Elmore I, Kumar M, Koiwa H, Kang H-G (2021) Imaging-Based Resistance Assay Using Enhanced Luminescence-Tagged Pseudomonas

syringae Reveals a Complex Epigenetic Network in Plant Defense Signaling Pathways. Mol Plant-Microbe Interact 34: 990–1000

Qin C, Li B, Fan Y, Zhang X, Yu Z, Ryabov E, Zhao M, Wang H, Shi N, Zhang P, Jackson S, Tör M, Cheng Q, Liu Y, Gallusci P, Hong Y (2017) Roles of dicer-like proteins 2 and 4 in intra- and intercellular antiviral silencing. Plant Physiol 174: 1067–1081

Rambani A, Pantalone V, Yang S, Rice JH, Song Q, Mazarei M, Arelli PR, Meksem K, Stewart CN, Hewezi T (2020) Identification of introduced and stably inherited DNA methylation variants in soybean associated with soybean cyst nematode parasitism. New Phytol 227: 168–184

Ramirez-Prado JS, Abulfaraj AA, Rayapuram N, Benhamed M, Hirt H (2018a) Plant Immunity: From Signaling to Epigenetic Control of Defense. Trends Plant Sci 23: 833–844

Ramirez-Prado JS, Abulfaraj AA, Rayapuram N, Benhamed M, Hirt H (2018b) Plant Immunity: From Signaling to Epigenetic Control of Defense. Trends Plant Sci 23: 833–844

Ramirez-Prado JS, Piquerez SJM, Bendahmane A, Hirt H, Raynaud C, Benhamed M (2018c) Modify the histone to win the battle: Chromatin dynamics in plant–pathogen interactions. Front Plant Sci 9: 1–18

Ramos-Cruz D, Troyee AN, Becker C (2021) Epigenetics in plant organismic interactions. Curr Opin Plant Biol 61: 1–10

Ramu VS, Dawane A, Lee S, Oh S, Lee HK, Sun L, Senthil-Kumar M, Mysore KS (2020) Ribosomal protein QM/RPL10 positively regulates defence and protein translation mechanisms during nonhost disease resistance. Mol Plant Pathol 21: 1481–1494

Rasmussen EMK, Seier KL, Pedersen IK, Kreibich C, Amdam G V., Münch D, Dahl JA (2021) Screening bioactive food compounds in honey bees suggests curcumin blocks alcohol-induced damage to longevity and DNA methylation. Sci Rep 11: 1–11

Rojas-Rojas FU, Vega-Arreguín JC (2021) Epigenetic insight into regulatory role of chromatin covalent modifications in lifecycle and virulence of Phytophthora. Environ Microbiol Rep 13: 445–457

Salas-Marina MA, Isordia-Jasso MI, Islas-Osuna MA, Delgado-Sánchez P, Jiménez-Bremont JF, Rodríguez-Kessler M, Rosales-Saavedra MT, Herrera-Estrella A, Casas-Flores S

(2015) The Epl1 and Sm1 proteins from Trichoderma atroviride and Trichoderma virens differentially modulate systemic disease resistance against different life style pathogens in Solanum lycopersicum. Front Plant Sci 6: 1–13

Sánchez AL, Stassen JHM, Fursi l., Smith LM, Ton J (2016) The role of DNA (de)methylation in immune responsiveness of Arabidopsis. Plant J 88: 361–374

Santos RB, Nascimento R, Coelho A V., Figueiredo A (2020) Grapevine – Downy Mildew Rendezvous : Proteome Analysis of the First Hours of an Incompatible Interaction. Plants 9: 1–17

Sari E, Cabral AL, Polley B, Tan Y, Hsueh E, Konkin DJ, Knox RE, Ruan Y, Fobert PR (2019) Weighted gene co-expression network analysis unveils gene networks associated with the Fusarium head blight resistance in tetraploid wheat. BMC Genomics 20: 1–24

Schumann U, Lee JM, Smith NA, Zhong C, Zhu JK, Dennis ES, Millar AA, Wang MB (2019) DEMETER plays a role in DNA demethylation and disease response in somatic tissues of Arabidopsis. Epigenetics 14: 1074–1087

Serra H, Choi K, Zhao X, Blackwell AR, Kim J, Henderson IR (2018) Interhomolog polymorphism shapes meiotic crossover within the Arabidopsis RAC1 and RPP13 disease resistance genes. PLOS Genet 14: e1007843

Shah J (2003) The salicylic acid loop in plant defense. Curr Opin Plant Biol 6: 365–371

Shannon P, Markiel A, Ozier O, Baliga N, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T (2003) Cytoscape: A Software Environment for Integrated Models. Genome Res 13: 2498–2504

Sheikh AH, Nawaz K, Tabassum N, Almeida-Trapp M, Mariappan KG, Alhoraibi H, Rayapuram N, Aranda M, Groth M, Hirt H (2023) Linker histone H1 modulates defense priming and immunity in plants. Nucleic Acids Res 51: 4252–4265

Song J, Bent AF (2014) Microbial Pathogens Trigger Host DNA Double-Strand Breaks Whose Abundance Is Reduced by Plant Defense Responses. PLoS Pathog 10: 1–11

Song ZT, Liu JX, Han JJ (2021) Chromatin remodeling factors regulate environmental stress responses in plants. J Integr Plant Biol 63: 438–450

Stotz HU, Pittendrigh BR, Kroymann J, Weniger K, Fritsche J, Bauke A, Mitchell-Olds T (2001) Erratum: Induced plant defense responses against chewing insects. Ethylene

signaling reduces resistance of arabidopsis against Egyptian cotton worm but not diamondback moth (Plant Physiology (2000) 124 (1007-1017)). Plant Physiol 125: 2203

Tian S, Yin X, Fu P, Wu W, Lu J (2020) Ectopic expression of grapevine gene VaRGA1 in arabidopsis improves resistance to downy mildew and pseudomonas syringae pv. Tomato DC3000 but increases susceptibility to botrytis cinerea. Int J Mol Sci 21: 1–19

Toth Z, Winterhagen P, Kalapos B, Su Y, Kovacs L, Kiss E (2016) Expression of a Grapevine NAC Transcription Factor Gene Is Induced in Response to Powdery Mildew Colonization in Salicylic Acid-Independent Manner. Sci Rep 6: 1–14

Tsuda K, Mine A, Bethke G, Igarashi D, Botanga CJ, Tsuda Y, Glazebrook J, Sato M, Katagiri F (2013) Dual Regulation of Gene Expression Mediated by Extended MAPK Activation and Salicylic Acid Contributes to Robust Innate Immunity in Arabidopsis thaliana. PLoS Genet 9: 1–14

Unger S, Büche C, Boso S, Kassemeyer H-H (2007) The course of colonization of two different Vitis genotypes by Plasmopara viticola indicates compatible and incompatible host-pathogen interactions. Phytopathology 97: 780–786

Vicente J, Cascón T, Vicedo B, García-Agustín P, Hamberg M, Castresana C (2012) Role of 9-lipoxygenase and α-dioxygenase oxylipin pathways as modulators of local and systemic defense. Mol Plant 5: 914–928

Vyse K, Faivre L, Romich M, Pagter M, Schubert D, Hincha DK, Zuther E (2020) Transcriptional and Post-Transcriptional Regulation and Transcriptional Memory of Chromatin Regulators in Response to Low Temperature. Front Plant Sci 11: 1–18

Walley JW, Rowe HC, Xiao Y, Chehab EW, Kliebenstein DJ, Wagner D, Dehesh K (2008) The chromatin remodeler SPLAYED regulates specific stress signaling pathways. PLoS Pathog 4: 1–8

Wang C, He X, Wang X, Zhang S, Guo X (2017) Ghr-miR5272a-mediated regulation of GhMKK6 gene transcription contributes to the immune response in cotton. J Exp Bot 68: 5895–5906

Wang M, Weiberg A, Lin FM, Thomma BPHJ, Huang H Da, Jin H (2016) Bidirectional cross-kingdom RNAi and fungal uptake of external RNAs confer plant protection. Nat Plants 2: 1–10

Wang P, Liu C, Wang D, Liang C, Zhao K, Fan J (2013) Isolation of resistance gene analogs

from grapevine resistant to downy mildew. Sci Hortic (Amsterdam) 150: 326–333

Wang S, Durrant WE, Song J, Spivey NW, Dong X (2010) Arabidopsis BRCA2 and RAD51 proteins are specifically involved in defense gene transcription during plant immune responses. Proc Natl Acad Sci U S A 107: 22716–22721

Wang S, Gu Y, Zebell SG, Anderson LK, Wang W, Mohan R, Dong X (2014) A noncanonical role for the CKI-RB-E2F cell-cycle signaling pathway in plant effector-triggered immunity. Cell Host Microbe 16: 787–794

Warren RF, Henk A, Mowery P, Holub E, Innes RW (1998) A mutation within the leucine-rich repeat domain of the arabidopsis disease resistance gene RPS5 partially suppresses multiple bacterial and downy mildew resistance genes. Plant Cell 10: 1439–1452

Weiberg A, Wang M, Lin FM, Zhao H, Zhang Z, Kaloshian I, Huang H Da, Jin H (2013) Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. Science (80-) 342: 118–123

Wight WD, Kim KH, Lawrence CB, Walton JD (2009) Biosynthesis and role in virulence of the histone deacetylase inhibitor depudecin from alternaria brassicicola. Mol Plant-Microbe Interact 22: 1258–1267

Xiao D, Zhou K, Yang X, Yang Y, Ma Y, Wang Y (2021) Crosstalk of DNA Methylation Triggered by Pathogen in Poplars With Different Resistances. Front Microbiol 12: 1–15

Xie H, Konate M, Sai N, Tesfamicael KG, Cavagnaro T, Gilliham M, Breen J, Metcalfe A, Stephen JR, De Bei R, Collins C, Lopez CMR (2017) Global DNA Methylation Patterns Can Play a Role in Defining Terroir in Grapevine (Vitis vinifera cv. Shiraz). Front Plant Sci 8: 1–16

Xu X, Liu X, Yan Y, Wang W, Gebretsadik K, Qi X, Xu Q, Chen X (2019) Comparative proteomic analysis of cucumber powdery mildew resistance between a single-segment substitution line and its recurrent parent. Hortic Res 6: 1–13

Yan L, Fan G, Li X (2019) Genome-wide analysis of three histone marks and gene expression in Paulownia fortunei with phytoplasma infection. BMC Genomics 20: 1–14

Yan X, Qiao H, Zhang X, Guo C, Wang M, Wang Y, Wang X (2017) Analysis of the grape (Vitis vinifera L.) thaumatin-like protein (TLP) gene family and demonstration that TLP29 contributes to disease resistance. Sci Rep 7: 1–14

Yang X, Xie Y, Raja P, Li S, Wolf JN, Shen Q, Bisaro DM, Zhou X (2011) Suppression of methylation-mediated transcriptional gene silencing by βc1-sahh protein interaction during geminivirus-betasatellite infection. PLoS Pathog 7: 1–13

Yu Y, Wan Y, Jiao Z, Bian L, Yu K, Zhang G, Guo D (2019) Functional characterization of resistance to powdery mildew of VvTIFY9 from vitis vinifera. Int J Mol Sci 20: 1–11

Zeng W, Huang H, Lin X, Zhu C, Kosami K ichi, Huang C, Zhang H, Duan CG, Zhu JK, Miki D (2021) Roles of DEMETER in regulating DNA methylation in vegetative tissues and pathogen resistance. J Integr Plant Biol 63: 691–706

Zhang Y, Wang HL, Li Z, Guo H (2020) Genetic network between leaf senescence and plant immunity: Crucial regulatory nodes and new insights. Plants 9: 1–22

Zhao H, Yin CC, Ma B, Chen SY, Zhang JS (2021) Ethylene signaling in rice and Arabidopsis: New regulators and mechanisms. J Integr Plant Biol 63: 102–125

Zheng X, Xing J, Zhang K, Pang X, Zhao Y, Wang G, Zang J, Huang R, Dong J (2019) Ethylene response factor erf11.Activates bt4.transcription to regulate immunity to pseudomonas syringae. Plant Physiol 180: 1132–1151

Zhi P, Chang C (2021) Exploiting Epigenetic Variations for Crop Disease Resistance Improvement. Front Plant Sci 12: 1–13

Zhu Q-H, Shan W-X, Ayliffe MA, Wang M-B (2016) Epigenetic Mechanisms: An Emerging Player in Plant-Microbe Interactions. Mol Plant Microbe Interact 29: 187–96

Chapter IV – Final Remarks

IV.1. Conclusion

In this PhD dissertation, the research performed was an intent to deepen the knowledge on the plant epigenetic machinery involved in the grapevine defense responses after the biotic stress created by the pathogen *P. viticola*.

So far, an in-depth analysis of the response of table grape genotypes with different susceptibilities against *P. viticola* has been reported for the first time. The transcriptomic characterisation allowed us to observe the modulation of important classes of defence genes during *V. vinifera–P. viticola* interaction, as those encoding for proteins involved in PAMP perception, phytohormone signalling and response, PR proteins and defensin-like proteins.

In this study, an observation of the epigenetic associated machinery was also performed. Our work indicates that the DNA methylation is affected by *P. viticola* inoculation and suggests that differences in the DNA methylation levels might be related to the different susceptibility to *P. viticola.* Moreover, a differential regulation of genes involved in chromatin and histone modification, small RNA biogenesis and DNA damage and reparation processes were also observed, suggesting that these processes may also play a role in the grapevine defence responses to this pathogen.

Finally, we found that the tolerant *V. vinifera* genotype analysed for the early modulation of defence genes and the effect on global DNA methylation resemble what has been observed in the resistant grapevine genotypes derived from other *Vitis* species, showing an intermediate behaviour between resistance and susceptibility. These data support the presence of tolerance mechanisms in *V. vinifera* and the presence of similar strategies to counteract *P. viticola* among different *Vitis* species.

IV.2. Future Perspectives

The focus of this thesis is to deepen the knowledge of the involvement between epigenetic machinery and grapevine immune system specifically while *V. vinifera* responds to *P. viticola* infection.

For the continuation and improvement of knowledge in epigenetics in this work, one topic that was not tackled within this PhD thesis was the involvement in-dept of chromatin/histones alteration on grapevines after *P. viticola*. Chromatin has been observed to be an important

player in the epigenetic machinery from its conformation alteration, the chromatin remodeling complexes influence and the histones alteration (Palma et al. 2010; Yan et al. 2019). The impact and intricate relationship of these chromatin regulation factors on genes leads to an active or repressing modulation of the gene's expression (Palma et al. 2010; Crespo-Salvador et al. 2018; Yan et al. 2019; Atighi et al. 2020). The fine tuning of the gene expression helps the defense plasticity of the plants against pathogens attacks known as priming state (Palma et al. 2010; Yan et al. 2019). Chromatin immunoprecipitation technique (ChIP) has been shown to be one of the best approaches to study histone modifications in grapevine responses to pathogens as shown in other plant-pathogens interaction studies (Alvarez-Venegas et al. 2007; Chakraborty et al. 2018; Crespo-Salvador et al. 2018). ChIP is an important tool to observe the interactions between genomic DNA and DNA-binding proteins therefore a good approach regarding epigenetic research to observe gene expression and histone modification relationship (Alvarez-Venegas et al. 2007; Gómez-Díaz et al. 2013; Lee et al. 2017; Chakraborty et al. 2018; Crespo-Salvador et al. 2018). Moreover, when combined with other techniques it also allows to study in-depth DNA-binding motifs, putative target genes, proteins biological roles and provide for a genome-wide map of epigenetic modifications (Lee et al. 2017).

Another interesting point of view that this research observed is the presence of RNA machinery and the potential role in *V. vinifera* - *P. viticola* interaction which also has been seen by Brilli and colleagues (Brilli et al. 2018). The presence of this mechanism could be considered a bidirectional process since in the plant-pathogens interactions an interactive impact of the pathogen with the targeted hosts cells has an effector-like structure, to delay all processes, or a host defense strategy on the surrounding infection (Wang et al. 2016; Brilli et al. 2018). To confirm this hypothesis small RNA silencing techniques should be performed focusing on either the RNA machinery of the host and/or the pathogen (Brilli et al. 2018). Recent techniques have been developed to analyse possible cross-kingdom RNA such as spray-induced gene silencing (SIGS) and Host-induced gene silencing (HIGS) (Brilli et al. 2018; Chen et al. 2023). In regard of SIGS, Haile and colleagues already observed on the *V. vinifera* - *P. viticola* interaction which showed that *Pv*DCLs silencing through engineered dsRNA affects the disease though its control (Haile et al. 2021). Meanwhile HIGS technique could be an interesting technique to observe since for it an RNAi construct with a focus on target genes that are pathogen restrictive which therefore can help in the day-by-day crop management (Capriotti et al. 2020).

With the results obtained in this work regarding the epigenetic machinery, the promising outcomes could be applied in breeding programs identified has *epibreeding* programs (Clippinger et al. 2024). Breeding program has one of its main goals the obtention of grapevine populations with resistance to different pathogen (Clippinger et al. 2024). This is possible with the creation of crossing hybrids where there is the introgression of QTLs (one or more) from pathogens resistant cultivars (Clippinger et al. 2024). However, one of the major concerns of this processes is the lack of quality of the hybrid cultivars as well as the process length since it needs more than one generation to be able to achieve goal (Clippinger et al. 2024). The use of epigenetic machinery in breeding programs seems promising since the use of a variety traits without changing the genomic structure and with the results being visible in one generation and possible transgenerational allows breeders to fight different stressful stimuli (Dalakouras and Vlachostergios 2021; Gupta and Salgotra 2022).

IV.3. References

Alvarez-Venegas R, Abdallat A Al, Guo M, Alfano JR, Avramova Z (2007) Epigenetic control of a transcription factor at the cross section of two antagonistic pathways. Epigenetics 2: 106–113

Atighi MR, Verstraeten B, De Meyer T, Kyndt T (2020) Genome-wide DNA hypomethylation shapes nematode pattern-triggered immunity in plants. New Phytol 227: 545–558

Brilli M, Asquini E, Moser M, Bianchedi PL, Perazzolli M, Si-Ammour A (2018) A multi-omics study of the grapevine-downy mildew (Plasmopara viticola) pathosystem unveils a complex protein coding and noncoding-based arms race during infection. Sci Rep 8: 1–12

Capriotti L, Baraldi E, Mezzetti B, Limera C, Sabbadini S (2020) Biotechnological approaches: Gene overexpression, gene silencing, and genome editing to control fungal and oomycete diseases in grapevine. Int J Mol Sci 21: 1–29

Chakraborty J, Ghosh P, Sen S, Das S (2018) Epigenetic and transcriptional control of chickpea WRKY40 promoter activity under Fusarium stress and its heterologous expression in Arabidopsis leads to enhanced resistance against bacterial pathogen. Plant

Sci 276: 250–267

Chen A, Halilovic L, Shay JH, Koch A, Mitter N, Jin H (2023) Improving RNA-based crop protection through nanotechnology and insights from cross-kingdom RNA trafficking. Curr Opin Plant Biol 76:

Clippinger JI, Dobry EP, Laffan I, Zorbas N, Hed B, Campbell MA (2024) Traditional and Emerging Approaches for Disease Management of Plasmopara viticola, Causal Agent of Downy Mildew of Grape. Agriculture 14: 1–24

Crespo-Salvador Ó, Escamilla-Aguilar M, López-Cruz J, López-Rodas G, González-Bosch C (2018) Determination of histone epigenetic marks in Arabidopsis and tomato genes in the early response to Botrytis cinerea. Plant Cell Rep 37: 153–166

Dalakouras A, Vlachostergios D (2021) Epigenetic approaches to crop breeding: Current status and perspectives. J Exp Bot 72: 5356–5371

Gómez-Díaz E, Jordà M, Peinado MA, Rivero A (2013) Epigenetics of host-pathogen interactions: The road ahead and the road behind. Epigenetics and Pathology: Exploring Connections between Genetic Mechanisms and Disease Expression. Apple Academic Press, pp. 2–22

Gupta C, Salgotra RK (2022) Epigenetics and its role in effecting agronomical traits. Front Plant Sci 13: 1–19

Haile ZM, Gebremichael DE, Capriotti L, Molesini B, Negrini F, Collina M, Sabbadini S, Mezzetti B, Baraldi E (2021) Double-Stranded RNA Targeting Dicer-Like Genes Compromises the Pathogenicity of Plasmopara viticola on Grapevine. Front Plant Sci 12: 1–7

Lee JH, Jin S, Kim SY, Kim W, Ahn JH (2017) A fast, efficient chromatin immunoprecipitation method for studying protein-DNA binding in Arabidopsis mesophyll protoplasts. Plant Methods 13: 1–12

Palma K, Thorgrimsen S, Malinovsky FG, Fiil BK, Nielsen HB, Brodersen P, Hofius D, Petersen M, Mundy J (2010) Autoimmunity in arabidopsis acd11 is mediated by epigenetic regulation of an immune receptor. PLoS Pathog 6:

Wang, Gao, Bian, Zhao, Zhao, Xia, Song, Hou, Wan, Wang (2016) Genome-Wide Identification and Comparative Analysis of Cytosine-5 DNA Methyltransferase and

Demethylase Families in Wild and Cultivated Peanut. Front Plant Sci 7:

Yan L, Fan G, Li X (2019) Genome-wide analysis of three histone marks and gene expression in Paulownia fortunei with phytoplasma infection. BMC Genomics 20: 1–14

Chapter V – Supporting Information

V.1. Supplementary Tables

Table S 1 - Rating scale applied for the phenotypic evaluation of *Plasmopara viticola* sporulation in Gape bunches analysis.

Scale	Bunch damage surface signs
0	without symptoms
1	5 - 20%
2	25 - 40%
3	45 - 60%
4	65 - 80%
5	85 - 100%

Table S 2 - Microsatellites profiling of Red Globe x Regal Seedless progenies. Analysis of two Italian microsatellite markers (ISV2, ISV4) and one real assistance selection marker for sedlessness (VVagl11).

Samples	Microsatellites markers												Presence of seeds
	ISV2				**ISV4**				**VVagl11**				
	R.G.		R.S.		R.G.		R.S.		R.G.		R.S.		
	Nr+44	Nr+48	Nr+22	NR+22	Nr+18	NR+24	Nr+8	Nr+22	206	206	206	216	
N23/018	Nr + 44		Nr + 22		Nr + 24		Nr + 22		206		216		False
N20/029	Nr + 48		Nr + 22		Nr + 24		Nr + 8		206		216		False
N20/020	Nr + 48		Nr + 22		Nr + 24		Nr + 22		206		216		False
N20/012	Nr + 48		Nr + 22		Nr + 18		Nr + 22		206		216		False

R.G. - Red Globe; R.S. - Regal Seedless

Nr – Shorter allele find in the microsatellite

Table S 3 - Percentage of Disease Incidence (DI) and Severity (DS) of *P. viticola* in different assays performed between 2016 and 2018 in different table grape varieties and crossings. Data on grapevine varieties and crossings derived from the cross ♀Red Globe x ♂ Regal Seedless are reported. For grape bunch, a total of 115 new genotypes from the cross Red Globe x Regal Seedless (5 plants/genotype) were observed. The grape bunch analysis of the entire cross population and its statistical assessment is reported in the Figure S 2. Genotypes belonging to C1 cluster showed higher susceptibility to *P. viticola*, compared to C2 (lower susceptibility), as assessed by cluster analysis. In the leaf disk assay, the four crossing genotypes represent a selection of 40 individuals analysed. The values followed by different letters are statistically different according to non-parametric Conover's test.

	Table Grape	**Grape Bunch***			**Leaf disk assay****			
		DI (%)	**DS (%)**		**DI (%)**		**DS (%)**	
Varieties	**Regal Seedless**	90.5	74.0	*C1*	100.0	*a*	93.6	*a*
	Italia	21.5	1.6	*C2*	71.4	*ab*	64.2	*bc*
	Crimson Seedless	6.7	1.1	*C2*	100.0	*a*	85.7	*ab*
	Red Globe	37.1	12.6	*C2*	61.9	*ab*	52.4	*bc*
Crossin	**N20/012**	34.0	16.6	*C2*	62.0	*ab*	25.2	*c*
	N20/020	79.3	46.5	*C1*	85.7	*ab*	80.3	*ab*
	N20/029	76.0	47.9	*C1*	100.0	*a*	79.6	*abc*
	N23/018	38.3	19.1	*C2*	35.7	*b*	27.6	*c*

*Field observation performed in 2018.

** Leaf disk assay performed on varieties (in 2016) and cross progenies (in 2017).

V.2. Supplementary Figures

Figure S 1 - Rating scale applied for the phenotypic evaluation of *Plasmopara viticola* sporulation in leaf disc assays. 1: absence of sporangiophores; 3: sparse sporangiophores, localised in the inoculation point; 5: patches of sparse sporulation intermixed with necrotic flecks underneath sporulating area; 7: dense sporulation limited to inoculation point; 9: dense sporulation beyond the point of inoculation

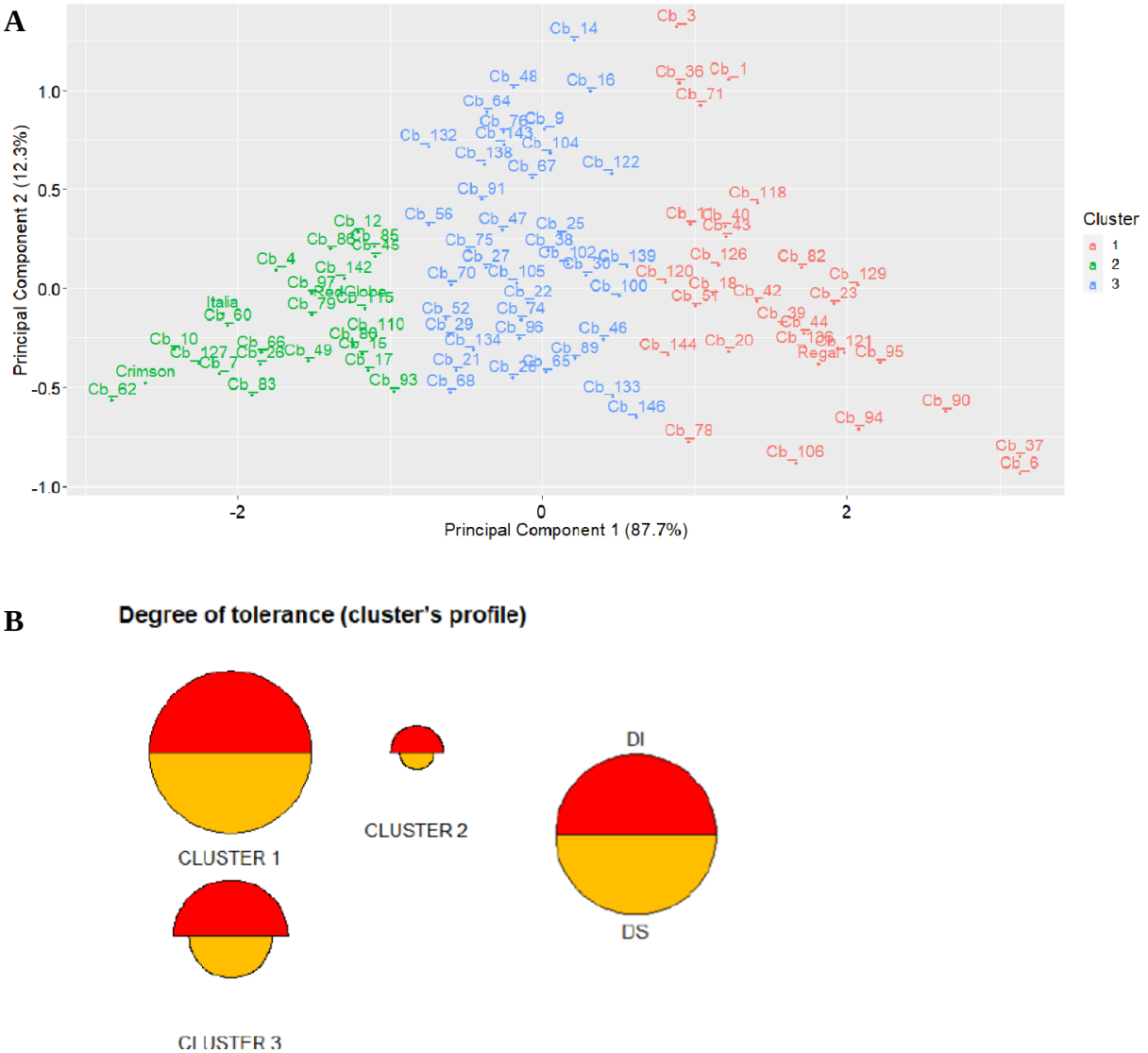


Figure S 2 - Degree of tolerance/susceptibility to *P. viticola* within the cross population Red Globe x Regal. Cluster analysis (k-means) (A) and cluster profile (B) performed on 115 new genotypes (Red Globe x Regal seedless) based on their tolerance/susceptibility to *P. viticola* infections on grape bunches. Genotypes belonging to C1 cluster showed higher susceptibility to *P. viticola*, compared to C3 (middle susceptibility) and C2 (lower susceptibility). Cb_12 = N20/012 (C2); Cb_18 = N20/020 (C1); Cb_20 = N20/029 (C1); Cb_115 = N23/018 (C2). DI = Disease index; DS = Disease severity. Circle dimension is proportional to indices values.