



**HAL**  
open science

# Comparative Transcriptomics Suggests Early Modifications by Vintec® in Grapevine Trunk of Hormonal Signaling and Secondary Metabolism Biosynthesis in Response to *Phaeomoniella chlamydospora* and *Phaeoacremonium minimum*

Ana Romeo-Oliván, Justine Chervin, Coralie Breton, Thierry Lagravère, Jean Daydé, Bernard Dumas, Alban Jacques

## ► To cite this version:

Ana Romeo-Oliván, Justine Chervin, Coralie Breton, Thierry Lagravère, Jean Daydé, et al.. Comparative Transcriptomics Suggests Early Modifications by Vintec® in Grapevine Trunk of Hormonal Signaling and Secondary Metabolism Biosynthesis in Response to *Phaeomoniella chlamydospora* and *Phaeoacremonium minimum*. *Frontiers in Microbiology*, 2022, 13, pp.898356. 10.3389/fmicb.2022.898356 . hal-03687473

**HAL Id: hal-03687473**

**<https://ut3-toulouseinp.hal.science/hal-03687473>**

Submitted on 3 Jun 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



# Comparative Transcriptomics Suggests Early Modifications by Vintec® in Grapevine Trunk of Hormonal Signaling and Secondary Metabolism Biosynthesis in Response to *Phaeomoniella chlamydospora* and *Phaeoacremonium minimum*

## OPEN ACCESS

### Edited by:

Rajarshi Kumar Gaur,  
Deen Dayal Upadhyay Gorakhpur  
University, India

### Reviewed by:

Vivek Sharma,  
Chandigarh University, India  
Arup Kumar Mukherjee,  
National Rice Research Institute  
(ICAR), India

### \*Correspondence:

Alban Jacques  
alban.jacques@purpan.fr

### Specialty section:

This article was submitted to  
Microbe and Virus Interactions with  
Plants,  
a section of the journal  
Frontiers in Microbiology

Received: 17 March 2022

Accepted: 08 April 2022

Published: 17 May 2022

### Citation:

Romeo-Oliván A, Chervin J,  
Breton C, Lagravère T, Daydé J,  
Dumas B and Jacques A (2022)  
Comparative Transcriptomics  
Suggests Early Modifications by  
Vintec® in Grapevine Trunk  
of Hormonal Signaling and Secondary  
Metabolism Biosynthesis in Response  
to *Phaeomoniella chlamydospora*  
and *Phaeoacremonium minimum*.  
Front. Microbiol. 13:898356.  
doi: 10.3389/fmicb.2022.898356

Ana Romeo-Oliván<sup>1</sup>, Justine Chervin<sup>2,3,4</sup>, Coralie Breton<sup>1</sup>, Thierry Lagravère<sup>1</sup>,  
Jean Daydé<sup>1</sup>, Bernard Dumas<sup>2</sup> and Alban Jacques<sup>1\*</sup>

<sup>1</sup> Unité de Recherche Physiologie, Pathologie, et Génétique Végétales (PPGV), INP PURPAN, Université de Toulouse, Toulouse, France, <sup>2</sup> Laboratoire de Recherche en Sciences Végétales, CNRS, UPS, Toulouse INP, Université de Toulouse, Toulouse, France, <sup>3</sup> Metatoul-AgromiX Platform, MetaboHUB, National Infrastructure for Metabolomics and Fluxomics, LRSV, CNRS, UPS, Toulouse INP, Université de Toulouse, Toulouse, France, <sup>4</sup> MetaboHUB-MetaToul, National Infrastructure of Metabolomics and Fluxomics, Toulouse, France

Given their well-known antifungal abilities, species of the genus *Trichoderma* are of significant interest in modern agriculture. Recent studies have shown that *Trichoderma* species can induce plant resistance against different phytopathogens. To further extend this line of investigation, we investigate herein the transcriptomic response of grapevine trunk to Vintec®, which is a *Trichoderma atroviride* SC1-based commercial formulation for biological control of grapevine trunk diseases and which reduces wood colonization. The aim of the study is to understand whether the biocontrol agent Vintec® modifies the trunk response to *Phaeoacremonium minimum* and *Phaeomoniella chlamydospora*, which are two esca-associated fungal pathogens. An analysis of transcriptional regulation identifies clusters of co-regulated genes whose transcriptomic reprogramming in response to infection depends on the absence or presence of Vintec®. On one hand, the results show that Vintec® differentially modulates the expression of putative genes involved in hormonal signaling, especially those involved in auxin signaling. On the other hand, most significant gene expression modifications occur among secondary-metabolism-related genes, especially regarding phenylpropanoid metabolism and stilbene biosynthesis. Taken together, these results suggest that the biocontrol agent Vintec® induces wood responses that counteract disease development.

**Keywords:** biocontrol, *Trichoderma atroviride* SC1, *Phaeomoniella chlamydospora*, *Phaeoacremonium minimum*, esca, grapevine trunk diseases

## INTRODUCTION

Crops worldwide face multiple diseases that cause huge economic loss (Savary et al., 2019). Over the past decades, pest management has relied mostly on chemical control, which has caused serious harm to environmental and human health. An alternative to these conventional phytosanitary products is the use of living organisms or natural substances; a strategy known as biological control (Eilenberg et al., 2001). Although biological control offers numerous advantages over chemical pesticides, its main advantages are that it is environmentally friendly, cost-effective, and human safe technology (Cook, 1988; Bhat et al., 2013; Brodeur et al., 2018; Ram et al., 2018).

The main approach to managing plant disease *via* biological control consists in using antagonistic fungi or bacteria to combat plant pathogens (Thambugala et al., 2020). In this regard, the genus *Trichoderma* is of great interest (Vinale et al., 2008; Zin and Badaluddin, 2020; Ferreira and Musumeci, 2021). *Trichoderma* is a genus of fungi inhabiting a large spectrum of soils and its species are opportunistic plant symbionts (Vinale et al., 2008) that attack or compete with other fungi by using various direct mechanisms, such as the production of antimicrobial compounds or lytic enzymes or the competition for resources (Kubicek et al., 2001; Benítez et al., 2004). To date, 25 *Trichoderma* species have been identified as potential biological control agents (BCAs) and several commercial formulations based on different strains of *Trichoderma* are available (Ferreira and Musumeci, 2021).

*Trichoderma* spp. also act indirectly by reprogramming gene expression in the host (Shaw et al., 2016). The associations between *Trichoderma* spp. and the host plant often improve plant resistance to disease, augment abiotic stress tolerance, and enhance other developmental processes. For instance, Zhao et al. (2021) reported increased growth of tomato plants upon inoculation by *T. afroharzianum*. In addition, *T. longibrachiatum* reportedly enhances the salt tolerance of wheat (Zhang et al., 2016) and the water-stress response on tomato (De Palma et al., 2019). *T. harzianum* enhances the antioxidant response (Mastouri et al., 2012) and also stimulates the production of jasmonic acid and salicylic acid, which reduces the incidence of root nematodes on tomato seedlings (Yan et al., 2021).

In viticulture, biological control has attracted significant interest, especially against pathogens associated with grapevine trunk diseases (GTDs). GTDs are a growing concern because of their increasing incidence in vineyards worldwide, which reduces yield and productivity, degrading significantly the economics of the viticulture industry (Fontaine et al., 2016; Mondello et al., 2018a,b). Esca, Botryosphaeria dieback, and Eutypa dieback are currently the three main GTDs in European vineyards. Esca is a complex disease with symptoms that vary depending on the age of the vine and the infecting pathogen (Mugnai et al., 1999; Graniti et al., 2000; Bertsch et al., 2013). Numerous pathogenic agents are associated with Esca; the principal causing agents are *Phaeoaniella chlamydospora*, *Phaeoacremonium minimum* (previously *Phaeoacremonium aleophyllum*) (Larignon et al., 2000), *Fomitiporia mediterranea*, and other *Phaeoacremonium* spp. (Gramaje et al., 2015). Since the ban of sodium arsenate and benzimidazoles in the early 2000s, no effective treatment

has been found to eradicate these diseases. Instead, an integrated pest-management strategy has been established that consists of different prophylactic treatments both in nurseries and in vineyards (Mondello et al., 2018a,b).

In recent years, the use of various strains of *Trichoderma* has produced good results against pathogens associated with these diseases. In grapevine nurseries, the application of *T. harzianum* during propagation proved as effective in combating these diseases as the use of conventional phytosanitary products (Fourie et al., 2001). Similar results were obtained by Di Marco et al. (2004); Di Marco and Osti (2007), who reported a reduced incidence of esca-associated pathogens and improved morphological features. In vineyards, the application of various *Trichoderma* spp. for protecting pruning wounds reduced the presence of pathogens (Kotze et al., 2011), decreasing the percentage of infected plants and reducing the severity of symptoms (Bigot et al., 2020).

In France, three *Trichoderma*-based biocontrol commercial solutions certified against GTDs are currently available. One is a combination of *T. asperellum* ICC012 and *T. gamsi* ICC080 (Escalator®, De Sangosse); the other two are formulations of *T. atroviride* I1237 (Esquive WP®, Agrauxine by Lesaffre) and *T. atroviride* SC1 (Vintec®, Belchim Crop Protection). Recent studies designed to evaluate the efficacy of Vintec® showed promising results in the battle against GTDs. Pertot et al. (2016) reported that the application of the BCA Vintec® at different stages of the propagation process decreases vine infections by *P. minimum* and *P. chlamydospora*. In addition, Berbegal et al. (2020) confirmed that the application of the BCA Vintec® in the nursery reduces the incidence of diverse GTDs and showed that subsequent treatments in vineyards provide long-term protection against GTDs.

The results of a previous study show that the application of the BCA Vintec® on grapevine wood triggers significant metabolic changes upon infection with *P. chlamydospora* and *P. minimum*. Three weeks after infection, the different classes of stilbenoid and flavonoid compounds, known for their antifungal properties, are differently produced in plants pre-inoculated with Vintec® compared with non-pre-inoculated plants (Chervin et al., 2022). In the present work, we investigate rapid transcriptomic reprogramming in grapevine trunk in response to inoculation with the BCA Vintec®, which is a commercial *T. atroviride* SC1-based solution. The aims of this study are thus (i) to investigate the plant molecular mechanisms modified by the presence of the BCA Vintec® and (ii) to determine whether the Vintec® treatment enhances plant response against the two esca-associated pathogens *P. chlamydospora* and *P. minimum*.

## MATERIALS AND METHODS

### Fungal Material

This study used the fungal strains *P. minimum* CBS 100398 and *P. chlamydospora* CBS 239.74 from the Westerdijk Fungal Biodiversity Institute (Utrecht, Netherlands). Both fungi were grown in Malt Extract-Agar (MEA) at 26°C in the dark. The BCA Vintec® (*T. atroviride* SC1,  $2 \times 10^{10}$  conidia per gram of

formulated product) was provided by Belchim Crop Protection, and the Vintec<sup>®</sup> suspension was prepared at 2 g/L in autoclaved distilled water.

## Plant Material

One-year-old canes of Cabernet Sauvignon clone 15 were collected from Daydé Nurseries (Pépinières Daydé, Montans, Occitanie, France) in January 2019 and divided into two-node dormant cuttings. The cuttings were surface-disinfected in a 10 L bath of 0.05% bleach (2.6% active chloride) for 30 s, then in a 10 L bath of 0.05% 8-hydroxyquinoline sulfate (Beltanol<sup>®</sup>, Syngenta) for 12 h in a cold chamber (4°C). After the bleach disinfection, the cuttings were rinsed twice with clear water, and after the 8-hydroxyquinoline sulfate disinfection, the cuttings were rinsed three times with clear water. Cuttings were first planted in autoclaved (121°C, 15 min) mineral wool to allow rooting and budding. Three weeks after the emergence of the first leaf, cuttings were transferred to individual pots filled with potting substrate (PAM2, PROVEEN Substrates). The plants were then grown in plant growth tents (photoperiod 12 h/12 h, 45% humidity, 25°C).

## Plant Inoculation

The plants were drilled at the internode and inoculated with 20 µL of Vintec<sup>®</sup> suspension or 20 µL of sterile water. Five days after Vintec<sup>®</sup> inoculation, the plants were infected with two plugs of colonized agar, one of each fungus. The injuries were protected with parafilm (American National Can, Chicago, United States). After the infection, five different conditions were studied: non-injured/non-infected plants (NINi,  $n = 25$ ), injured/non-infected plants (INi,  $n = 25$ ), inoculated with Vintec<sup>®</sup> (IV,  $n = 25$ ), infected with both fungi (IPP,  $n = 25$ ), and inoculated with Vintec<sup>®</sup> and infected with both fungi (IVPP,  $n = 25$ ). Wood samples of 1 cm above and below the inoculation site were taken at 48 h post-infection (hpi) and 6 weeks post-infection (wpi). Four biological replicates consisting of a pool of four plants were collected for each condition at 48 hpi. At the latest time (i.e., 6 wpi), only three pools of three plants were taken. Immediately after collection, the samples were frozen in liquid nitrogen and stored at -80°C. Before RNA and DNA extraction, the samples were lyophilized and ground. The samples collected 6 wpi were used to confirm pathogen infection *via* qPCR quantification of the Pm/Pch  $\beta$ -tubulin gene as per Pouzoulet et al. (2013).

## RNA Extraction, DNase Treatment, and Reverse Transcription

One milliliter of RNA extraction buffer (CTAB 2%, PVPP 2%, Tris 300 mM, EDTA 25 mM, NaCl 2 M, pH 8, 2%  $\beta$ -mercaptoethanol) was added to one hundred milligrams of wood powder and the mix was incubated 10 min at 65°C under continuous agitation (9,000 rpm). The samples were then centrifuged for 15 min at 10,000 rpm, 4°C. One volume of chloroform: isoamyl alcohol (24/1, v/v) solution was added to the supernatant, following which the solution was centrifuged for 15 min at 10,000 rpm, 4°C to form two phases. The upper aqueous phase contains nucleic acids. This stage was repeated twice.

To allow the nucleic acids to precipitate, 0.6 volume of isopropanol and 0.1 volume of CH<sub>3</sub>COONa 3M were added to the upper phase, following which the samples were stored overnight at -80°C. The next day, the samples were centrifuged for 30 min at 10,000 rpm, 4°C. The resulting pellet was redissolved in 300 µL of SSTE buffer (NaCl 1 M, SDS 0.5%, Tris 10 mM, EDTA 1 mM, pH 7) and 600 µL of pure ethanol. The mixture was transferred to a mini spin column (RNeasy mini kit, Qiagen, United States). The successive steps were done as described in the kit handbook. RNA was eluted in 30 µL of RNase-free water, aliquoted in 6 µL samples, and stored at -80°C. RQ1 RNase-Free DNase kit (Promega, United States) was used to eliminate the possible co-purified genomic DNA.

## RNA Sequencing and Data Analysis

cDNA library preparation and sequencing were done by using the genomic platform GeT-Plage (GenoToul, INRA, Toulouse, France). The cDNA libraries were prepared with the help of TruSeq Stranded mRNA (Illumina) and then sequenced by using an Illumina HiSeq 3000 sequencer as paired-end reads of 150 bp (Illumina, CA, United States). Quality control was done by using fastqc (v0.11.9; Andrews, 2010). If necessary, quality trimming was done by using sickle-trim (v1.33; Joshi and Fass, 2011) or cutadapt (v2.10; Martin, 2011) to remove adapters. Mapping to the reference genome 12X (Canaguier et al., 2017) was done by using STAR (v2.7.5a; Dobin et al., 2013), and mapped counts were extracted by using featureCounts (subread v2.0.1; samtools v1.9; Liao et al., 2014). Differential expression analysis was performed using the R package SARTools<sup>1</sup>. All conditions were compared with the NINi control (i.e., INi vs NINi, IV vs NINi, IPP vs NINi, IVPP vs NINi) and differentially expressed genes (DEGs;  $p$ -value < 0.05) were extracted. DEGs induced by the injury (INi vs NINi) were discarded for the remainder of the analysis. DEG functional annotations were assessed by using the VitisNet functional annotation database (Grimplet et al., 2009). Clustering was analyzed by using R with the *heatmap* package, and functional enrichment was calculated in R by using Fisher's exact test.

## RT-qPCR

Five genes were tested by RT-qPCR to validate RNA seq results. Gene candidates (listed in **Table 1**) satisfied the following criteria: (i) they were differentially expressed compared with the control condition, (ii) they were specific to a single condition, and (iii) they served an interesting putative biological function. For RT-qPCR analysis, cDNA was first synthesized from RNA samples by reverse transcriptase reaction using the GoScript<sup>®</sup> reverse transcriptase Kit (Promega, United States). RT-qPCR reactions were done in an ABI 7500 Real-Time PCR cycler (Applied Biosystems, Foster City, United States) using GoTaq RT-qPCR systems (Promega, United States) in a final volume of 10 µL. Primers were designed by using Primer Blast (Ye et al., 2012; Optimal T<sub>m</sub> = 62°C, 50–60% GC) and were used at a concentration of 0.5 µM. The cycling program consisted of 95°C for 5 min (initial denaturation); 40 cycles of 15 s at 95°C (denaturation) followed by 45 s at 62°C (annealing and

<sup>1</sup><https://github.com/PF2-pasteur-fr/SARTools>



**TABLE 1** | List of primers for RT qPCR designed for validating the RNA sequencing analysis.

Gene ID	Primer	Sequence	Tm
VIT_04s0023g03010	Glyco Fw	5'-GGTCTCTCCTGCTCGGATGT-3'	61.0
	Glyco Rv	5'-ATTCAGGCACCAACCTGGGG-3'	62.0
VIT_12s0028g00690	AUXR Fw	5'-GAAGGTCCCTGTGCTCGTTG-3'	60.2
	AUXR Rv	5'-TGCTTGAACAGCTTTGTGCGGT-3'	60.7
VIT_05s0049g00390	E8 Fw	5'-AGACGTGCGGTTGCCATTC-3'	61.9
	E8 Rv	5'-GAGTGAGCCCAGCATCCAGT-3'	61.9
VIT_05s0077g01570	PATHO10 Fw	5'-CTCTGCAAACCAACCATTCTCC-3'	61.9
	PATHO10 Rv	5'-CACTCTCGTAAGTGAAAACACCCA-3'	61.0
VIT_16s0100g00860	ChalcS Fw	5'-GGAAGCAGCATTGAAGGCC-3'	61.9
	ChalcSRv	5'-GCAGTTTCTGCATAGCACCC-3'	61.0

extension); and an additional melting analysis of 40 min from 60 to 95°C. The ABI SDS software v.1.4 (Applied Biosystems, Foster City, United States) was used to collect the amplification data. Gene expression was calculated by using the  $2^{-ddCt}$  method (Zhang et al., 2020).

## RESULTS

### Transcriptomic Woody Response to BCA Vintec®, Pathogens, and Wound

We performed RNA sequencing on wood samples inoculated with the BCA Vintec® (IV), the two pathogens (IPP), or the BCA Vintec® in combination with pathogens (IVPP). An injured control (INi) was added to the analysis to select DEGs affected only by the treatment and not by the injury. We obtained an average of 20.4 M reads per condition and pool. The percentage of reads aligned to the reference genome of *Vitis vinifera* (12X.0 version, cv PN40024) varies depending on the condition (see **Supplementary Table 1**). The transcriptome of reference (*Vitis vinifera.12X.48.chr.gtf.gz*, from Ensembl plants genomes) was used for gene annotation. Differential analysis was applied to transcript normalized counts. All test conditions were compared with the non-injured/non-inoculated control (i.e., INi vs NINi; IPP vs NINi; IV vs NINi; and IVPP vs NINi).

The injury alone modified the expression of 1,869 genes ( $p$ -value < 0.05), whereas the number of DEGs induced by the BCA Vintec® alone, the two pathogens, and the BCA Vintec® in combination with the two pathogens was 2,114, 3,748, and 3,519, respectively. From these sets of DEGs, we discarded those that were in response to injury, which left 1,442 DEGs in response to the BCA Vintec®, 3204 DEGs in response to the two pathogens, and 2,739 DEGs in response to the two pathogens in the presence of the BCA Vintec® (**Figure 1A**). The pattern expression of five candidate genes was confirmed by qRT-PCR analysis (**Supplementary Figures 1, 2**) as validation of the RNAseq analysis.

The comparison of the three sets of DEGs revealed that only 787 genes were differentially expressed in all three conditions, which represents 17.3% of the total DEGs. The presence of Vintec® specifically induced the differential expression of 448 genes, from which 146 genes were also differentially expressed in the presence of the two pathogens. Infection with the two pathogens modified the expression of 2,210 genes, of which 917

genes overlapped with infection with the two pathogens in the presence of the BCA Vintec®. Interestingly, the combination of the two pathogens with Vintec® modifies the expression of 889 genes, which represents 19.6% of the total DEGs (**Figure 1B**). In other words, 889 genes were differentially expressed when the plants were inoculated with BCA Vintec® prior to pathogen infection.

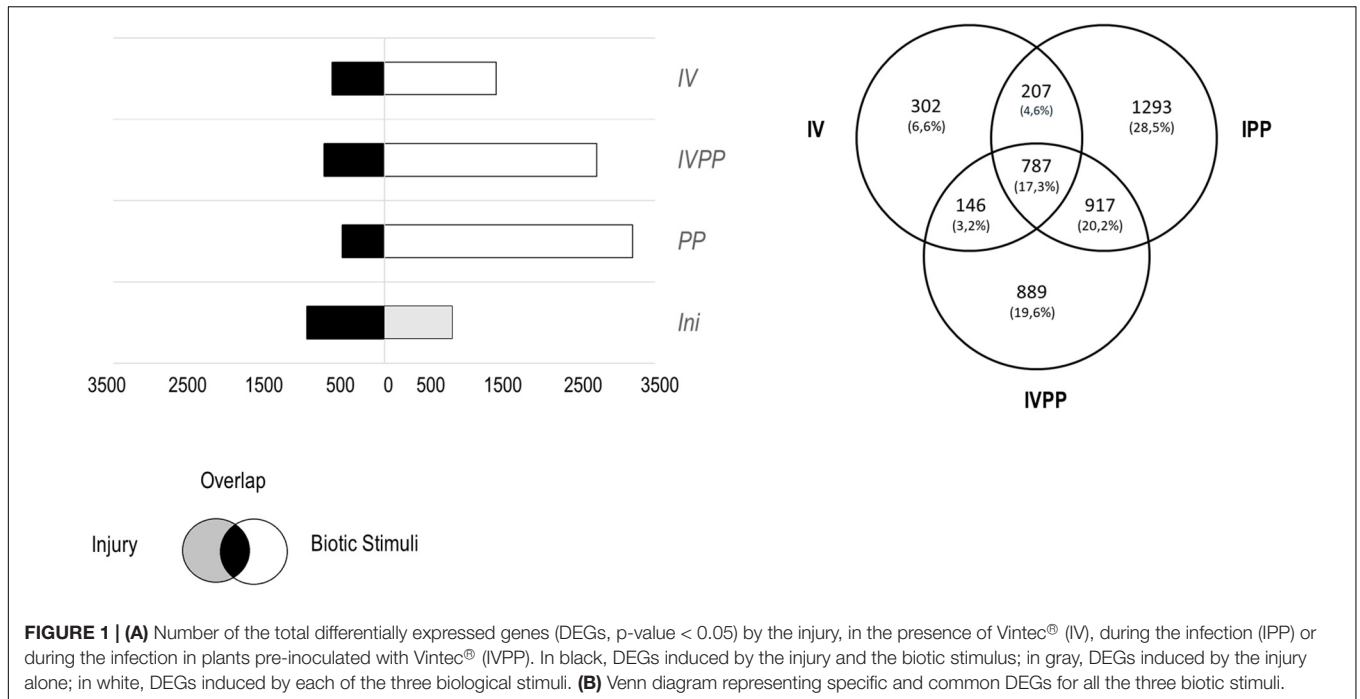
### Gene Clustering Leads to Identification of Three Groups of Co-regulated Genes

For further analysis, the DEGs were separated into three groups based on their expression pattern (**Figure 2**). Each group was composed of two clusters of co-regulated genes showing patterns of expression. Group I contained genes whose expression was modified by one of the three biotic stimuli, but more strongly by the two pathogens (Cluster 1 and 3). Group II contained genes whose expression was modified by the presence of the two pathogens in plants pre-inoculated with the BCA Vintec® (Clusters 4 and 5), and Group III contained genes whose expression was modified by the presence of the BCA Vintec® (Clusters 6 and 2).

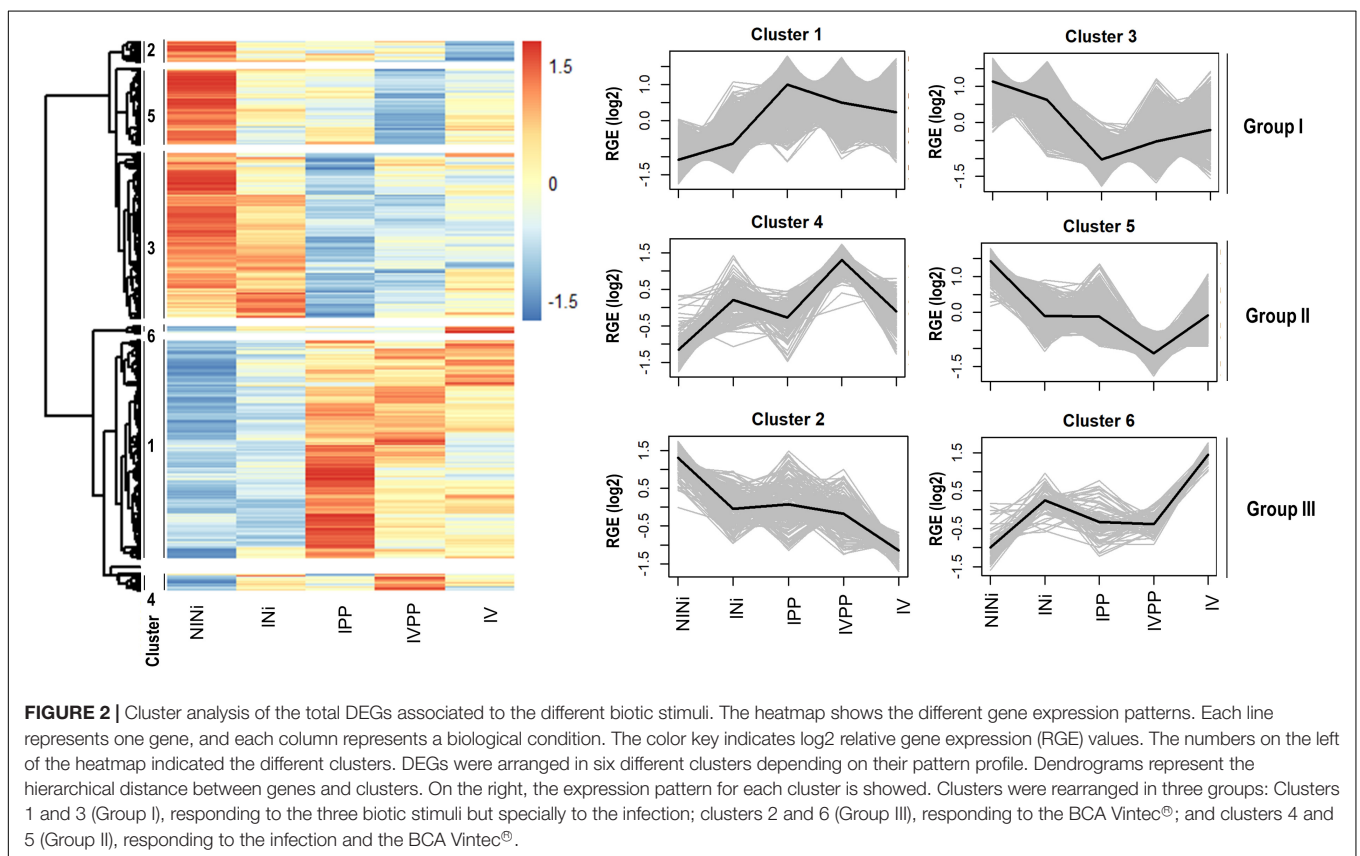
Group I contains the majority of DEGs (76.5%). The expression pattern of the DEGs in this group is thus very heterogeneous. Group III regroups the smallest portion of total DEGs (5%). Based on their expression pattern, the induction or repression of these genes seem to specifically respond to the presence of Vintec®. The rest of the DEGs (18.5%) appears in clusters 4 and 5 (Group II). The expression of these genes is up- or downregulated by the presence of the two pathogens in plants pre-inoculated with the BCA Vintec®. The greater percentages of annotated DEGs in the three groups (23.6, 18.0, and 20.5%, respectively) were associated with “Primary Metabolism” functions, followed by “Signaling” (7.6%) and “Regulation” (7.4%) in Group I, “Transport elements” (10.6%) and “Regulation” (8.8%) in Group II, and “Transport elements” (12%) and “Cellular process” (8.1%) in Group III.

### Functional Enrichment Gives Insights Into Different Perception of Vintec® and Pathogen Infection

Functional enrichment analysis (Fisher’s exact test,  $p$ -value < 0.05) was applied to the three groups (see



**FIGURE 1 | (A)** Number of the total differentially expressed genes (DEGs,  $p$ -value < 0.05) by the injury, in the presence of Vintec® (IV), during the infection (IPP) or during the infection in plants pre-inoculated with Vintec® (IVPP). In black, DEGs induced by the injury and the biotic stimulus; in gray, DEGs induced by the injury alone; in white, DEGs induced by each of the three biological stimuli. **(B)** Venn diagram representing specific and common DEGs for all the three biotic stimuli.



**FIGURE 2 |** Cluster analysis of the total DEGs associated to the different biotic stimuli. The heatmap shows the different gene expression patterns. Each line represents one gene, and each column represents a biological condition. The color key indicates log<sub>2</sub> relative gene expression (RGE) values. The numbers on the left of the heatmap indicated the different clusters. DEGs were arranged in six different clusters depending on their pattern profile. Dendrograms represent the hierarchical distance between genes and clusters. On the right, the expression pattern for each cluster is showed. Clusters were rearranged in three groups: Clusters 1 and 3 (Group I), responding to the three biotic stimuli but specially to the infection; clusters 2 and 6 (Group III), responding to the BCA Vintec®; and clusters 4 and 5 (Group II), responding to the infection and the BCA Vintec®.

results in **Tables 2–4**). The “Glutathione metabolism” ( $p$ -value =  $3.291 \times 10^{-10}$ ), “Phenylpropanoid biosynthesis” ( $p$ -value =  $2.481 \times 10^{-5}$ ), and “Tyrosine metabolism”

( $p$ -value =  $2.53 \times 10^{-4}$ ) categories are enriched for upregulated DEGs in Group I. Among upregulated functions, “ABA biosynthesis” and “ethylene-mediated signaling pathway” are

**TABLE 2 |** Functional enrichment in Group I. DEGs up- and down-regulated by the two pathogens (IPP).

Functional category	Regulation	n DEGs	p-value
Glutathione metabolism	UP	15	$3.29 \times 10^{-10}$
Phenylpropanoid biosynthesis	UP	12	$2.48 \times 10^{-5}$
Tyrosine metabolism	UP	5	$2.53 \times 10^{-4}$
Transcription factor: Zinc finger B-box family	UP	3	$1.02 \times 10^{-3}$
ABA biosynthesis	UP	2	$1.49 \times 10^{-3}$
Wounding	UP	3	$1.59 \times 10^{-3}$
Pentose glucuronate interconversion	UP	6	$1.74 \times 10^{-3}$
Photosynthesis, Photosystem II	UP	4	$1.81 \times 10^{-3}$
Flavonoid biosynthesis (Anthocyanin)	UP	6	$2.00 \times 10^{-3}$
Ethylene-mediated signaling pathway	UP	9	$3.00 \times 10^{-3}$
Cell wall organization and biogenesis	DOWN	7	$6.12 \times 10^{-5}$
Calcium sensors and signaling	DOWN	5	$1.35 \times 10^{-4}$
DNA metabolism	DOWN	4	$7.73 \times 10^{-4}$
Major Facilitator Superfamily. Anion:Cation Symporter	DOWN	1	$1.50 \times 10^{-2}$
Methionine metabolism	DOWN	2	$2.26 \times 10^{-2}$
Protein metabolism and modification: Protein folding	DOWN	3	$2.30 \times 10^{-2}$
Channels and pores: A-Type channels.	DOWN	1	$2.49 \times 10^{-2}$
CorA Metal Ion Transporter	DOWN	1	$4.44 \times 10^{-2}$
Photosynthesis, antenna proteins	DOWN	1	$4.44 \times 10^{-2}$
Cytokinin inactivation	DOWN	1	$4.92 \times 10^{-2}$

also enriched ( $p$ -value =  $1.49 \times 10^{-3}$  and  $3 \times 10^{-3}$ , respectively), whereas “Cytokinin inactivation” ( $p$ -value =  $4.92 \times 10^{-2}$ ) joins the downregulated categories in Group I. No significant enrichment for categories associated with “Hormone signaling” occurs among DEG functions of Group II. In contrast, in Group III, auxin-mediated signaling appears to be repressed because auxin-responsive elements (VIT\_03s0038g01130, VIT\_03s0038g00950, VIT\_19s0085g00010) are strongly downregulated compared with the NINi control, and auxin inactivation-related genes are upregulated.

Auxins, cytokinins, and gibberellins control plant vegetative development and growth by coordinating cell proliferation and differentiation (Vanstraelen and Benkov, 2012). No DEGs appear related to gibberellin signaling. However, in addition to auxins, differential regulation of genes is associated with cytokinin biosynthesis and signaling (Figure 3). The analysis of gene expression associated with “auxin-mediated signaling pathways” in the presence of Vintec® reveals an inverse gene expression pattern compared with the NINi control. Most auxin-responsive putative genes (ARFs) are repressed by the presence of Vintec®, and this repression is more significant under the IPPV condition. In addition, auxin inactivation elements seem to be over-expressed in IV and IVPP. Altogether, these results suggest that auxin signaling pathways are repressed by the presence of Vintec®. These statements are also valid for cytokinin-mediated signaling pathways. Cytokinin type-A responsive elements, which negatively regulate type-B responsive elements, are induced in both IV and IVPP conditions, as well as cytokinin catabolic pathways. Furthermore, the category “cell wall organization and

**TABLE 3 |** Functional enrichment in Group II. DEGs up- and down-regulated by the two pathogens and Vintec® (IVPP).

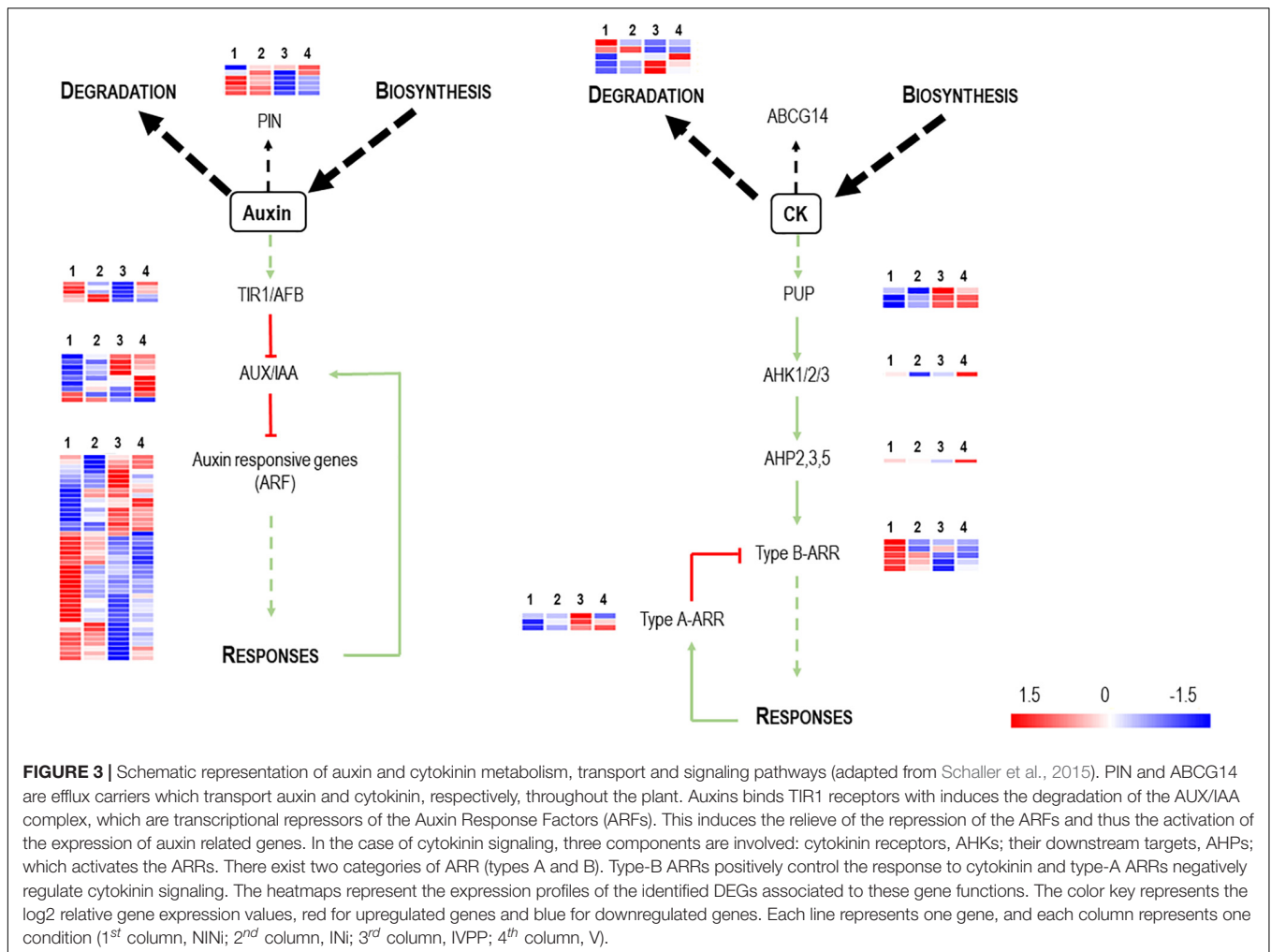
Functional category	Regulation	n DEGs	p-value
Phenylpropanoid biosynthesis	UP	5	$2.57 \times 10^{-4}$
Porters. Phosphate Permease (Pho1)	UP	2	$9.18 \times 10^{-4}$
Sphingolipid biosynthesis	UP	1	$1.29 \times 10^{-2}$
Glycolysis Gluconeogenesis	UP	3	$1.65 \times 10^{-2}$
Transcription factor: BSD family	UP	1	$1.93 \times 10^{-2}$
Aerolysin Channel-forming Toxin	UP	1	$2.87 \times 10^{-2}$
Biotic stress response	UP	3	$3.04 \times 10^{-2}$
Protein transport, Tethering factors	UP	2	$3.38 \times 10^{-2}$
Monoterpenoid metabolism	UP	2	$3.38 \times 10^{-2}$
Glycan catabolism	DOWN	1	$3.27 \times 10^{-3}$
Plastid outer envelope protein 16 kDa	DOWN	1	$6.53 \times 10^{-3}$
Regulation of translation (activation)	DOWN	1	$6.53 \times 10^{-3}$
DNA recombination and repair	DOWN	3	$8.07 \times 10^{-3}$
Drug/Metabolite Transporter	DOWN	3	$1.16 \times 10^{-2}$
Biotic stress response: Plant-pathogen interaction (R-prot)	DOWN	4	$1.50 \times 10^{-2}$
Pentatricopeptide domain family	DOWN	5	$1.59 \times 10^{-2}$
Fatty acid metabolism, Alpha-linolenic acid metabolism	DOWN	2	$1.93 \times 10^{-2}$
Ammonia Channel Transporter	DOWN	1	$2.91 \times 10^{-2}$

biogenesis” is enriched among upregulated genes of Group III ( $p$ -value =  $8.01 \times 10^{-4}$ ) but downregulated in Group I ( $p$ -value =  $6.12 \times 10^{-5}$ ), which could be linked to these differences in hormonal signaling.

### BCA Vintec® Enhances Reprogramming of Secondary Metabolism Gene Expression During Pathogen Infection

Among the up-regulated DEGs in Group II, the category “Phenylpropanoid biosynthesis” is significantly enriched ( $p$ -value =  $2.57 \times 10^{-4}$ ), along with “Monoterpenoid metabolism” ( $p$ -value =  $3.38 \times 10^{-2}$ ), which is also related to “Secondary metabolism.” Interestingly, when studying the expression pattern of DEGs associated with “Flavonoid biosynthesis,” “Terpenoid biosynthesis,” or “Phenylpropanoid biosynthesis,” we observe specific signatures (Figure 4). Some genes are up-regulated in the presence of the two pathogens, but their expression remains unchanged in plants pre-inoculated with Vintec®. In contrast, we observe another set of genes responding specifically to the infection only in the presence of Vintec®.

Secondary metabolites such as flavonoids, terpenoids, or stilbenes are known to play important roles in plant protection against biotic and abiotic stresses (Samanta et al., 2011). Interestingly, the upregulation of some of these genes is exclusive to the IVPP condition. The results show that genes VIT\_10s0042g00920 and VIT\_16s0100g00940 (ENTREZ ID 100266562 and 100853526, respectively) are strongly upregulated in plants inoculated with Vintec® before infection. These genes code for two putative stilbene synthases, which are enzymes associated with the biosynthesis of resveratrol and



pterostilbene. In contrast, VIT\_110037g00440 (ENTREZ ID 100250337), another gene associated with this same metabolic pathway, is significantly repressed during the infection in the presence of the BCA. The first two genes code for the stilbene synthase, which is implicated in the conversion of coumaroyl-CoA into *trans*-resveratrol, whereas the third gene codes for a shikimate *O*-hydroxycinnamoyltransferase, which catalyzes the interconversion of coumaroyl-CoA into caffeoyl-CoA (Figure 5).

## DISCUSSION

*Trichoderma* spp. have been known since the 1920s for their ability to compete with other fungi *via* direct mechanisms such as competition for nutrients and space, mycoparasitism, or antibiosis (Harman, 2006). However, the ability of *Trichoderma* spp. to trigger modifications in plant response was not studied until the 1990s (De Meyer et al., 1998). Since then, the way in which *Trichoderma* spp. affect plant species in response to diverse abiotic and biotic stimuli has been widely studied (Tucci et al., 2011; De Palma et al., 2019; Wang et al., 2020; Yan et al., 2021). For instance, several studies report

that *Trichoderma* spp. modulates growth promotion while enhancing salt and water stress tolerance *via* plant transcriptome reprogramming in *Arabidopsis thaliana* (Brotman et al., 2013), tomato (Mastouri et al., 2012; De Palma et al., 2019; Zhao et al., 2021), wheat (Zhang et al., 2016), and cacao (Bae et al., 2009). In another example involving tomatoes, the application of *T. harzianum* helps activate plant defense reactions against root-knot nematodes and stink bugs (Alinç et al., 2021) by enhancing secondary metabolite production and the activation of defense-related enzymes (Yan et al., 2021). Defense induction by *Trichoderma* spp. is also reported in cucumber (Yedidia et al., 2003; Shoresh et al., 2005), *A. thaliana* (Mathys et al., 2012), rice, and cotton (Djonović et al., 2006). In the present work, we study the transcriptome reprogramming in grapevine trunk triggered by the commercial biocontrol solution Vintec® (*Trichoderma atroviride* SC1) both alone and during infection with *P. chlamydospora* and *P. minimum*, the two main pathogens associated with esca disease.

Recent studies have shown that pathogen infection triggers the reprogramming of gene expression in grapevine trunk (Pierron et al., 2016; Massonnet et al., 2017; Gonçalves et al., 2019; Labois et al., 2020). Here, we confirm that the combined infection

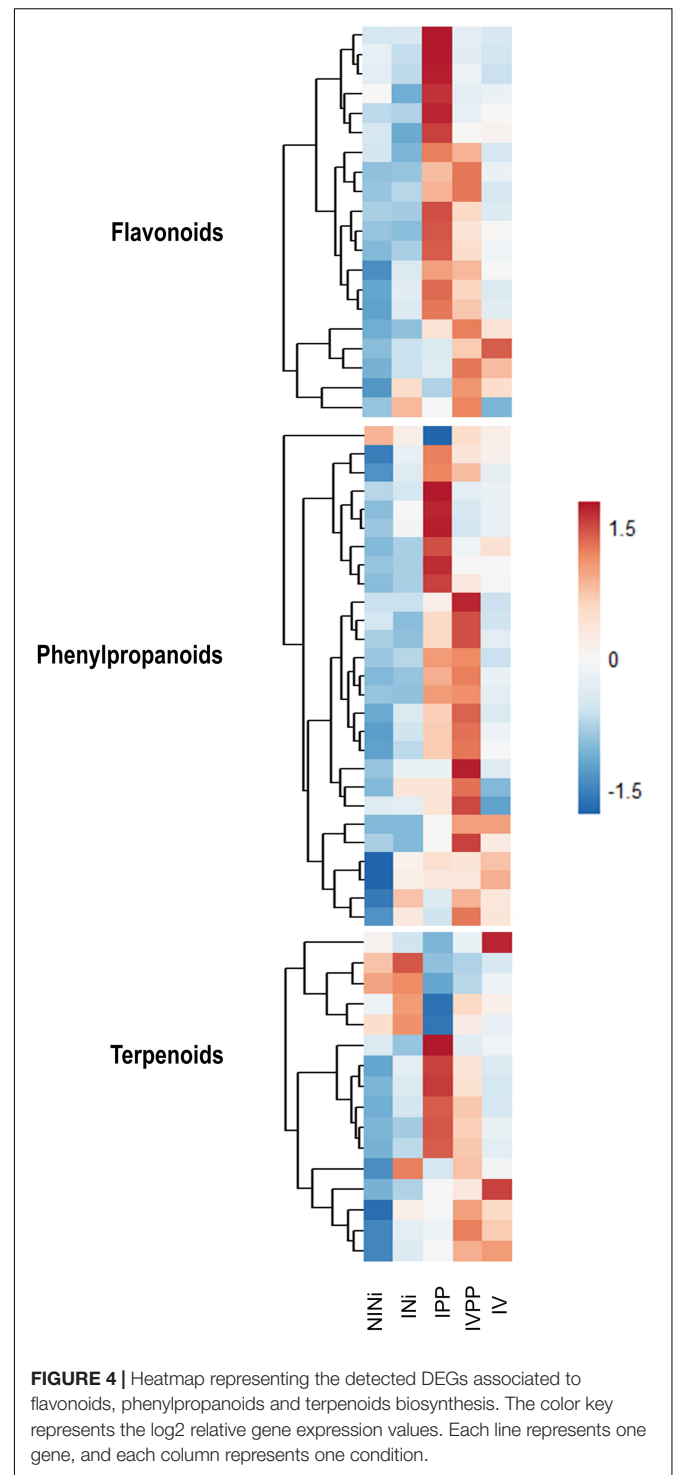


**TABLE 4** | Functional enrichment in Group III. DEGs up- and down-regulated by the BCA Vintec®.

Functional category	Regulation	n DEGs	p-value
Cell wall organization and biogenesis	UP	10	$8.01 \times 10^{-4}$
Channels and pores: beta-barrel porins	UP	3	$3.05 \times 10^{-3}$
Photosynthetic-chain phosphorylation	UP	3	$3.52 \times 10^{-3}$
Auxin inactivation	UP	2	$7.81 \times 10^{-3}$
Transcription factor: WRKY family	UP	3	$8.03 \times 10^{-3}$
Phenylalanine metabolism	UP	4	$9.62 \times 10^{-3}$
Porters. Oligopeptide Transporter	UP	2	$1.26 \times 10^{-2}$
Protein Kinase Signaling	UP	16	$1.32 \times 10^{-2}$
Abiotic stress response: UV	UP	1	$1.48 \times 10^{-2}$
Propanoate metabolism	UP	2	$1.71 \times 10^{-2}$
Glycerolipid biosynthesis	DOWN	1	$6.75 \times 10^{-3}$
Transcription factor: FHA-like family.	DOWN	1	$1.05 \times 10^{-2}$
Light signaling	DOWN	1	$1.20 \times 10^{-2}$
Auxin-mediated signaling pathway	DOWN	2	$1.39 \times 10^{-2}$
Protein folding	DOWN	2	$1.40 \times 10^{-2}$
G-protein signaling. RAB-GTPase regulation	DOWN	1	$1.72 \times 10^{-2}$
Senescence	DOWN	1	$1.72 \times 10^{-2}$
Transcription factor: Trihelix family	DOWN	1	$2.09 \times 10^{-2}$
Riboflavin metabolism	DOWN	1	$2.45 \times 10^{-2}$
Transcription factor: G2-like family.	DOWN	1	$2.60 \times 10^{-2}$

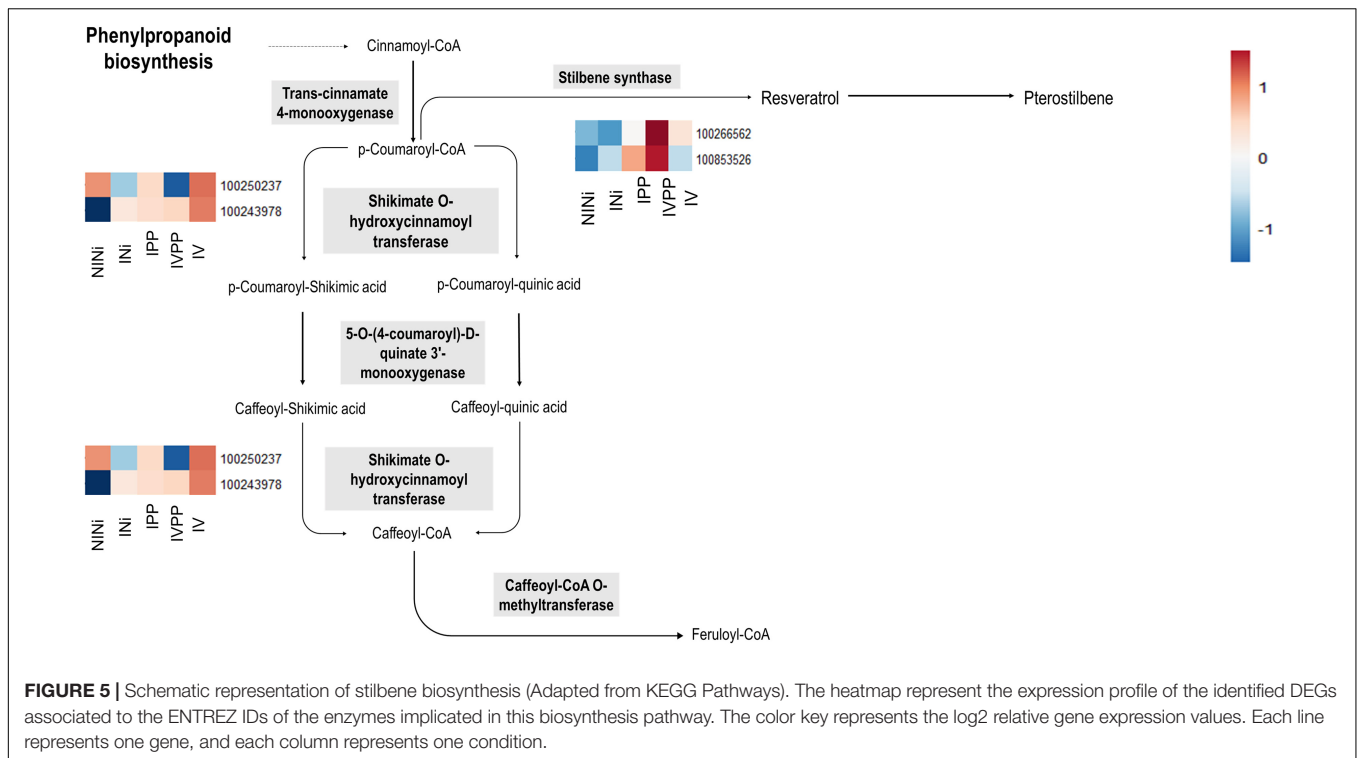
of *P. chlamydospora* and *P. minimum* leads to the modification of a large set of genes. The presence of the BCA Vintec® alone also modifies gene expression of grapevine trunk but to a lesser extent than pathogen infection, suggesting that grapevine trunk perceives and differentially responds to the BCA or the pathogens. Functional enrichment analysis also shows dissimilarities between the BCA Vintec® and the two pathogens.

Hormone signaling depends on the biotic stimuli. Plant hormones orchestrate plant development over different stages, and are vital for maintaining the balance between defense and growth when a plant is stressed (Pieterse et al., 2012; Naseem et al., 2015). Several studies show that the establishment of the plant-*Trichoderma* interaction and the ability to activate systemic resistance depends on different mechanisms employing jasmonic acid (JA) and ethylene (ET) signaling pathways (Shoresh et al., 2005, 2010; De Palma et al., 2019). The role of auxin signaling has been widely studied in connection with growth promotion induced by *Trichoderma*. Initially, *Trichoderma* was thought to modulate plant growth via the production of hormone-like compounds (Gravel et al., 2007; Contreras-Cornejo et al., 2009; Wang et al., 2020). Recent studies reveal that *Trichoderma* may activate the internal molecular mechanisms of plants, thereby altering hormonal signaling (Martínez-Medina et al., 2017; Patel et al., 2019; Wang et al., 2020). Wang et al. (2020) report

**FIGURE 4** | Heatmap representing the detected DEGs associated to flavonoids, phenylpropanoids and terpenoids biosynthesis. The color key represents the log<sub>2</sub> relative gene expression values. Each line represents one gene, and each column represents one condition.

that, in AUXR1, overexpressing poplar plantlets in which auxin is constitutively overproduced the level of indole-3-acetic acid decreases upon inoculation by *T. asperellum*.

The present results showed that the BCA Vintec® alone negatively regulates auxin signaling and differentially regulates cytokinin signaling in grapevine trunk. The cross-talk between auxin and cytokinin signaling reportedly plays a major role



in coordinating cell enlargement and cell division in meristem tissues (Aloni et al., 2006; Immanen et al., 2016). These studies show that auxin and cytokinin signaling act in an antagonistic but partially overlapping way (Schaller et al., 2015). Studies done on poplar provide insight into the distribution profiles of these two phytohormones during the activity of the vascular cambium, which is the lateral meristem where wood formation starts. Immanen et al. (2016) show that cell division in poplar vascular cambium increases significantly in transgenic trees, overexpressing a cytokinin biosynthesis-related gene from *Arabidopsis thaliana* (AtIP7). They also report that the levels of auxin and auxin-related genes also increase, which suggests that cytokinin functions as a major regulator of cambial activity.

During infection, no hormone-signaling-related functional category is enriched among genes up- or downregulated in plants pre-inoculated with Vintec®. On the contrary, the categories “Ethylene-signaling pathway” and “ABA biosynthesis” are enriched among DEGs in the presence of the two pathogens. Ethylene intervenes in many physiological processes; for instance, its cross-talk with auxin signaling regulates diverse plant developmental processes (Zemlyanskaya et al., 2018) and its interaction with jasmonic acid signaling modulates plant defense responses (Pieterse et al., 2012). Abscisic acid (ABA) is associated with abiotic stress responses, mainly drought and water stresses (Xie et al., 2005; Chen et al., 2020), but can play a role in the biotic stress response, which, in most cases, is considered to be a negative regulation of plant response (Mauch-Mani and Mauch, 2005; Asselbergh et al., 2008; Ueno et al., 2015). Various studies demonstrate that ABA interacts negatively with SA and JA/ET signaling (Anderson et al., 2004;

Mauch-Mani and Mauch, 2005; Cao et al., 2011; Ueno et al., 2015). Altogether, this evidence combined with the present results suggests that both the pathogens and the BCA Vintec® may interact with hormone signaling, altering hormone homeostasis in the trunk. Pathogens might use this mechanism to interfere with plant immunity, whereas the presence of Vintec® could counteract this strategy.

Conversely, numerous studies report that *Trichoderma* spp. stimulate plant natural defenses before or during pathogen infection (Shoresh et al., 2010; Shaw et al., 2016). Mathys et al. (2012) show that *T. hamatum* T382 activates Induced Systemic Resistance (ISR) in *A. thaliana*, resulting in accelerated activation of the defense response to *Botrytis cinerea*. They postulated that the stimulation of the phenylpropanoid pathway by *T. hamatum* plays an essential role in ISR. Yan et al. (2021) also report that another species of *Trichoderma*, *T. harzianum*, enhances the accumulation of secondary metabolites such as phenols and flavonoids and suppresses root-knot nematode infections in tomato. Pratap Singh et al. (2021) also report a higher total phenolic content in plants pre-inoculated with *T. harzianum* and *T. asperellum* after *Sclerotinia sclerotium* infection in brinjal. The present study also reveals an enrichment in the secondary metabolism biosynthesis pathways during the infection, mostly associated with phenylpropanoids, flavonoids, and phenols.

Several studies highlight the accumulation of this type of metabolite in diseased grapevine wood (Amalfitano et al., 2000; Lima et al., 2012; Rusjan et al., 2017). Remarkably, the pattern of expression of genes associated with the biosynthesis of phenylpropanoids, flavonoids, or phenols depends on the

presence or the absence of the BCA Vintec® during the infection. Our results suggest that the presence of the BCA Vintec® enhances the accumulation of stilbenes, which have antifungal properties, in accordance with parallel metabolomic work on the same pathosystem (Chervin et al., 2022). Stilbenes are described as phytoalexins in grapevine, so they are implicated in plant defense (Langcake and Pryce, 1977). For instance, the protective role of a number of stilbenes against *Plasmopara viticola*, the causal agent of downy mildew in grapevine, is described by Pezet et al. (2015) and Gabaston et al. (2017). In addition, a number of studies investigate how elicitation with chitosan affects grapevine gray mold (Iriti et al., 2004) and powdery mildew (Ait Barka et al., 2004; Romanazzi et al., 2021). The mechanism underlying the protection of *V. vinifera* by chitosan elicitation is related to the enhanced production of stilbenes in grapes (Lucini et al., 2018). Recently, a study comparing tolerant *V. vinifera* subspecies (*V. vinifera* subs. *Sylvestris*) and susceptible *V. vinifera* subs. *vinifera* showed that, after infection by *Neofusicoccum parvum*, tolerant subspecies contained more resveratrol and its derivatives than susceptible subspecies (Labois et al., 2020). Although the role of these compounds in esca disease has not yet been completely elucidated, all this evidence suggests that stilbenes play a key role in the protection of grapevine against trunk diseases.

## CONCLUSION

This study shows that the application of the BCA Vintec®—a *Trichoderma atroviride* SC1 commercial solution—enhances modifications in the gene response to grapevine trunk diseases, both alone and during pathogen infection with two pathogens associated with esca disease. The BCA Vintec® seems to alter in different ways the hormone homeostasis both alone and during infection with *P. minimum* and *P. chlamydospora*. Only during the infection does the BCA Vintec® promote the expression of genes related to the biosynthesis of stilbenes, phenols, and flavonoids, which are metabolites known for their antifungal properties. Altogether, these results suggest that the BCA Vintec® interferes in the plant response to esca-associated pathogens by enhancing the primary defense response.

## REFERENCES

- Ait Barka, E., Eullaffroy, P., Clément, C., and Vernet, G. (2004). Chitosan improves development, and protects *Vitis vinifera* L. against *Botrytis cinerea*. *Plant Cell Rep.* 22, 608–614. doi: 10.1007/s00299-003-0733-3
- Alinç, T., Cusumano, A., Peri, E., Torta, L., and Colazza, S. (2021). *Trichoderma harzianum* Strain T22 modulates direct defense of tomato plants in response to *nezaravirus* feeding activity. *J. Chem. Ecol.* 47:455. doi: 10.1007/S10886-021-01260-3
- Aloni, R., Aloni, E., Langhans, M., and Ullrich, C. I. (2006). Role of cytokinin and auxin in shaping root architecture: regulating vascular differentiation, lateral root initiation, root apical dominance and root gravitropism. *Ann. Bot.* 97, 883–893. doi: 10.1093/AOB/MCL027
- Amalfitano, C., Evidente, A., Mugnai, L., Tegli, S., Bertelli, E., and Surico, G. (2000). Phenols and stilbene polyphenols in the wood of Esca-diseased grapevines. *Phytopathol. Mediterr.* 39, 178–183. doi: 10.1400/57841

## DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The name of the repository and accession number can be found below: NCBI; PRJNA817518.

## AUTHOR CONTRIBUTIONS

AR-O and AJ conceived the experimental plan. AJ coordinated the study. AR-O conducted the experiments (plant production, RNA and DNA extractions, qRT-PCR, and qPCR reactions), conducted RNA sequencing bioinformatics analysis, and wrote the manuscript. CB assisted with plant production and qRT-PCR reactions. AJ, TL, JC, and JD contributed to the data analysis and interpretation. All co-authors revised and contributed modifications to the final manuscript.

## FUNDING

We are grateful to the French Public Bank of Investment (BPI France) for funding this work within the framework of the PSCP Project “SOLSTICE” (SOLutionS pour des Traitements Intégrés dans une Conduite Environnementale).

## ACKNOWLEDGMENTS

We thank Belchim Crop Protection for providing the BCA Vintec® and especially Wilfried Remus-Borel, Thomas Besnier, and Gabriel D’Enjoy for their expertise and fruitful discussions related to this work. Finally, we thank the GeT-Plage platform for their work and the high-quality sequences that they generated.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmicb.2022.898356/full#supplementary-material>

- Anderson, J. P., Badruzsaufari, E., Schenk, P. M., Manners, J. M., Desmond, O. J., Ehlert, C., et al. (2004). Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in arabidopsis. *Plant Cell* 16, 3460–3479. doi: 10.1105/tpc.104.025833
- Andrews, S. (2010). Babraham bioinformatics – FastQC a quality control tool for high throughput sequence data. *Soil* 5, 47–81. doi: 10.1016/0038-0717(73)90093-X
- Asselbergh, B., De Vleeschauwer, D., and Höfte, M. (2008). Global switches and fine-tuning—ABA modulates plant pathogen defense. *Mol. Plant Microbe Interact.* 21, 709–719. doi: 10.1094/MPMI-21-6-0709
- Bae, H., Sicher, R. C., Kim, M. S., Kim, S. H., Strem, M. D., Melnick, R. L., et al. (2009). The beneficial endophyte *Trichoderma hamatum* isolate DIS 219b promotes growth and delays the onset of the drought response in *Theobroma cacao*. *J. Exp. Bot.* 60, 3279–3295. doi: 10.1093/JXB/ERP165

- Benítez, T., Rincón, A. M., Limón, M. C., and Codón, A. C. (2004). Biocontrol mechanisms of *Trichoderma* strains. *Int. Microbiol.* 7, 249–260. doi: 10.2436/im.v7i4.9480
- Berbegal, M., Ramón-Albalat, A., León, M., and Armengol, J. (2020). Evaluation of long-term protection from nursery to vineyard provided by *Trichoderma atroviride* SC1 against fungal grapevine trunk pathogens. *Pest Manag. Sci.* 76, 967–977. doi: 10.1002/ps.5605
- Bertsch, C., Ramírez-Suero, M., Magnin-Robert, M., Larignon, P., Chong, J., Abou-Mansour, E., et al. (2013). Grapevine trunk diseases: complex and still poorly understood. *Plant Pathol.* 62, 243–265. doi: 10.1111/j.1365-3059.2012.02674.x
- Bhat, A., Junaid, J. M., Dar, N. A., Bhat, T. A., Hussain Bhat, A., and Bhat, M. A. (2013). Commercial biocontrol agents and their mechanism of action in the management of plant pathogens. *Int. J. Mod. Plant Anim. Sci.* 1, 39–57.
- Bigot, G., Sivilotti, P., Stecchina, M., Lujan, C., Freccero, A., and Mosetti, D. (2020). Long-term effects of *Trichoderma asperellum* and *Trichoderma gamsii* on the prevention of esca in different vineyards of Northeastern Italy. *Crop. Prot.* 137:105264. doi: 10.1016/j.cropro.2020.105264
- Brodeur, J., Abram, P. K., Heimpel, G. E., and Messing, R. H. (2018). Trends in biological control: public interest, international networking and research direction. *BioControl* 63, 11–26. doi: 10.1007/s10526-017-9850-8
- Brotman, Y., Landau, U., Cuadros-Inostroza, Á., Takayuki, T., Fernie, A. R., Chet, I., et al. (2013). *Trichoderma*-plant root colonization: escaping early plant defense responses and activation of the antioxidant machinery for saline stress tolerance. *PLoS Pathog.* 9:e1003221. doi: 10.1371/JOURNAL.PPAT.1003221
- Canaguier, A., Grimplet, J., Di Gaspero, G., Scalabrin, S., Duchêne, E., Choisine, N., et al. (2017). A new version of the grapevine reference genome assembly (12X.v2) and of its annotation (VCost.v3). *Genomics Data* 14, 56–62. doi: 10.1016/j.gdata.2017.09.002
- Cao, F. Y., Yoshioka, K., and Desveaux, D. (2011). The roles of ABA in plant-pathogen interactions. *J. Plant Res.* 124, 489–499. doi: 10.1007/s10265-011-0409-y
- Chen, K., Li, G. J., Bressan, R. A., Song, C. P., Zhu, J. K., and Zhao, Y. (2020). Abscisic acid dynamics, signaling, and functions in plants. *J. Integr. Plant Biol.* 62, 25–54. doi: 10.1111/JIPB.12899
- Chervin, J., Romeo-Oliván, A., Fournier, S., Puech-Pages, V., Dumas, B., Jacques, A., et al. (2022). Modification of early response of *Vitis vinifera* to pathogens relating to esca disease and biocontrol agent vintec® revealed by untargeted metabolomics on woody tissues. *Front. Microbiol.* 13:234. doi: 10.3389/fmicb.2022.835463
- Contreras-Cornejo, H. A., Macías-Rodríguez, L., Cortés-Penagos, C., and López-Bucio, J. (2009). *Trichoderma virens*, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in *Arabidopsis*. *Plant Physiol.* 149, 1579–1592. doi: 10.1104/PP.108.130369
- Cook, R. J. (1988). Biological control and holistic plant-health care in agriculture. *Am. J. Altern. Agric.* 3, 51–62. doi: 10.1017/S0889189300002186
- De Meyer, G., Bigirimana, J., Elad, Y., and Höfte, M. (1998). Induced systemic resistance in *Trichoderma harzianum* T39 biocontrol of *Botrytis cinerea* Geert. *Eur. J. Plant Pathol.* 104, 279–286.
- De Palma, M., Salzano, M., Villano, C., Aversano, R., Lorito, M., Ruocco, M., et al. (2019). Transcriptome reprogramming, epigenetic modifications and alternative splicing orchestrate the tomato root response to the beneficial fungus *Trichoderma harzianum*. *Hortic. Res.* 6:15. doi: 10.1038/s41438-018-0079-1
- Di Marco, S., Cesari, A., and Osti, F. (2004). Experiments on the control of Esca by “*Thricoderma*”. *Phytopathol. Mediterr.* 43, 1000–1008. doi: 10.1400/14580
- Di Marco, S., and Osti, F. (2007). Applications of *Trichoderma* to prevent *Phaeoconiella chlamydospora* infections in organic nurseries. *Phytopathol. Mediterr.* 46, 73–83.
- Djonović, S., Pozo, M. J., Dangott, L. J., Howell, C. R., and Kenerley, C. M. (2006). Sm1, a proteinaceous elicitor secreted by the biocontrol fungus *Trichoderma virens* induces plant defense responses and systemic resistance. *Mol. Plant Microbe Interact.* 19, 838–853. doi: 10.1094/MPMI-19-0838
- Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., et al. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 29, 15–21. doi: 10.1093/BIOINFORMATICS/BTS635
- Eilenberg, J., Hajek, A., and Lomer, C. (2001). Suggestions for unifying the terminology in biological control. *BioControl* 46, 387–400.
- Ferreira, F. V., and Musumeci, M. A. (2021). *Trichoderma* as biological control agent: scope and prospects to improve efficacy. *World J. Microbiol. Biotechnol.* 37:90. doi: 10.1007/s11274-021-03058-7
- Fontaine, F., Gramaje, D., Armengol, J., Smart, R., Nagy, Z. A., Borgo, M., et al. (2016). *Grapevine Trunk Diseases. A review*. Available online at: <https://www.oiv.int/public/medias/4650/trunk-diseases-oiv-2016> (accessed 1 April, 2019).
- Fourie, P. H., Halleen, F., Van der Vyver, J., and Schreuder, W. (2001). Effect of *Trichoderma* treatments on the occurrence of decline pathogens in the roots and rootstocks of nursery grapevines. *Phytopathol. Mediterr.* 40, 473–478. doi: 10.14601/PHYTOPATHOL\_MEDITERR-1619
- Gabaston, J., Cantos-Villar, E., Biais, B., Waffo-Teguo, P., Renouf, E., Corio-Costet, M. F., et al. (2017). Stilbenes from *Vitis vinifera* L. Waste: a sustainable tool for controlling *Plasmopara viticola*. *J. Agric. Food Chem.* 65, 2711–2718. doi: 10.1021/acs.jafc.7b00241
- Gonçalves, M. F. M., Nunes, R. B., Tilleman, L., Van De Peer, Y., Deforce, D., Van Nieuwerburgh, F., et al. (2019). Dual RNA sequencing of *Vitis vinifera* during *Lasiodiplodia theobromae* infection Unveils host–pathogen interactions. *Int. J. Mol. Sci.* 20:6083. doi: 10.3390/IJMS20236083
- Gramaje, D., Mostert, L., Groenewald, J. Z., and Crous, P. W. (2015). *Phaeoacremonium*: from esca disease to phaeoohyphomycosis. *Fungal Biol.* 119, 759–783. doi: 10.1016/j.funbio.2015.06.004
- Graniti, A., Surico, G., and Mugnai, L. (2000). Esca of grapevine: a disease complex or a complex of diseases? *Phytopathol. Mediterr.* 39, 16–20. doi: 10.14601/Phytopathol\_Mediterr-1539
- Gravel, V., Antoun, H., and Tweddell, R. J. (2007). Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: possible role of indole acetic acid (IAA). *Soil Biol. Biochem.* 39, 1968–1977. doi: 10.1016/J.SOILBIO.2007.02.015
- Grimplet, J., Cramer, G. R., Dickerson, J. A., Mathiason, K., van Hemert, J., and Fennell, A. Y. (2009). Vitisnet: “Omics” integration through grapevine molecular networks. *PLoS One* 4:e8365. doi: 10.1371/journal.pone.0008365
- Harman, G. E. (2006). Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathology* 96, 190–194. doi: 10.1094/PHYTO-96-0190
- Immanen, J., Nieminen, K., Smolander, O. P., Kojima, M., Alonso Serra, J., Koskinen, P., et al. (2016). Cytokinin and auxin display distinct but interconnected distribution and signaling profiles to stimulate cambial activity. *Curr. Biol.* 26, 1990–1997. doi: 10.1016/j.cub.2016.05.053
- Iriti, M., Rossoni, M., Borgo, M., and Faoro, F. (2004). Benzothiadiazole enhances resveratrol and anthocyanin biosynthesis in grapevine, meanwhile improving resistance to *Botrytis cinerea*. *J. Agric. Food Chem.* 52, 4406–4413. doi: 10.1021/JF049487B
- Joshi, N. A., and Fass, J. N. (2011). *Sickle: A Sliding-Window, Adaptive, Quality-based Trimming Tool for FastQ Files (Version 1.33) [Software]*. Available online at <https://github.com/najoshi/sickle>
- Kotze, C., Van Niekerk, J., Mostert, L., Halleen, F., and Fourie, P. (2011). *Evaluation of Biocontrol Agents for Grapevine Pruning Wound Protection against Trunk Pathogen Infection*. Firenze: Firenze University Press Mediterranean Phytopathological Union. doi: 10.2307/26458725
- Kubicek, C. P., Mach, R. L., Peterbauer, C. K., and Lorito, M. (2001). *Trichoderma*: from genes to biocontrol. *J. Plant Pathol.* 83, 11–23.
- Labois, C., Wilhelm, K., Laloue, H., Tarnus, C., Bertsch, C., Goddard, M. L., et al. (2020). Wood metabolomic responses of wild and cultivated grapevine to infection with *Neofusicoccum parvum*, a trunk disease pathogen. *Metabolites* 10:232. doi: 10.3390/METABO10060232
- Langcake, P., and Pryce, R. J. (1977). A new class of phytoalexins from grapevines. *Experientia* 33, 151–152. doi: 10.1007/BF02124034
- Larignon, P., Dupont, J., and Dubos, B. (2000). Esca disease. The biological background of two agents of the disease, *Phaeoacremonium aleophilum* and *Phaeoconiella chlamydospora*. *Phytoma* 527, 30–35.
- Liao, Y., Smyth, G. K., and Shi, W. (2014). featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* 30, 923–930.
- Lima, M. R. M., Ferreres, F., and Dias, A. C. P. (2012). Response of *Vitis vinifera* cell cultures to *Phaeoconiella chlamydospora*: changes in phenolic production, oxidative state and expression of defence-related genes. *Eur. J. Plant Pathol.* 132, 133–146. doi: 10.1007/s10658-011-9857-4



- Lucini, L., Baccolo, G., Roupael, Y., Colla, G., Bavaresco, L., and Trevisan, M. (2018). Chitosan treatment elicited defence mechanisms, pentacyclic triterpenoids and stilbene accumulation in grape (*Vitis vinifera* L.) bunches. *Phytochemistry* 156, 1–8. doi: 10.1016/j.phytochem.2018.08.011
- Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. journal* 17, 10–12. doi: 10.14806/EJ.17.1.200
- Martínez-Medina, A., Fernández, I., Lok, G., Pozo, M., Pieterse, C., and Van Wees, S. (2017). Shifting from priming of salicylic acid- to jasmonic acid-regulated defences by *Trichoderma* protects tomato against the root knot nematode *Meloidogyne incognita*. *New Phytol.* 213, 1363–1377. doi: 10.1111/NPH.14251
- Massonnet, M., Figueroa-Balderas, R., Galarneau, E. R. A. A., Miki, S., Lawrence, D. P., Sun, Q., et al. (2017). *Neofusicoccum parvum* colonization of the grapevine woody stem triggers asynchronous host responses at the site of infection and in the leaves. *Front. Plant Sci.* 8:1117. doi: 10.3389/fpls.2017.01117
- Mastouri, F., Björkman, T., and Harman, G. (2012). *Trichoderma harzianum* enhances antioxidant defense of tomato seedlings and resistance to water deficit. *Mol. Plant. Microbe Interact.* 25, 1264–1271. doi: 10.1094/MPMI-09-11-0240
- Mathys, J., De Cremer, K., Timmermans, P., Van Kerckhove, S., Lievens, B., Vanhaecke, M., et al. (2012). Genome-wide characterization of ISR induced in *Arabidopsis thaliana* by *Trichoderma hamatum* T382 against *Botrytis cinerea* infection. *Front. Plant Sci.* 3:108. doi: 10.3389/fpls.2012.00108
- Mauch-Mani, B., and Mauch, F. (2005). The role of abscisic acid in plant–pathogen interactions. *Curr. Opin. Plant Biol.* 8, 409–414. doi: 10.1016/J.PBI.2005.05.015
- Mondello, V., Larignon, P., Armengol, J., Kortekamp, A., Vaczy, K., Prezman, F., et al. (2018a). Management of grapevine trunk diseases: knowledge transfer, current strategies and innovative strategies adopted in Europe. *Phytopathol. Mediterr.* 57, 369–383. doi: 10.14601/Phytopathol\_Mediterr-23942
- Mondello, V., Songy, A., Battiston, E., Pinto, C., Coppin, C., Trotel-Aziz, P., et al. (2018b). Grapevine trunk diseases: a review of fifteen years of trials for their control with chemicals and biocontrol agents. *Plant Dis.* 102, 1189–1217. doi: 10.1094/PDIS-08-17-1181-FE
- Mugnai, L., Graniti, A., and Surico, G. (1999). Esca (Black measles) and brown wood-streaking: two old and elusive diseases of grapevines. *Plant Dis.* 83, 404–418. doi: 10.1094/PDIS.1999.83.5.404
- Naseem, M., Kaldorf, M., and Dandekar, T. (2015). The nexus between growth and defence signalling: auxin and cytokinin modulate plant immune response pathways. *J. Exp. Bot.* 66, 4885–4896. doi: 10.1093/JXB/ERV297
- Patel, J., Teli, B., Bajpai, R., Meher, J., Rashid, M., Mukherjee, A., et al. (2019). *Trichoderma-Mediated Biocontrol and Growth Promotion in Plants: An Endophytic Approach*. Amsterdam: Elsevier Inc., doi: 10.1016/b978-0-12-817004-5.00013-0
- Pertot, I., Prodanutti, D., Colombini, A., and Pasini, L. (2016). *Trichoderma atroviride* SC1 prevents *Phaeoconiella chlamydospora* and *Phaeoacremonium aleophilum* infection of grapevine plants during the grafting process in nurseries. *BioControl* 61, 257–267. doi: 10.1007/s10526-016-9723-6
- Pezet, R., Gindro, K., Viret, O., and Richter, H. (2015). Effects of resveratrol, viniferins and pterostilbene on *Plasmopara viticola* zoospore mobility and disease development. *Vitis J. Grapevine Res.* 43, 145–145. doi: 10.5073/VITIS.2004.43.145-148
- Pierron, R. J. G., Pouzoulet, J., Couderc, C., Judic, E., Compant, S., and Jacques, A. (2016). Variations in early response of grapevine wood depending on wound and inoculation combinations with *Phaeoacremonium aleophilum* and *Phaeoconiella chlamydospora*. *Front. Plant Sci.* 7:268. doi: 10.3389/fpls.2016.00268
- Pieterse, C. M. J., Van Der Does, D., Zamioudis, C., Leon-Reyes, A., and Van Wees, S. C. M. (2012). Hormonal modulation of plant immunity. *Annu. Rev. Cell Dev. Biol.* 28, 489–521. doi: 10.1146/ANNUREV-CELLBIO-092910-154055
- Pouzoulet, J., Mailhac, N., Couderc, C., Besson, X., Daydé, J., Lummerzheim, M., et al. (2013). A method to detect and quantify *Phaeoconiella chlamydospora* and *Phaeoacremonium aleophilum* DNA in grapevine-wood samples. *Appl. Microbiol. Biotechnol.* 97, 10163–10175. doi: 10.1007/s00253-013-5299-6
- Pratap Singh, S. S., Keswani, C., Pratap Singh, S. S., Sansinenea, E., Xuan Hoat, T., Singh, S. P., et al. (2021). *Trichoderma* spp. mediated induction of systemic defense response in brinjal against *Sclerotinia sclerotiorum*. *Curr. Res. Microb. Sci.* 2:100051. doi: 10.1016/J.CRMICR.2021.100051
- Ram, R. M., Keswani, C., Bisen, K., Tripathi, R., Singh, S. P., and Singh, H. B. (2018). “Biocontrol technology: eco-friendly approaches for sustainable agriculture,” in *Omics Technologies and Bio-Engineering: Towards Improving Quality of Life*, Vol. Volume 2, eds D. Barh and V. Azevedo (Amsterdam: Elsevier), 177–190. doi: 10.1016/B978-0-12-815870-8.00010-3
- Romanazzi, G., Mancini, V., Foglia, R., Marcolini, D., Kavari, M., and Piancatelli, S. (2021). Use of chitosan and other natural compounds alone or in different strategies with copper hydroxide for control of grapevine downy mildew. *Plant Dis.* 105, 3261–3268. doi: 10.1094/PDIS-06-20-1268-RE
- Rusjan, D., Persic, M., Likar, M., Biniari, K., and Mikulic-Petkovsek, M. (2017). Phenolic responses to Esca-associated fungi in differently decayed grapevine woods from different trunk parts of “Cabernet Sauvignon”. *J. Agric. Food Chem.* 65, 6615–6624. doi: 10.1021/acs.jafc.7b02188
- Samanta, A., Das, G., and Das, S. (2011). Roles of flavonoids in plants. *Carbon N. Y.* 100:6.
- Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N., and Nelson, A. (2019). The global burden of pathogens and pests on major food crops. *Nat. Ecol. Evol.* 3, 430–439. doi: 10.1038/s41559-018-0793-y
- Schaller, G. E., Bishopp, A., and Kieber, J. J. (2015). The Yin-Yang of hormones: cytokinin and auxin interactions in plant development. *Plant Cell* 27, 44–63. doi: 10.1105/TPC.114.133595
- Shaw, S., Le Cocq, K., Paszkiewicz, K., Moore, K., Winsbury, R., de Torres Zabala, M., et al. (2016). Transcriptional reprogramming underpins enhanced plant growth promotion by the biocontrol fungus *Trichoderma hamatum* GD12 during antagonistic interactions with *Sclerotinia sclerotiorum* in soil. *Mol. Plant Pathol.* 17, 1425–1441. doi: 10.1111/MPP.12429
- Shoresh, M., Harman, G. E., and Mastouri, F. (2010). Induced systemic resistance and plant responses to fungal biocontrol agents. *Annu. Rev. Phytopathol.* 48, 21–43. doi: 10.1146/ANNUREV-PHYTO-073009-114450
- Shoresh, M., Yedidia, I., and Chet, I. (2005). Involvement of jasmonic acid/ethylene signaling pathway in the systemic resistance induced in cucumber by *Trichoderma asperellum* T203. *Phytopathology* 95, 76–84. doi: 10.1094/PHYTO-95-0076
- Thambugala, K. M., Daranagama, D. A., Phillips, A. J. L., Kannangara, S. D., and Promputtha, I. (2020). Fungi vs. fungi in biocontrol: an overview of fungal antagonists applied against fungal plant pathogens. *Front. Cell. Infect. Microbiol.* 10:604923. doi: 10.3389/fcimb.2020.604923
- Tucci, M., Ruocco, M., De Masi, L., De Palma, M., and Lorito, M. (2011). The beneficial effect of *Trichoderma* spp. on tomato is modulated by the plant genotype. *Mol. Plant Pathol.* 12, 341–354. doi: 10.1111/J.1364-3703.2010.00674.X
- Ueno, Y., Yoshida, R., Kishi-Kaboshi, M., Matsushita, A., Jiang, C. J., Goto, S., et al. (2015). Abiotic stresses antagonize the rice defence pathway through the tyrosine-dephosphorylation of OsMPK6. *PLoS Pathog.* 11:e1005231. doi: 10.1371/JOURNAL.PPAT.1005231
- Vanstraelen, M., and Benkov, E. (2012). Hormonal interactions in the regulation of plant development. *Annu. Rev. Cell Dev. Biol.* 28, 463–487. doi: 10.1146/ANNUREV-CELLBIO-101011-155741
- Vinale, F., Sivasithamparan, K., Ghisalberti, E. L., Marra, R., Woo, S. L., and Lorito, M. (2008). *Trichoderma*-plant-pathogen interactions. *Soil Biol. Biochem.* 40, 1–10. doi: 10.1016/j.soilbio.2007.07.002
- Wang, Y., Hou, X., Deng, J., Yao, Z., Lyu, M., and Zhang, R. (2020). *AUXIN RESPONSE FACTOR 1* acts as a positive regulator in the response of poplar to *Trichoderma asperellum* inoculation in overexpressing plants. *Plants (Basel)* 9:272. doi: 10.3390/PLANTS9020272
- Xie, Z., Ruas, P., and Shen, Q. J. (2005). Regulatory networks of the phytohormone abscisic acid. *Vitam. Horm.* 72, 235–269. doi: 10.1016/S0083-6729(05)72007-0
- Yan, Y., Mao, Q., Wang, Y., Zhao, J., Fu, Y., Yang, Z., et al. (2021). *Trichoderma harzianum* induces resistance to root-knot nematodes by increasing secondary metabolite synthesis and defense-related enzyme activity in *Solanum lycopersicum* L. *Biol. Control* 158:104609. doi: 10.1016/J.BIOCONTROL.2021.104609
- Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., and Madden, T. L. (2012). Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics* 13:134. doi: 10.1186/1471-2105-13-134
- Yedidia, I., Shoresh, M., Kerem, Z., Benhamou, N., Kapulnik, Y., and Chet, I. (2003). Concomitant induction of systemic resistance to *Pseudomonas syringae* pv. lachrymans in cucumber by *Trichoderma asperellum* (T-203) and

- accumulation of phytoalexins. *Appl. Environ. Microbiol.* 69, 7343–7353. doi: 10.1128/AEM.69.12.7343-7353.2003
- Zemlyanskaya, E. V., Omelyanchuk, N. A., Ubogoeva, E. V., and Mironova, V. V. (2018). Deciphering auxin-ethylene crosstalk at a systems level. *Int. J. Mol. Sci.* 19:4060. doi: 10.3390/IJMS19124060
- Zhang, J. D., Ruschhaupt, M., and Biczok, R. (2020). *ddCt Method for qRT-PCR Data Analysis*.
- Zhang, S., Gan, Y., and Xu, B. (2016). Application of plant-growth-promoting fungi *Trichoderma longibrachiatum* T6 enhances tolerance of wheat to salt stress through improvement of antioxidative defense system and gene expression. *Front. Plant Sci.* 7:1405. doi: 10.3389/FPLS.2016.01405
- Zhao, J., Liu, T., Liu, W. C., Zhang, D. P., Dong, D., Wu, H. L., et al. (2021). Transcriptomic insights into growth promotion effect of *Trichoderma afroharzianum* TM2-4 microbial agent on tomato plants. *J. Integr. Agric.* 20, 1266–1276. doi: 10.1016/S2095-3119(20)63415-3
- Zin, N. A., and Badaluddin, N. A. (2020). Biological functions of *Trichoderma* spp. for agriculture applications. *Ann. Agric. Sci.* 65, 168–178. doi: 10.1016/j.aos.2020.09.003

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher's Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Romeo-Oliván, Chervin, Breton, Lagravère, Daydé, Dumas and Jacques. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.