Title: Exploring the Impact of Various Treatments on Gene Expression in Olive (*Olea europaea* L.) Drupes Affected by *Phytophthora oleae*: Insights from RNA sequencing-based transcriptome analysis.

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Abstract

Phytophthora oleae is a pathogen recently reported to cause fruit rot on olive orchards in Italy and root rot in a natural wild-olive forest in Spain. RNAseq analysis was conducted to gain insight into the molecular mechanisms that trigger a plant defense response upon the inoculation of drupes with *P. oleae* and the pre-treatment with the antagonistic yeast *Candida oleophila* or with culture filtrates of the antagonistic filamentous fungus *Trichoderma atroviride*. Both treatments were applied to the olive drupe 24 h before the inoculation with the pathogen. Although no full resistance was observed, the virulence of *P. oleae* was reduced when the drupes were co-inoculated with the yeast or treated with culture filtrates of *Trichoderma*. Severity of *Phytophthora* rots in olive drupes was assessed at 24, 72, and 168 hours post pathogen inoculation (hpi) and rated based on an empirical scale. The most effective in reducing the disease severity of *P. oleae* infection on olive fruit was the treatment with *T. atroviride* filtrate (56% reduction), followed by *C. oleophila* (52%). Results showed that 2,466, 1,883, and 1,757 genes were differentially expressed in response to *P. oleae*, to the binary pathosystem *C. oleophila* and *P. oleae*, and *T. atroviride* and *P. oleae*, respectively, as compared to wound. Differential RNAseq by DESeq2, performed at 72 hours post-inoculation, and qPCR validation, at 24,

72, and 168 hpi, of the top differentially expressed genes defined a new pattern of plant defense mechanisms involving both PAMP and ETI immunity, with production of ROS and PRs.

Keywords: RNAseq, Gene expression, Oomycete, Pathogenesis, Defense responses, Biological control agent, *Candida oleophila*, *Trichoderma atroviride*.

Abbreviations: BCA, biological control agent; PAMP, pathogen-associated molecular pattern; ETI, effector triggered immunity; ROS, reactive oxygen species; PRs, pathogenesis related proteins.

1. Introduction

Phytophthora oleae, the only known species comprised in subclade 2d of *Phytophthora* clade 2d[1,2], was for the first time reported as an emergent pathogen of olive (*Olea europaea* L.) in southern Italy[3]. Infection by this oomycete causes a soft rot of mature drupes whose symptoms often overlap with those of anthracnose caused by diverse *Colletotrichum* species, which is regarded as the most serious disease of olive fruit worldwide[4–8]. Other *Phytophthora* species were reported to cause rots of olive fruit[9]. Pre- and post-harvest rots of olive drupes incited by fungi and oomycetes may cause severe yield losses and have detrimental effects on oil quality[10–12]. Management strategies to prevent spoilage of olive production, caused by infectious diseases of fruit, have mainly focused on anthracnose. Although those strategies have prevalently relied on the use of synthetic fungicides, alternative means, such as the natural substances and biological control agents (BCAs) have been also considered[4,13,14]. By contrast, minor fungal diseases and emerging diseases of olive fruit caused by oomycetes have so far received little attention. The search for more sustainable alternatives to conventional synthetic fungicides is a new frontier of modern plant pathology. In the olive oil supply chain, the objective of substituting or reducing the use of synthetic fungicides is of particular relevance due to the risk that residues on fruit pass into the oil during the extraction process. It is noteworthy the olive fruit itself is a reservoir of microorganisms that can be exploited as BCAs[15,16]. Among BCAs, *Trichoderma* species have been extensively tested against pre- and post-harvest fruit pathogens, including diverse species of *Phytophthora*[17–23]. It has been also envisaged the possibility of using bioactive metabolites produced by *Trichoderma* species as natural fungicides[22,24]. In field applications, the efficacy of metabolites would be less conditioned by environmental factors than the living microorganism. A more rapid effect would be an additional advantage of metabolites

over the living microorganism, as in the olive oil supply chain the post-harvest phase preceding the oil extraction is very brief. Yeasts are other effective BCAs of post-harvest fruit diseases[25–27]. A very interesting antagonistic yeast is *Candida oleophila*, which has showed a biocontrol activity against several post-harvest diseases of diverse fruit crops. Strains of this yeast have been exploited as commercial bioproducts[19,28–30].

Several lines of evidence indicate multiple modes of action contribute to the effectiveness of BCAs in suppressing plant diseases, including the elicitation of plant immune system[31]. However, few studies have addressed the transcriptomic analysis of the complex interaction pathogen/host/BCA. *Phytophthora* species are known to secrete a large array of effectors during infection of the plant host. In recent decades, significant progresses have been made in identifying numerous *Phytophthora* effectors, broadly categorized into two main types: CRN and RxLR effectors.[32] The CRN proteins are a family of effectors that cause necrosis in the cells of the host and also induce further intracellular effectors that target the host nucleus during infection. RxLR effectors play a central role in pathogen–plant interactions. They are secreted proteins that manipulate the host's immune responses, allowing the pathogen to establish infection[33,34]. Although several studies have already elucidated the role of CRN and RxLR proteins in several *Phytophthora* spp., there is a lack of knowledge about the role of these effectors in the pathosystem *P. oleae*/olive drupes.

Successful *Phytophthora* pathogens have evolved and adapted to a specific host range, developing a set of effector proteins and phytotoxins that either directly induce plant cell death or suppress pattern-triggered immunity (PTI) and trigger susceptibility of the host. Nevertheless, some pathogenesis-related proteins (PR-proteins) are constitutive in some plants and represent a first barrier to the pathogen conveying a sort of innate immunity. Most of the time, even such constitutive levels of PR-proteins might be triggered by the ecto-symbiosis with microorganisms, such as *Trichoderma* spp., or their metabolites known to trigger both growth promotional effects and plant defense mechanisms by the elicitation of salicylic acid (SA)-, ethylene (ET)-, and jasmonic acid (JA)-dependent processes[22,35].

The aims of this study were: i. to evaluate the effectiveness of either *Trichoderma atroviride* culture filtrates or *C. oleophila* strain O in suppressing rot of olive drupes inoculated with *P. oleae*; ii. to examine the differential gene expression in *P. oleae* and olive drupes, treated/non treated with *C. oleophila* or *T. atroviride*-culture filtrate by high throughput technologies (*i.e.* Illumina RNA sequencing).

85

86 **2. Results**

87 **2.1. Evaluating the effectiveness of *C. oleophila* strain O and *T. atroviride*-culture filtrate in controlling**
88 **the rot of olive drupes incited by *P. oleae***

89 This section reports the results of tests aimed at evaluating the effectiveness of either *C. oleophila* strain O cell
90 suspension or *T. atroviride*-culture filtrate in controlling the rot of olive drupes inoculated with *P. oleae* isolate
91 VK10A. Overall, drupes treated with *T. atroviride*-culture filtrate (IDB) or *C. oleophila* cell suspension (IDC)
92 reported values of disease severity significantly lower than control drupe treated with sterile distilled water
93 (sdw) (IDA) (Figure 1). In particular, the mean value of the relative area under the disease progress curve
94 (rAUDPC) was 0.43 for the control (IDA), 0.21 (i.e. 52% reduction compared to the control) and 0.19 (i.e.
95 56% reduction compared to the control) for the drupes treated with *C. oleophila* (IDC) and *T. atroviride*-culture
96 filtrate (IDB), respectively. *In vitro* inhibitory activity of liquid culture extracts of *T. atroviride* against
97 *Phytophthora* species (*P. nicotianae* and *P. parvispora*) had been demonstrated in a previous study[22]. By
98 contrast, no previous studies reported any data on the efficacy of *C. oleophila* against *Phytophthora* species.

99 **2.2. Differential RNA analysis at 72 hours post inoculation with *P. oleae***

100 This section describes the transcriptomic responses of both olive drupes and *P. oleae* in the system olive drupe-
101 *P. oleae*-*C. oleophila* or olive drupe-*P. oleae*-*T. atroviride*-culture filtrate. To this purpose, the following seven
102 treatments were established: (i) non-wounded drupes treated with 20 µl of sdw (ID1); (ii) wounded drupes
103 treated with 20 µl of sdw (ID2); (iii) wounded drupes inoculated with *P. oleae* (ID3); (iv) wounded drupes
104 treated with *T. atroviride*-culture filtrate and inoculated with *P. oleae* (ID4); (v) wounded drupes treated with
105 *C. oleophila* and inoculated with *P. oleae* (ID5); (vi) wounded drupes treated with *T. atroviride*-culture filtrate
106 (ID6); (vii) wounded drupes treated with *C. oleophila* (ID7). Pre-treatment with *C. oleophila* cell suspension
107 or *T. atroviride*-culture filtrate was performed 24h before *P. oleae* inoculation. Drupe fragments were collected
108 at 24-, 72- and 168-hours post inoculation of *P. oleae* (hpi). However, as in the experiment described at
109 paragraph 2.1., 72 hpi was found to be the best timing for the RNAseq evaluation, as the symptoms of *P. oleae*
110 infection were evident (values of rot severity of about 25%).

111 The following paragraphs describe the RNAseq analysis of olive fruit performed by analyzing binary
112 conditions (see paragraphs: comparisons 1, 2, 3, 4, 6 and 7). A cluster analysis was also carried out among all

113 the treatments for the olive genes in all the conditions analyzed (see paragraph: comparison 5). Finally, a
114 RNAseq analysis was carried out in the same samples to highlight the differential mRNA changes of *P. oleae*
115 versus the different treatments with either *C. oleophila* cell suspension or *T. atroviride*-culture filtrate; to this
116 aim, pairwise comparisons (see paragraphs: comparisons 8 and 9) or PCA plots were elaborated.

117 **2.2.1. Comparison 1: intact olive drupes (treatment ID1) vs. wounded drupes treated with sterile distilled**
118 **water (treatment ID2)**

119 Only 243 (192 upregulated and 51 downregulated) differentially expressed genes (DEGs) of the olive fruit
120 were detected after filtering with a false discovery rate (FDR) p-value of 0.001 (Figure 2). The upregulated
121 genes included enzymes involved in secondary metabolite production, particularly berberine bridge enzymes
122 (BBE; LOC111391703 and LOC111391702), a subgroup of the superfamily of FAD-linked oxidases that
123 could either be involved in isoquinone alkaloid biosynthesis or in the oxidation of carbohydrates at the
124 anomeric center to the appropriate lactones. Besides, oligogalacturonides (OGs), small fragments coming from
125 the hydrolysis of pectin-homogalacturonan, represent the best example of damage-associated molecular
126 patterns (DAMP) which activate immunity[36]. However, once the DAMP immunity has been established, the
127 excess of OG accumulation has a negative impact on the plant recovery, which could lead to cell death. BBEs
128 are probably involved in oxidizing oligosaccharide fragments that might be released during mechanical
129 wounds or pathogenesis and could act as DAMPs[37]. Thus, BBE can efficiently neutralize the OGs by oxidation
130 to the lactone counterparts[38], terminating the DAMP activation response[39].

131 Other top DEGs include the ethylene biosynthetic isogenes of 1-aminocyclopropane-1-carboxylate synthase-
132 like (ACC synthases; LOC111390291, LOC111392037, and LOC111370655). Indeed, their enzyme activity
133 is involved in the synthesis of the precursor, 1-aminocyclopropane 1-carboxylic acid (ACC), synthesized from
134 S-adenosyl-L-methionine (SAM) by ACC synthases (ACSs) and finally oxidized to ethylene (ET) by ACC
135 oxidases (ACOs)[40]. It is known that gaseous ET can trigger and enhance host resistance and basal resistance
136 to fungal pathogens[41]. The ET perception and activation of the defense mechanism pass through the ET
137 receptors and ET transcription factors (TFs) that mediate the signal transduction until the ethylene-insensitive
138 EIN TF (EIN2 and EIN3) hubs mediated by the F-box genes (e.g. EBF1 and EBF2)[42]. ET receptors are
139 represented by a family of receptors localized in the (ER) membrane. Two main types of ethylene receptors
140 are known; Ethylene response1 (ETR1) and ERS1, belong to subfamily 1c, and ETR2, EIN4, and ERS2 belong

141 to subfamily 2[43]. Other TFs are activated by ET, such as APETALA2-ethylene responsive factors (AP2-ERFs)
142 which are mediators of stress responses and developmental programs[44]. Many ET-related TFs here were DEGs
143 as the ETR TF 1B-like (LOC111378715) which belong to the AP2 DNA-binding TFs in plants such as
144 APETALA2 and EREBPs[45]. The elicitin INF-1 of *P. infestans* induced several defense responses in *Nicotiana*
145 spp., including reactive oxygen species (ROS) and ET production with consequent hypersensitive cell death
146 (HCD) and accumulation of the sesquiterpenoid phytoalexin capsidiol[46]. In this specific case, the production
147 of phytoalexins is regulated by the AP2/ERF TF, NbERF-IX-33[46].
148 Dirigent (DIR) protein 22-like (LOC111383984) was also a highly upregulated DEG, probably involved in
149 catalyzing regio- and stereoselectivity of bimolecular phenoxy radical coupling during lignin or lignan
150 biosynthesis. Dirigent proteins in plants modulate cell wall homeostasis during abiotic and biotic stress[47].
151 Another top DEG, which is typically expressed after wounding, is beta-amyrin 28-oxidase-like
152 (LOC111396592), a cytochrome CYP716A244 which is implicated in the biosynthesis of oleanane-type
153 saponin biosynthesis[48]. Beta-amyrin synthase and beta-amyrin oxidase were characterized in a yeast, where
154 they were able to form not only α-amyrin 6, but also β-amyrin 1, butyrospermol 23, and Ψ-taraxasterol 19[48].
155 Nonsterol triterpenoids of *O. europaea* are then metabolized into multioxygenated compounds and these could
156 represent the precursors of triterpene saponins[49]. Triterpenoids as well as saponins isolated from various plant
157 organs from different species are known to possess antimicrobial as well as antioxidant activities[50].
158 Among top DEGs, it is noteworthy the presence of elicitor-responsive protein 1-like (LOC111406007), which
159 encodes a protein possessing C2 domains which confer a Ca^{2+}-dependence or binding activity. Usually, such
160 C2 domains bind a wide variety of substances including bound phospholipids, inositol polyphosphates, and
161 intracellular proteins probably acting as signal transduction enzymes. The excess of calcium release during the
162 wound or infection can disrupt the calcium signaling. In *Arabidopsis*, its homologue, NP_001078296.1, is
163 annotated as a calcium-dependent lipid-binding (CaLB domain) family protein, and it acts as a novel repressor
164 of abiotic stress response by chelating the excess free Ca^{2+} [51].
165 Among the innate immunity activated defense genes/proteins which have been detected here as DEGs are
166 protease inhibitors (e.g., glu, ase, PSI, potato proteinase inhibitor I, kunitz trypsin inhibitor 2), endo-1,3-beta-
167 glucosidases, endo- and exo-chitinases, pathogenesis-related protein STH-2-like, thaumatin-like protein,
168 cysteine proteinase inhibitor B-like, and defensin-like protein (Supplementary Table 1).

169 **2.2.2. Comparison 2: *Phytophthora oleae*-inoculated wounded drupes (treatment ID3) vs. sterile distilled**
170 **water-treated wounded drupes (treatment ID2)**
171 About 2466 (854 upregulated and 1612 downregulated) genes were differentially expressed with a FDR p-
172 value of 0.001; top upregulated genes are shown in Figure 2. However, when differentially expressed genes
173 are analyzed by the DESeq2 algorithm, a base PFKM mean is also given. Both log2FoldChange and the
174 baseMean of the FPKM counts are not high enough to have a real biological significance in the differential
175 RNAseq data. Therefore, both log2FoldChange (L2FC) and PFKM (baseMean) have been further filtered by
176 a baseMean value above 2.5 (Figure 3).
177 The top differentially expressed transcripts were mRNA encoding many EXORDIUM-like proteins (e.g.
178 LOC111408846, LOC111402133, LOC111389937, etc.), presumably involved in cell proliferation.
179 EXORDIUM proteins were first identified as mediators of brassinosteroid (BR)-promoted growth in
180 *Arabidopsis*[52], but they can be negatively regulated by exogenous cytokinin[53]. EXORDIUM 2 like (EXL2,
181 Glyma15g06010) was upregulated and classified among the incompatible interaction genes (IIGs) in the case
182 of *P. sojae* infecting soya bean near isogenic lines (NILs) and associated with resistance[54].
183 Another differentially expressed gene was the BON1-associated protein 2 (LOC111391572) which is part of
184 the regulatory complex formed by the BONZAI1 (BON1):BON1-associated protein (BAP1) proteins[55].
185 BON1-associated proteins seem to exert a negative regulator role related to cell death and defense responses.
186 In plants, BON1 and BAP1 protein-protein interaction sends a negative activation signal to a suppressor of
187 npr1-1, constitutive 1 (SNC1), a nuclear R protein[56]. The product of R gene SNC1 represents a hub of different
188 signaling events coming from many different protein receptor kinases at the plasma membrane, which mediate
189 the final event leading to the activation, or not, of plant PTI or effector-triggered immunity[56].
190 Ethylene biosynthesis is also highly upregulated in the case of *P. oleae* infection, since the final enzyme
191 involved in its biosynthesis, the 1-aminocyclopropane-1-carboxylate oxidase 1-like (ACO, LOC111366119),
192 is markedly upregulated as compared to wounded fruit treated with sdw (L2FC≥ 7.6). Two other ACO isogenes
193 were detected as differentially expressed genes (e.g. LOC111370655 LF2C≥4 and LOC111370659
194 L2FC≥1.9), suggesting that this final step of ET biosynthesis occurs to a high degree during infection[57,58].
195 As mentioned before, Ca^{2+} signaling is very active during wounding. Coupled with pathogen attack, Ca^{2+}
196 signaling is further amplified, as inferred by the fact that a higher number of upregulated calcium binding

197 proteins are differentially expressed during *P. oleae* infection: calcium-binding protein CML38-like
198 (LOC111365847), calmodulin-like protein 6 (LOC111406833), calcium-binding protein CML19
199 (LOC111378369), probable calcium-binding protein CML30 (LOC111406832), calcium-binding protein
200 PBP1-like (LOC111402783), calmodulin-binding protein 60 C-like (LOC111409968), and others. In
201 particular, calmodulin-binding protein 60 C-like (CBP60, LOC111409968) and calmodulin-binding protein
202 60 C-like (LOC111370556), as well as the transcription activator SAR DEFICIENT 1-like (LOC111402349)
203 and protein SAR DEFICIENT 1 (SARD1) (LOC111377821) are known to bind directly to the promoter of
204 ISOCHORISMATE SYNTHASE 1 (ICS1), increasing the production of salicylic acid (SA) and/or binding to
205 the promoter of ABERRANT GROWTH AND DEATH2 (AGD2)-LIKE DEFENSE RESPONSE PROTEIN
206 1 (ALD1) and SAR DEFICIENT 4 (SARD4), which leads to the biosynthesis of pipecolic acid (Pip)[59]. In turn,
207 SA and Pip lead to the activation of immune-related genes, such as PATHOGENESIS-RELATED GENES 1
208 (PRs)[60]. CBP60 is also known to bind the promoter region of SARD1, suggesting that it directly promotes
209 SARD1 expression[61]. Activation of PTI and effector-triggered immunity at local infection sites leads to the
210 development of systemic acquired resistance (SAR) in distal parts of the plant, of which SA and Pip represent
211 the signaling molecules[62].
212 Another differentially expressed gene, which encodes the protein SRC2 homolog (LOC111386812), is a
213 calcium binding protein localized in the plasma membrane which possesses a C2 domain. This latter is known
214 to be involved in protein–protein interactions, binding of phospholipids, and targeting of proteins to the
215 membrane in response to Ca^{2+} signaling[63]. However, in the case of pepper infection, when *Phytophthora*
216 *capsici* secretes a protein elicitor 1 (PcINF1) with homology to the elicitor of the hypersensitive reaction (HR),
217 it induces a cell death response in pepper leaves mediated by the SRC2 protein[64]. In this case, the upregulation
218 of olive SRC2 could represent a negative factor for plant immunity, but act as a positive mediator of the *P.*
219 *oleae* INF1 effector by increasing its cell death efficacy.
220 A huge transcriptional remodeling during *P. oleae* infection is suggested by the massive upregulation of olive
221 histones (Supplementary Table 2): histone H3.2 (LOC111381491), histone H2AX-like (LOC111369819),
222 histone H4 (LOC111408016), histone H2AX-like (LOC111379587), and histone H2B.5-like
223 (LOC111399700). Genome remodeling via epigenetics (changes in methylation level) and transposases
224 activity are known to occur during pathogen attack and the development of plant immunity[65]. In plants,

225 acetylation of histones H3 and H4 (H3ac and H4ac), trimethylation on lysine residues, and mono-
226 ubiquitination of H2B (H2Bub1) are normally signs of heterochromatin formation with enhanced
227 transcriptional activation[66]. Transcriptional remodeling and genome rearrangement including retrotransposon
228 jumping within the chromatin play a central role in plant defense mechanisms[67,68]. Based on *de-novo* synthesis
229 of histones alone, it cannot be possible to discern their epigenetic post-translational modification, but certainly,
230 artificial treatment of plants with SA induces acetylation of histones H3 and H4[69].
231 Other differentially expressed genes are the product of the genes encoding olive E3 ubiquitin-protein ligases.
232 Some of those could be the homologue of Arabidopsis RING E3 ligase HUB1 which mediates histone mono-
233 ubiquitination with the subsequent activation of heterochromatin and probably contributes to enhancement of
234 the defense mechanism[70].
235 That genome rearrangement could be linked to the induction of plant resistance that is evident when DDE-type
236 transposases (containing a DD[E/D]-motif) are involved[71]. In this study, it is highly probable that *P. oleae*
237 induced a high expression of the protein ALP1-like (LOC111383726), which possesses a N-terminal RNase
238 H domain, a DNA binding domain and a known transposase DDE C-terminal domain. Demethylation of
239 transposable elements (TEs) makes them jump in heterochromatin genes, which, in this case, could be
240 associated with mediating a 'long-term memory', since their insertion into the promoter of these genes makes
241 them constitutively expressed. This epigenetic stress memory can be transmitted to subsequent generations
242 and involves changes in the silencing of transposable elements (TEs) by DNA methylation, histone
243 modifications, and non-coding RNAs. At late infection stages, the DNA jumping activities of functional class
244 I ('copy and paste') and class II ('cut and paste') TEs, might result in small and large mutations at the sites of
245 excision (class 2) and insertion (class 1 and 2). TE-induced insertion/excision accelerates the evolution of
246 novel defense regulatory genes and, because TEs are somewhat regulated by epigenetic mechanisms, the
247 derived defense genes will remain under stress-dependent epigenetic control and potentially able to resist to
248 biotic stresses[72].
249 Transcription factors (TFs) known to be involved during both wound response and pathogen attack are the
250 WRKY TFs[73]. These TFs contain a 60-70 amino acid protein domain composed of a conserved WRKYGQK
251 motif and a zinc-finger region (C2HC). Both domains contribute to the capability of DNA to bind with W-box
252 and/or other cis-acting elements, such as in R-genes[74] or biosynthetic genes related to specialized metabolism[75].

253 Upon *P. oleae* infection, it has been recorded an upregulated (L2FC≥ 3.4) olive WRKY TF 33
254 (LOC111405948) homologue of AtWRKY33, which functions as a positive regulator of resistance toward
255 some necrotrophic fungi[76]. However, the olive WRKY TF 18-like (LOC111377241) was even more
256 differentially expressed (L2FC≥ 4.6) and, according to the homology function with AtWRKY18, could
257 enhance certain defense signaling, boosting the transcription of PR genes and resistance against certain
258 pathogens[77]. Other upregulated WRKY TFs include WRKY TF 53 (LOC111367778. L2FC>6.1), WRKY
259 transcription factor 23 (LOC111388449, L2FC>4.7), WRKY transcription factor 70 (LOC111392938,
260 L2FC>4.3), and WRKY transcription factor 57 (LOC111373803, L2FC>1.2).
261 The main differentially upregulated R-genes were the LEAF RUST 10 DISEASE-RESISTANCE LOCUS
262 RECEPTOR-LIKE PROTEIN KINASE-like 1.1 (LOC111390435, L2FC>5.2), the protein ENHANCED
263 DISEASE RESISTANCE 2 (LOC111389201, L2FC>5), the disease resistance protein At5g63020
264 (LOC111397442, L2FC>5), receptor-like protein kinase HERK 1 (LOC111407351, L2FC>5.5;
265 LOC111407714, L2FC>2.3), the rust resistance kinase Lr10-like (LOC111411655, L2FC>3.6), the disease
266 resistance RPP13-like protein 1 (LOC111400669, L2FC>3.6), the disease resistance protein RGA1
267 (LOC111392054, L2FC>3.2), and the disease resistance RPP13-like protein 4 (LOC111379833, L2FC>2.2).
268 These plasma membrane-located receptors possess a nucleotide-binding site–leucine-rich repeat (NBS-LRR)
269 and a protein kinase C domain for intracellular signal transduction and are probably involved in the signaling
270 cascade of pathogen sensing[78]. On the other hand, upregulated F-box/LRR-repeat proteins might be associated
271 with resistance to *Phytophthora* species, since for instance, in cacao (*Theobroma cacao*), similar genes were
272 differentially upregulated in response to *P. megakarya* only in a resistant cultivar[79]. Here, upregulated F-
273 box/LRR-repeat proteins were represented by F-box/FBD/LRR-repeat protein At1g13570-like
274 (LOC111386658) and the F-box/kelch-repeat protein At1g67480-like (LOC111406244) (Supplementary
275 Table 2). Other differentially expressed TFs during *P. oleae* infection are the ethylene-responsive TFs
276 ERF104-like (LOC111386758), ERF017-like (LOC111373863), ERF017-like (LOC111373875), ERF118-
277 like (LOC111403203), ERF071-like (LOC111406367), RAP2-3-like (LOC111403560), ERF4-like
278 (LOC111395465), and ERF04 (LOC111381486). Ethylene induced immunity involves sensing and TF-
279 responsive transcription; this ET-pathway interacts both positively and negatively with the SA-pathway, in
280 relation to the type of plant and pathogen[80]. The APETALA2 (AP2)/ETHYLENE RESPONSE FACTOR

281 (ERF) transcription factor ORA59 is a major positive switch of the ET/JA-mediated defense pathway in *A.*
282 *thaliana*. In *Arabidopsis*, biosynthetic genes responsible for the formation of hydroxycinnamic acid amides
283 (HCAAs), known as phytoalexins, are regulated by ORA59 and other ET-responsive TFs[81].
284 Jasmonic acid (JA) and its precursors and derivatives, referred to as jasmonates (Jas), are important molecules
285 in the regulation of plant responses to biotic and abiotic stresses[82]. JA-dependent defense responses are
286 predominantly effective against necrotrophic pathogens and their downstream signaling pathway is typically
287 targeted by fungal effectors to prevent the expression of JA-related defense genes. Coronatine-insensitive
288 protein 1 (COI1) is an F-box protein essential for all the jasmonate responses. COI1 interacts with multiple
289 proteins to form the SCF:COI1:E3 ubiquitin ligase complex which recruits jasmonate ZIM-domain (JAZ)
290 proteins for degradation by the 26S proteasome. *Phytophthora sojae* uses an RXLR effector, Avh94, to
291 manipulate host JA signaling to promote infection hijacking-JA signaling[83]. Zhao et al. have demonstrated that
292 Avh94 interacts with soybean JAZ1 or JAZ2, which is a repressor of jasmonic acid (JA) signaling[83]. Methyl
293 jasmonate (MeJA)-triggered JAZ1 protein degradation could be inhibited by the proteasome inhibitor MG132,
294 suggesting that JAZ1 is consistently degraded by the 26S proteasome. Avh94 bind JAZ1 and protect it against
295 methyl jasmonate-induced degradation. In turn, the JAZ proteins act as repressors of JA TFs such as AtMYC2,
296 which is involved in the induction of JA biosynthetic genes. In the case of *P. oleae* infection, the coronatine-
297 insensitive protein 1-like (LOC111390662) is a differentially expressed gene and could represent a jasmonate
298 receptor. The highly differential expression of COI1 is accompanied by other upregulated differentially
299 expressed genes encoding F-box proteins: F-box protein At3g07870-like (LOC111409106), F-box protein
300 At1g61340-like (LOC111407155), and F-box protein PP2-A12-like (LOC111408420).
301 Among differentially expressed genes related to the PR proteins, in the case of *P. oleae* infection, two genes
302 are predominating, the PR proteins STH-2-like (LOC111388408, L2FC≥5) and STH-1-like (LOC111388885,
303 L2FC≥2.3). LOC111388408 was a gene upregulated in response to wounding, but it is even more upregulated
304 in response to the pathogen. Furthermore, another upregulated gene, as compared to the wound condition itself,
305 is the cysteine proteinase inhibitor B-like (LOC111390855, L2FC≥6) and the cysteine proteinase inhibitor A-
306 like (LOC111411928, L2FC>3) which could act in defense, trying to block several *P. oleae* cysteine proteases.
307 An upregulated gene related to the apoplastic immunity/defense protease was the cysteine protease RD19B
308 (LOC111394575) of the *O. europaea* homologue of RD19, an *Arabidopsis* cysteine protease that modulates

309 RESISTANT TO RALSTONIA SOLANACEARUM 1-R (RRS1-R)[84]. Other differentially expressed genes
310 related to defense response, from highest to lowest L2FC, respectively, are pollen allergen Che a 1-like
311 (LOC111394816, L2FC≥4.7), subtilisin inhibitor CLSI-I-like (LOC111374455, L2FC≥4.5), and kirola-like
312 proteins (LOC111411103, and LOC111410001). On the other hand, a defense response can be represented by
313 the upregulated expression of proteases such as aspartic proteinase Asp1-like (LOC111394357, L2FC>2.2),
314 subtilisin-like protease SBT1.6 (LOC111374885, L2FC>2.1), and SBT3.15 (LOC111386418, L2FC>1.7).
315 For most of the common differentially expressed genes detected in this comparison, a higher level of
316 upregulation was generally observed. Importantly, tetraspanin-2 (LOC111399506) and tetraspanin-8-like
317 (LOC111403728, and LOC111383284) are genes related to the wounding condition itself (Figure 1).
318 Tetraspanins are transmembrane specific proteins of extracellular vesicles (EVs) or exosomes (EXOs)[85], which
319 possess the function of cargo vesicle shipping molecules involved in plant–pathogen interaction. Exosomes
320 transport lipids, proteins (e.g. PR proteins), messenger RNA, and especially microRNAs[86]. Silencing of the
321 pathogens by miRNA is a recent trend in pathogen resistance[87]. Indeed, plant EVs inhibit fungal growth and
322 virulence by transferring their cargo content into fungal cells at the appressoria/haustoria[88]. Another role of
323 tetraspanins is in moving the EVs through infection hot spots and allowing the exosomal NADPH oxidase
324 (NOX) to release ROS molecules locally (e. g. H_2O_2 production).
325 It is also worth mentioning the differential expression of the gene encoding for the receptor-like protein kinase
326 FERONIA (FER; LOC111374777, L2FC≥5.5) (Supplementary Table 2). FER negatively regulates plant
327 immunity by inhibiting JA and coronatine (COR) signaling. It phosphorylates and destabilizes MYC2, the
328 master regulator of JA signaling, which otherwise activates SA biosynthesis[89]. FER, in response to abiotic and
329 biotic stress, acts as a sensor of cell wall integrity which could be challenged by pathogen secreted enzymes[90].
330 FER may negatively regulate plant immunity to biotrophic pathogens[91]. The plant rapid alkalinization factor
331 (RALF) are secreted peptides that were first identified through their ability to trigger a rapid increase in
332 extracellular pH [a]. Their mechanism of action is through binding to FERONIA, that once activated triggers
333 extracellular alkalinization, to favor pathogenic subtiliases (alkaline proteases) or alkaline cysteine proteases
334 and inhibits the defense response[91].

335 **2.2.3. Comparison 3: wounded olive drupes pre-treated with *C. oleophila* and inoculated with *P. oleae***
336 **(treatment ID5) vs. wounded olive drupes inoculated with *P. oleae* (treatment ID3).**

337 A total of 1883 (1018 upregulated and 865 downregulated) olive differentially expressed genes were detected
338 in this comparison (Supplementary Table 3). It is known that *C. oleophila* secretes various cell wall-degrading
339 enzymes, exo-β-1,3-glucanases, chitinases, and proteases, which are especially active against filamentous
340 fungi[92]. *C. oleophila* has an *in vitro* effect on the inhibition of mycelial growth of several *Phytophthora*
341 species[93]. Due to this beneficial effect, some of the genes in *O. europaea* related to the pathogen response
342 could be downregulated, since the pre-treatment ameliorates the pathogenic index (rAUDPC = 0.21; Figure 1,
343 IDC). Thus, the downregulated genes, in this comparison, could represent the olive genes that help to
344 counteract the pathogen invasion, or which are no longer needed since the pathogenic effect of *P. oleae*
345 decreased due to *C. oleophila* pre-treatment. Among the top downregulated genes with L2FC>-21 were ALA-
346 interacting subunit 1-like (LOC111368552), the ras-related protein Rab7 (LOC111380462), and protein
347 YIF1B-like (LOC111391136), all localized into the Golgi apparatus, regulating endomembrane dynamics and
348 involved in secretory vesicle formation[94]. In the specific case here, RABs, which are the largest family of small
349 guanosine triphosphate (GTP)-binding proteins, are involved in intracellular trafficking and in autophagy or
350 plant microbe interactions, and in biotic and abiotic stress responses[94], for instance, the secretion of
351 PATHOGENESIS-RELATED 1 (PR1) protein to the apoplast. *Phytophthora capsici* RxLR effector RxLR242
352 has been found to bind RABE1-7, a ras-related protein, and inhibits vesicle trafficking[95]. This detected decrease
353 of vesicle trafficking is accompanied by the strong downregulation of the WRKY TF 18-like (LOC111377241)
354 here with L2FC>-8.5 instead of +3.5 as for *P. oleae* infection itself. Otherwise, WRKY 18, as mentioned,
355 could have further enhanced another defense signaling[75].
356 One of the possible mechanisms by which *O. europaea* counteracts *P. oleae* infection is through synthesis of
357 sesquiterpenoid phytoalexins. In potato challenged by *P. infestans*, it has been found that the infected tissue
358 produces antimicrobial sesquiterpenoid phytoalexins (*e.g.* lubimin and rishitin) which might have been
359 implicated in a resistance mechanism in some cultivars[96]. Here, there is a clear downregulation of
360 sesquiterpenoid phytoalexin biosynthesis when drupes are pre-treated with *C. oleophila* and inoculated with
361 *P. oleae* (treatment ID5) as compared to drupes inoculated with *P. oleae* (treatment ID3) (Supplementary Table
362 3). Vetispiradiene synthases or sesquiterpene cyclases and cytochrome P450 premnaspirodiene oxygenases
363 catalyze several key biosynthetic steps in the synthesis of antimicrobial (phytoalexins) sesquiterpenes[97].
364 Indeed, the expression of olive vetispiradiene synthase 2-like (LOC111371938, L2FC>-7.1), vetispiradiene

365 synthase 2-like (LOC111372151, L2FC>-7), premnaspirodiene oxygenase-like (LOC111369993, L2FC>-6.5;
366 LOC111383354, L2FC>-5.9; LOC111375139, L2FC>-5.2; LOC111374442, L2FC>-4.6), vetispiradiene
367 synthase 2-like (LOC111394812, L2FC>-6.3), vetispiradiene synthase 2-like (LOC111394298, L2FC>-5.7),
368 and (-)-isopiperitenol/(-)-carveol dehydrogenase (LOC111388383, L2FC>-5.7; LOC111388380, L2FC>-5.0)
369 clearly points to the production of sesquiterpenoid defense compounds. These enzymes are upregulated in
370 treatment with the only inoculation of *P. oleae* (ID3) (Supplementary Table 3) but also during *P. infestants*
371 infection in potato. Associated with decreased infectivity of *P. oleae* due to *C. oleophila* pre-treatment,
372 production of these phytoalexins is downregulated. However, the amount of phytoalexins produced also
373 depends on the amount of the precursor mevalonate and hence, the expression level of 3-hydroxy-3-
374 methylglutaryl CoA reductase (HMGR)[98].
375 Lignan biosynthesis is also regulated differentially from that occurring during wounding and plant defense
376 responses in this comparison. Many phytoalexins in plants are represented by complex dimeric substituted
377 lignan[99]. Dimerization of two coniferyl alcohol units produces pinoresinol, which is then reduced to
378 lariciresinol and secoisolariciresinol by pinoresinol/lariciresinol reductase (PLR), and finally into matairesinol
379 by secoisolariciresinol dehydrogenase (SIRD)[100]. Here, opposite to what was observed in olive drupes only
380 inoculated with *P. oleae* (treatment ID3) (Supplementary Table 2), the biosynthetic enzymes of the lignan
381 biosynthesis are also downregulated with *C. oleophila* co-infection: dirigent protein 22-like (LOC111374881,
382 L2FC>-3.5), dirigent protein 19-like (LOC111376314, L2FC>-3), dirigent protein 22-like (LOC111383984,
383 L2FC>-2), and the secoisolariciresinol dehydrogenase-like (LOC111412270, L2FC>-3.6; LOC111412278,
384 L2FC>-1.3) (Supplementary Table 3).
385 On the other hand, *C. oleophila* pre-treatment also triggered the upregulation of beneficial genes/proteins that
386 help to counteract the *P. oleae* infection. For instance, the PR proteins STH-21-like (LOC111388400) and
387 STH-2-like (LOC111388410) showed L2FC≥1.09 and =1.7, respectively, but baseMean (PFKM) counts of
388 4032 and 2646. This means that even a small log2-fold increase with elevated high expression counts could be
389 significant if the *p*-value and *p*-adj value are small. The lowest *p*-value and *p*-adj value of the upregulated
390 mRNA/genes during *C. oleophila* co-infection were also filtered by high baseMean and L2FC (Figure 4).
391 Many differentially expressed genes related to defense proteins are also upregulated, such as proteinase
392 inhibitor PSI-1.2-like (LOC111373900), xylanase inhibitor thaumatin-like protein 1b (LOC111367584),

393 chitinase hevamine-A-like (LOC111402962), basic endochitinase-like (LOC111379188), aquaporin PIP1-3-
394 like (LOC111408442), TIP1-1 (LOC111403122), and PIP2-2-like (LOC111372815) (Figure 4,
395 Supplementary Table 3). The involvement of the aquaporins (AQPs) is not immediate, but it has been shown
396 that they also transport H_2O_2[101]. Hydrogen peroxide acts as a ROS signaling molecule and can be produced by
397 various oxidases, superoxide and superoxide dismutase, plasma membrane NADPH oxidases, and peroxisomal
398 oxidases, by electron transport in chloroplasts and mitochondria, or by other apoplastic oxidases. In wheat
399 (*Triticum aestivum* L.), TaPIP2;10 transports the pathogen-induced apoplastic H_2O_2 into the cytoplasm of the
400 infected cell and this signal induces PTI immunity[102]. Consequently, in this comparison, the olive AQPs
401 expressed here might have a function of stimulating the H_2O_2 signaling and accelerate the PTI response.
402 Besides, the non-symbiotic hemoglobins have been implicated in the scavenging of nitric oxide signaling[103].
403 In this comparison, the possible role of the upregulation of the non-symbiotic hemoglobin 2-like
404 (LOC111376313) could be that of neutralizing the excess of NO produced during the pre-treatment
405 (Supplementary Table 3).

406 **2.2.4. Comparison 4: wounded olive drupes pre-treated with *Trichoderma atroviride*-culture filtrate and**
407 **inoculated with *Phytophthora oleae* (treatment ID4) versus wounded drupes inoculated with *P. oleae***
408 **(treatment ID3).**

409 A total of 1757 (914 upregulated and 843 downregulated) olive DEGs differentially expressed genes were
410 detected in comparison 'olive drupes pre- treated with *Trichoderma atroviride*-culture filtrate (treatment ID4)
411 and inoculated with *Phytophthora oleae*' versus 'olive drupes inoculated with *P. oleae* (treatment ID3)'
412 (Supplementary Table 4). Biocontrol fungi, such as *Trichoderma* spp., are known to induce systemic resistance
413 (ISR) and prime their host plants to become more resistant to future attack from pathogenic microorganisms[104].
414 For example, *Trichoderma viride* produces the peptide antibiotic alamethicin[105], that forms PM-pores and
415 alters the membrane potential and ion permeability, and acts against other fungi or bacteria[106]. The *T.*
416 *atroviride*-culture filtrate used in this study was checked by proteomics for evaluating its composition in
417 proteins. To this aim, the culture filtrate was subjected to Liquid Chromatographic – Mass Spectrometry (LC-
418 MS) proteomic analysis and the top abundant proteins were identified (Table 1).

419 The most abundant protein was the Epl1 elicitin, which is a member of the Cerato-platanin family that contains
420 several phytotoxic proteins of about 150 AA residues. Cerato-platanin contains a cysteine-rich domain (four

421 cysteine residues that form two disulfide bonds). *Trichoderma viride* and *T. atroviride* secrete the proteins
422 Sm1 (XP_013943617.1) and Epl1 (XP_013937770.1), respectively, which elicit local and systemic disease
423 resistance in plants[107]. Deletion of epl1 in *T. atroviride* resulted in reduced systemic protection against
424 *Alternaria solani* and *Botritis cinerea*, whereas the *T. virens* sm1 KO strain was less effective in protecting
425 tomato against *Pseudomonas syringae* and *B. cinerea*. Upregulation of epl1 and sm1 led to enhanced basal
426 resistance in tomato i.e., systemic acquired resistance (SAR) and induced systemic resistance (ISR)[108].
427 Results from this study suggest that the impact of *T. atroviride*-culture filtrate pre-treatment on *P. oleae* was
428 significative, as it has been observed the lowest reduction in pathogenic index (rAUDPC = 0.19; Figure 1,
429 IDB). Furthermore, RNAseq results strongly suggest that the pre-treatment of olive drupes with this culture
430 filtrate determined a marked reduction of expression of the *P. oleae* infection marker proteins.
431 The data in Figure 5 many pairwise comparisons have been selected and filtered according to the most
432 significant upregulated differentially expressed genes (lane 7 describe the comparison 4).
433 By associating differentially expressed genes resulting from comparison 4 with results from LC-MS analysis,
434 it can be speculated that the pre-treatment of olive drupes with of *T. atroviride*-culture filtrate alerts the plant
435 tissues to pathogen attack. Indeed, one of the top upregulated gene, the HR protein-like protein 2, OeHIR-2
436 (LOC111404549) (Supplementary Table 4), is responsible for alerting the tissues[107]. In this study, two F-
437 box/LRR-repeat proteins 14 (LOC111382434) and (LOC111405202) were also upregulated, but not in
438 stoichiometric amounts as for OeHIR-2 (LOC111404549) (Supplementary Table 4). Therefore, OeHIR-2
439 should instead be responsible for the disease resistance response, perhaps triggered by *T. atroviride* culture
440 filtrate, which gave the lowest pathogenicity index in this comparison.
441 Unexpectedly, there were two possibly slightly upregulated resistance genes: LRR, leucine-rich repeat
442 receptor-like serine/threonine-protein kinase homologue of At1g06840 (LOC111371641) and MLO-like
443 protein 8 (LOC111401243) (Supplementary Table 4). Using the Arabidopsis eFP Browser[109], it is possible to
444 visualize that the expression of At1g06840 mRNA is very high 48 hpi after *Botrytis cinerea* inoculation of
445 leaves. MLO-like protein 8 (LOC111401243) is also overexpressed (Figure 5; Supplementary Table 4).In the
446 barley, the *Mlo* gene, is involved in particular mutation(s) that can cause the misfunction of the MLO protein,
447 resulting in a correlation with resistance to the fungal pathogen *Erysiphe graminis* f. sp. *hordei*[110]. It can be

448 inferred that the same mutations are also present in olive *MLO* germplasm and if those contribute to the
449 partial/total resistance to Oomycetes.

450 **2.2.5. Comparison 5: overall differential mRNA expression of olive genes**

451 Following the pairwise comparisons of olive mRNA in the previous conditions (Figures 2, 3, 4 and 5), it was
452 performed, in addition, the overall changes in mRNA, better summarized by cluster analysis (Figure 6). The
453 following three groups of treatments were used for this cluster analysis: (i) the control groups, including
454 wounded drupes treated with 20 µl of sterile distilled water (treatment ID2), wounded drupes treated with
455 either *T. atroviride*-culture filtrate (treatment ID6) or *C. oleophila* cell suspension (treatment ID7); (ii) olive
456 drupes inoculated with *P. oleae* (treatment ID3); and (iii) *P. oleae*-inoculated olive drupes pre-treated with
457 either *T. atroviride*-culture filtrate (treatment ID4) or *C. oleophila* cell suspension (treatment ID5). In olive
458 drupes inoculated with *P. oleae* (ID3), 739 olive genes were exclusively upregulated (in red in Figure 6) (L2FC
459 > 1) (Figure 6B; Supplementary Table 5).

460 The general trend of top upregulated genes triggered by *P. oleae* are described above (Figs. 3 and 4). However,
461 the plant response to the attack of the oomycete, even though this strain of *P. oleae* could not be fully blocked,
462 showed a differential defense response as indicated by the relative expressions of the pathogenic related (PR)
463 proteins (Supplementary Figure 1).

464 The expression level of PR proteins has been evaluated based on their L2FC (Supplementary Table 6), and
465 their overall comparison are shown in Supplementary Figure 1. The PR proteins were selected by blasting with
466 PR reported in the literature[111]. For the class of PR-1, results obtained here strongly suggest that some of them
467 are pre-activated by *T. atroviride*-culture filtrate or *C. oleophila* cells pre-treatment (e. g. LOC111385823,
468 LOC111385823, and LOC111384285); however, only the basic form of pathogenesis-related protein 1-like
469 (LOC111384285) remained expressed in drupes only inoculated with *P. oleae* (treatment ID3) or when the
470 pre-treatment with *C. oleophila* was also provided (treatment ID5) (Supplementary Figure 1). For β-1,3-
471 glucanase (PR-2), the trend is more complex, being LOC111378392 the only one shared among the treatments
472 and that could represent a threat to the target oomycete. The expression levels of the olive chitinases (PR-3,
473 PR-4, PR-8, and PR-11) seem to have a better trend: the chitinase 10 (LOC111407547) and endochitinase
474 EP3-like (LOC111393512) upregulation during the treatments with either *C. oleophila* or *T. atroviride*-culture
475 filtrate seems to be a marker for the PR-3 weapons against the oomycete.

476 Among the PR-5 highly induced by *P. oleae* infection, the pathogenesis-related protein 5-like
477 (LOC111397298) and the thaumatin-like protein (LOC111398526) were markedly differentially
478 overexpressed (Supplementary Figure 1), but the pre-treatment with either *C. oleophila* or *T. atroviride*-culture
479 filtrate slightly contributed to trigger the expression of the thaumatin-like protein (LOC111398526). The group
480 of PR-6 includes important proteinase inhibitors, such as the cysteine protease inhibitors (relevant for blocking
481 fungal cysteine proteases) and other enzyme inhibitors related to different classes of enzymes involved in the
482 defense response[112,113]. Among these PR-6, as shown in Supplementary Figure 1, the polygalacturonase
483 inhibitor LOC111382121 is the main PR-6 gene transcribed in response to fungal polygalacturonase. In this
484 study, *C. oleophila* resulted to be an effective inducer of the expression of this gene, in contrast with *T.*
485 *atroviride*-culture filtrate treatment. Neither *C. oleophila* or *T. atroviride*-culture filtrate could trigger the
486 expression of the cysteine proteinase inhibitor B-like (LOC111390855), which was only induced by *P. oleae*
487 infection itself. On the other hand, a set of inhibitors (proteinase inhibitor PSI-1.2-like LOC111390049 and
488 LOC111373900, pectinesterase inhibitor 9-like LOC111377531, and kunitz trypsin inhibitor 2-like
489 LOC111405411), including the cysteine proteinase inhibitor A-like (LOC111412304), were exclusively
490 induced by *C. oleophila*.
491 Among the subtilisin-like protease (PR-7), serine protease belonging to the peptidases_S8 subfamily, which
492 are already upregulated upon wounding alone, the highest upregulation was recorded for the gene encoding
493 the subtilisin-like protease SBT1.9 (LOC111394370) in all the treatments. Previous studies demonstrated that
494 the tomato P69 subtilase, which is a subtilisin, activates plant immunity since it can cut the pathogen-secreted
495 apoplastic "small cysteine-rich secreted protein" PC2[114]. Downstream fragments are alerting the plant
496 immunity by intracellular signaling. To evade the plant surveillance system, *Phytophthora* may have evolved
497 protease inhibitors, such as the Kazal-like, protease inhibitors (EPIs) to block PC2 extracellular processing.
498 Furthermore, *Phytophthora* may secrete cytoplasmic effectors to block the secretion of proteases (i.e.,
499 subtilisins and cysteine proteases) into the apoplast and, indirectly, block PC2-triggered immunity[115,116]. For
500 PR-8, the acidic endochitinase-like (LOC111391829) was the main upregulated PR protein in response to *P.*
501 *oleae* or *C. oleiphila* alone as compared the wound control. However, when both *P. oleae* and *C. oleophila*
502 were present then it was downregulated. Other PR-8 expressed were hevamine-A-like (LOC111402962 and

503 LOC111402963). The upregulation of PR-8 alone is hence not impressive to claim an active role in contrasting
504 the infection.
505 The PR-9 proteins are lignin-forming peroxidases that fortify the cell wall by increasing the crosslinking of
506 lignin. Results obtained here evidence that *P. oleae* infection determined the up-regulation of many PR-9
507 peroxidases (Supplementary Figure 1), but the treatment with either *C. oleophila* or *T. atroviride*-culture
508 filtrate also contributed to increase the PR-9 expression level, especially for the lignin-forming anionic
509 peroxidase-like (LOC111385717).
510 The PR-10 class is quite versatile in their biological action due to a loop that has small-chemical binding
511 capabilities and diverse role in stress signaling[117]. In this study, the upregulation of some of them (e.g.
512 pathogenesis-related protein STH-2-like - LOC111388404, LOC111388408, LOC111388411, and
513 LOC111388413) is significative in olive drupes pre-treated with either *C. oleophila* or *T. atroviride*-culture
514 filtrate as compared to olive drupes inoculated with *P. oleae* (Supplementary Figure 1). The highly expressed
515 PR-11 are the acidic mammalian chitinases-like (LOC111382479, LOC111382480, and LOC111382484), but
516 their expression is controversial, since wounding itself can trigger their higher expression and*T. atroviride*
517 culture filtrate downregulated their expression.
518 Among the PR-12 class, the defensins-like protein 1 were not expressed. Some genes encoding PR-13 and PR-
519 14 showed a slight upregulation in all the treatments.
520 The germin-like oxalate oxidases (PR-15) were also downregulated by *P. oleae* infection, even though
521 treatment with either *C. oleophila* or *T. atroviride*-culture filtrate had the effect of upregulating some of them
522 (Supplementary Figure 1).
523 Finally, the expression of PR-17 in olive drupes, which include important zinc apoplastic metalloproteinases,
524 was relevant for the LOC111379582 and LOC111391744 in case of olive drupes only inoculated with *P. oleae*
525 (treatment ID3) or drupes only pre-treated with *C. oleophila* (treatment ID7). Unfortunately, the combination
526 of the pretreatment with either *C. oleophila* or *T. atroviride*-culture filtrate and *P. oleae* infection had the effect
527 of reducing their expression (Supplementary Figure 1).
528 **2.2.6. Comparison 6: wounded olive drupes pre-treated with *C. oleophila* cell suspension (treatment ID7)**
529 **versus wounded drupes pre-treated with sterile distilled water (treatment ID2)**

530 In olive drupes, the treatment with *C. oleophila* (treatment ID7) triggered the differential upregulation of the
531 lignin/lignan biosynthetic pathway, possibly to fortify the cell wall and produce anti-fungal compounds.
532 Indeed, a lot of secoisolariciresinol dehydrogenase genes (LOC111412681, LOC111412273, LOC111412272,
533 and LOC111412275) were overexpressed (**Supplementary Table 7)**. Two kunitz trypsin inhibitors
534 (LOC111405411 and LOC111373900), two basic endochitinases (LOC111403475 and LOC111379188), and
535 two pathogenesis-related proteins STH-like (LOC111388400 and LOC111388410) were highly upregulated
536 as compared to the wound condition (treatment ID2). Meanwhile, the upregulation of metallothioneins
537 (LOC111369652, LOC111375946, LOC111385831, and LOC111385129), thioredoxins (LOC111370790
538 and LOC111370789) and aquaporins (LOC111368643, LOC111408442, LOC111368644, and
539 LOC111368645) might help the olive drupe to sustain a high level of ROS scavenging intracellularly and ROS
540 export to the extracellular environment, such as superoxide (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl
541 radical (•OH) [118,119].

542 **2.2.7. Comparison 7: wounded olive drupes pre-treated with *T. atroviride*-culture filtrate (treatment**
543 **ID6) versus wounded drupes pre-treated with sterile distilled water (treatment ID2)**

544 It is known that *Trichoderma* spp. produce metabolites able to trigger plant defense response against fungal
545 pathogens[35,120,121]. In olive drupes pre-treated with *T. atroviride*-culture filtrate only 60 genes were upregulated
546 and 176 downregulated (Supplementary Table 8). As for the treatment ID3 (*P. oleae* infection alone) also here
547 the biosynthetic enzymes of the lignan biosynthesis was highly upregulated in particular the
548 secoisolariciresinol dehydrogenase-like genes with L2FC≥3 (LOC111412681, LOC111412273,
549 LOC111412272).In addition, polygalacturonase inhibitor (PR-6, LOC111385823), proteinase inhibitor PSI-
550 1.2-like (LOC111390049), acidic glucan endo-1,3-beta-glucosidase (LOC111378392), kirola-like (PR-10,
551 LOC111392831) and a basic endochitinase-like (LOC111379188) were also induced (Supplementary Table
552 8). In this study, it has been also found the induction of ethylene biosynthesis (1-aminocyclopropane-1-
553 carboxylate oxidase-like LOC111370659). The activation of numerous disease-resistance- (Ethylene
554 Responsive Factor, ERF, NAC, bHLH, and STK) and defense-response genes (DRP, ABC, and HSP)
555 following treatment with *Trichoderma* spp. has been reported previously[122]. The NAC transcription factor 56-
556 like (LOC111393458) and the protein DOWNY MILDEW RESISTANCE 6-like (LOC111393985) were also
557 upregulated.

558 **2.2.8. Comparison 8: *P. oleae* differentially expressed genes during olive fruit infection (treatment ID3)**
559 **as compared to its *in vitro* growth in several culture media**
560 About 4513 genes were differentially expressed (2294 upregulated and 2219 downregulated) in *P. oleae* grown
561 during the infection on olive fruits as compared to *P. oleae* grown in PDA, MEA, or Czapek media
562 (Supplementary Table 9). A list of differentially expressed genes, identified as pathogen effectors, is given in
563 Table 2. Most of the recorded effectors, related to the RxLR–dEER domain type, typical oomycete virulence
564 proteins that should be translocated to the cytoplasm of the plant cell[123]. When secreted, RxLR proteins could
565 be internalized by the binding with plant plasma membrane receptors[124].Once internalized some RxLR proteins
566 might interacts with resistance proteins (R proteins), and as a result this binding might trigger an HR and
567 efficient callose deposition [i]. Crinkler (CRN) effectors play a significant role in the pathogenesis by
568 manipulating host cell functions (*i.e.* cell death) to facilitate infection and suppress host immune responses[125].
569 Genes encoding CRN effectors were also abundant and highly upregulated (Table 2). Some of these effectors
570 have been chosen for validation with RT-qPCR over the different time points (see Supplementary Table 15).
571 Elicitins are the main pathogenic effectors in *Phytophthora* species[126]. Elicitin INF-1 represents the main
572 important elicitin because it is known that induces a hypersensitive response (HR) and systemic acquired
573 resistance (SAR)[127]. *P. oleae* possess two genes, g8411.t1 and g8407.t1, both predicted to encode the same
574 proteins but with different length of leader peptide. A database of *P. oleae* proteins was made available as well
575 as its first genome draft and the relative nucleotide and protein sequences at Zenodo
576 (https://doi.org/10.5281/zenodo.11092245). *P. oleae* INF-1 were highly expressed in culture media but
577 downregulated during olive fruit infection maybe to reduce HR and SAR and favor infection (Table 2). Most
578 of the elicitins were downregulated except for a few that were upregulated (g1938.t1, g17483.t1, g18550.t1,
579 g13418.t1, and g20317.t1) (Table 2).
580 As for *P. oleae* proteases and protease inhibitors differentially expressed genes (statistically filtered;
581 FDR=0.001), including cysteine proteases and serine protease types (Peptidase S10, serine carboxypeptidase,
582 and chymotrypsin type), were downregulated. The cystatin cysteine protease inhibitor EPIC and the Kazal-
583 like serine protease inhibitor were also downregulated (Supplementary Table 9). Necrosis inducing protein
584 NPP1 or Nep-1 (g11296.t1) was upregulated while all the remaining homologues were downregulated
585 (Supplementary Table 9).

586 As already mentioned, *Phytophthora* species. also secrete elicitins, whose function is to facilitate the onset of
587 infection and its progress, because they are toxic proteins and most of the time induce necrotic and systemic
588 hypersensitive response (HR) in the infected tissues. The interpretation of the less expressed elicitin protein
589 INF1, known for its high toxicity, is that in certain cases INF-1 is responsible for the high virulence but it is
590 also known to be highly expressed in artificial media as compared to biotroph growth in infected tissues[128].
591 Virulent races of fungal and bacterial pathogens are generally defined by their failure to express particular
592 avirulence genes[129]. However, the loss or lack of elicitin production in *P. parasitica* was shown to increase its
593 virulence on sensitive plants[130]. Here, the downregulation of *P. oleae* INF-1 (g8411.t1 and g8407.t1) and others
594 (g8435.t1, g8431.t1, g8427.t1, g11723.t1, and g8405.t1) might instead have a strategic role to favor its
595 virulence, since, for example, the *in vitro* elicitin production by *Phytophthora* spp. is strongly negatively
596 correlated with their pathogenicity on tobacco[131]. The most upregulated elicitins, instead, were g8411.t1,
597 g8407.t1, g1938.t1, g17483.t1, and g18550.t1 (Supplementary Table 9). Other avirulent effectors were also
598 highly upregulated, like cysteine peptidase C1 domain containing protein (g14657.t1) and cystatin cysteine
599 protease inhibitor EPIC-like (g11549.t1) (Table 2).

600 **2.2.9. Comparison 9: differentially expressed genes of *P. oleae* during olive drupe infection (treatment**
601 **ID3) as compared to olive drupe inoculated with *P. oleae* and pre-treated with either *C. oleophila***
602 **(treatment ID5) or *T. atroviride*-culture filtrate (treatment ID4)**

603 Supplementary Table 10 shows 3716 (2116 upregulated and 1600 downregulated) and differentially expressed
604 mRNAs related to *P. oleae* during pre-treatment of olive drupes with either *C. oleophila* (treatment ID5) or *T.*
605 *atroviride*-culture filtrate (treatment ID4) versus olive drupes infected with *P. oleae* (treatment ID3), 72 hpi
606 (FDR =0.001). Supplementary Tables 11 and 12 show the *P. oleae* differential mRNA expression in the
607 comparisons 'olive drupes pre-treated with *C. oleophila* and inoculated with *P. oleae* (treatment ID5)' versus
608 'olive drupes only inoculated with *P. oleae* (treatment ID3)', and 'olive drupes pre-treated with *T. atroviride*-
609 culture filtrate and inoculated with *P. oleae* (treatment ID4)' versus 'olive drupes only inoculated with *P. oleae*
610 (treatment ID3)', respectively.

611 In the pre-treatment with *T. atroviride*-culture filtrate, the gene-encoding for the elicitin proteins INF-1
612 (g8411.t1 and g8407.t1) have been slightly upregulated as compared to drupes only inoculated with *P. oleae*;
613 however, *P. oleae*-inoculated drupes pre-treated with *C. oleophila* showed a slight downregulation (L2FC= -

614 0.8) (Supplementary Table 11). The differentially expressed elicitin genes that were not down-regulated in *P.*
615 *oleae*-inoculated olive drupes pre-treated with either *C. oleophila* or *T. atroviride*-culture filtrate were
616 g21198.t1, g10684.t1, g17483.t1, g24094.t1, and g4307.t1 (Supplementary Tables 10, 11); other genes-
617 encoding for elicitins were downregulated by pre-treatment with either *C. oleophila* or *T. atroviride*-culture
618 filtrate (e. g. g13418.t1, g1938.t1, g19255.t1).
619 To evaluate the efficacy of the antagonistic effect of the treatment with either *C. oleophila* or *T. atroviride*-
620 culture filtrate versus the infection of *P. oleae* alone and, for comparison purposes, genes, vs. axenic cultures
621 of *P. oleae* grown in PDA, MEA and CZ (CZAPEK) the DESeq2 results at 72 hpi were analyzed using the
622 Principal Component Analysis (PCA)(Figure 7). The sum of all these components (PC) accounted for 76% of
623 the total variance, while PC1 and PC2 represented 53% and 23%, respectively. Although PCA analysis is
624 usually significant when the percentage of variance in the first three components is at least 80%, it may be
625 used to find correlations based on the co-variance matrix. Figure 7 shows that four distinct groups were
626 grouped apart. Specifically, olive drupes inoculated with *P. oleae* were clearly separated from *P. oleae*-
627 inoculated drupes pre-treated with either *C. oleophila* or *T. atroviride*-culture filtrate, as well as from the axenic
628 cultures of *P. oleae* on CZ and MEA media. Only the culture of *Phytophthora oleae* on PDA medium is
629 singularly grouped in the quadrant with negative values on the abscissa and ordinate.
630 Furthermore, several cluster analyses have been performed to evaluate the changes in genes-encoding for a
631 number of pathogenic enzymes and effectors. To this aim, elicitins, crinkler, peptidases, and RxLR effectors
632 LF2C from DESeq2 data expression were chosen (Figure 8). Some examples include peptidases as the cysteine
633 peptidase C1 (g9173.t1), which are downregulated when *P. oleae* is inoculated in olive drupes, but upregulated
634 when either *C. oleophila* or *T. atroviride*-culture filtrate are included as pre-treatment. This could mean that
635 perhaps the cysteine peptidase C1 (g9173.t1) could serve as weapon against other surrounding fungi. An
636 opposite trend was observed for the Peptidase s59 (g4376.t1) (Figure 8A) and for the RxLR effectors (Figure
637 8C). The genes-encoding for the RxLR effector proteins g6343.t1, g19400.t1, g16251.t1, g19132.t1,
638 g24597.t1, g7454.t1, and g6774.t1, for example, were clearly upregulated in *P. oleae*-inoculated and non-
639 treated olive drupes (treatment ID3) but downregulated when either *C. oleophila* (ID5) or *T. atroviride*-culture
640 filtrate (ID4) where provided as pre-treatment (Figure 8C). Most of those highly expressed *P. oleae* RxLR
641 effectors might attenuates the responses to SA like including of PR1 as in the case of *Arabidopsis* infected

642 with *Hyaloperonospora arabidopsidis* (Hpa Emoy2)[132]. In facts, g6343.t1, and g7454.t1 are homologous
643 respectively to HaRxLL465b (BAP69127.1) and HaRxL24b (CCC55787.1) highly expressed in Hpa Emoy2
644 [p]. Besides, *P. oleae* RxLR effector g19132.t1 possesses also one WY motifs, known for being necessary for
645 the infection and RNA silencing suppression activity[133].
646 The trend of the *P. oleae* CRN78 homologous isogenes (g11433.t1, g676.t1, g3199.t1, g21724.t1, g24615.t1)
647 was characterized by an up-regulation when *P. oleae* was inoculated in non-pre-treated olive drupes (L2FC >
648 1.2) (ID3) and by a downregulation in drupes pre-treated with either *C. oleophila* (L2FC<-1.2) (ID5) or *T.*
649 *atroviride* (L2FC<0.4) (ID4) (Figure 6B). The Crinkler effector CRN78 of *P. soja* (XP_009521873.1) is
650 known to enhance oomycete pathogen infection by inhibiting plant immunity through a unique mechanism[134].
651 *P. sojae* effector CRN78 contains a secretion signal peptide (SP), a typical LXLFLAK and HVLVVVP motifs
652 at its N terminal; this latter is predicted to be a serine/threonine kinase domain. It has been demonstrated that
653 CRN78 mediates phosphorylation of NbPIP2;2 at Ser279 and induces its degradation via a 26S-dependent
654 pathway[134]. Since the aquaporin PIP2;2 acts as a H_2O_2 transporter into the apoplast, its degradation
655 substantially decreases extracellular ROS.

656 **2.2.10. RT-qPCR validation of selected genes of *Olea europaea* and *Phytophthora oleae*.**

657 A selection of differentially expressed genes of olive drupes (Supplementary Table 15), as analyzed by
658 RNAseq at 72 hpi, have been validated during the entire time course considered (24, 72, and 168 hpi). Figure
659 9 shows the trend of expression of those genes. At 24 hpi, the gene-encoding for the pathogenesis-related
660 protein STH-2-like (XM_023013103.1, LOC111388413) (Figure 9A) was significantly up-regulated in all the
661 treatments, but in wounded drupes; thus, indicating that this gene is positively transcribed as a consequence of
662 biotic stresses. Furthermore, *P. oleae* inoculated drupes pre-treated with either *T. atroviride*-culture filtrate or
663 *C. oleophila* showed the highest upregulation values. A similar trend was also observed at 72 hpi, although
664 less marked in olive drupes only inoculated with *P. oleae* (ID3) and those also pre-treated with *T. atroviride*-
665 culture filtrate (ID4). Finally, at 168 hpi, the most significant up-regulation of the gene-encoding for the
666 pathogenesis-related protein STH-2-like was recorded in *P. oleae*-inoculated olive drupes pre-treated with *T.*
667 *atroviride*-culture filtrate. Overall,, throughout all the infection period considered, it is constant the higher
668 upregulation of this PR protein in the presence of both *C. oleophila* and *T. atroviride* culture filtrate,
669 phenomenon already known for *T. harzianum* in case of sunflower challenged by *Rhizoctonia solani*[135]. The

670 gene-encoding for the BON1-associated protein 2-like (LOC111391572, XM_023016810.1) (Figure 9B)
671 showed a marked upregulation in drupes only inoculated with *P. oleae* (ID03) at the three time points of
672 measurement. BON1 is a calcium-dependent phospholipid-binding protein interacting with the leucine-rich-
673 repeat receptor-like kinases BIR1 (BAK1-interacting receptor-like kinase 1) and pathogen-associated
674 molecular pattern (PAMP) receptor regulator BAK1[136]. BON1-associated protein participates to the protein-
675 protein regulation of the PAMP signaling of which BAK1 represent a hub of signals functioning as negative
676 regulator role related to cell death and defense responses[137]. Results obtained in this study suggest that the
677 infection by *P. oleae* caused the upregulation of BON1-associated protein, probably suppressing both cell
678 death and reducing the PAMP response in activating the defense response. Notably, all the treatments that
679 included either *C. oleophila* or *T. atroviride*-culture filtrate showed a generalized low up-regulation or a down-
680 regulation. This study also analyzed the trend of the gene-encoding for FERONIA (Figure 9C), an LRK-
681 receptor-like protein kinase (LOC111374777, XM_022997515.1), involved in the plant immunity by
682 inhibiting JA signaling[89]. Hence, the transcription of this gene is supposed to exert a negative function toward
683 *P. oleae* pathogenicity. Results obtained here show that at 24 hpi the FERONIA encoding-gene was
684 significantly up-regulated in *P. oleae*-inoculated olive drupes pre-treated with *T. atroviride*-culture filtrate
685 (ID4) and in olive drupes only pre-treated with *C. oleophila* (ID5); at 72 hpi the modulation in the transcription
686 of the gene was significant just in *P. oleae*-inoculated olive drupes pre-treated with *T. atroviride*-culture filtrate
687 (ID4); at 168 hpi, no-significant differential transcriptions were recorded in drupes from any treatment.
688 As mentioned, the plant small extracellular vesicles (EVs) or exosomes (EXOs) that carry plant defense
689 enzymes, PRs and the ROS generating machine, were supposed accumulating toward the pathogen entrance
690 hot spot. Plant exosomes contain DADPH oxidases, PM-ATPases, and tetraspanins that mediate PM-fusion
691 (unloading of content). Many tetraspanins are involved in EXOs trafficking and here, the gene-encoding for
692 the tetraspanin-8-like (LOC111403728, XM_023032336.1) was chosen as a top differentially expressed gene
693 for RT-qPCR-analysis (Figure 9D). At the time points 24- and 72-hpi, the significant upregulation of the
694 tetraspanin-8-like in drupes only inoculated with *P. oleae* (ID3), was interpreted as the attempt of plant cells
695 to counteract the oomycete pathogen by ROS and PRs. Conversely, the downregulation in *P. oleae* inoculated
696 drupes pre-treated with either *C. oleophila* (ID5) or *T. atroviride*-culture filtrate (ID4), although non-
697 significant, suggests that the presence of either *C. oleophila* or *T. atroviride*-culture filtrate could have

698 downregulated the ROS and PRs mediated mechanism by their own extracellular enzymes/effectors to help
699 the plant immunity by by-passing the exosomes mechanism of defense. This interpretation of the results is
700 supported by the fact that at 168-hpi, drupes only inoculated with *P. oleae* (ID3) exhibited a downregulation
701 of tetraspanin-8-like gene, indicating that the advanced stage of infection could have completely compromised
702 any plant defense response. At this time point, the only significant up-regulation was recorded in olive drupes
703 treated with *T. atroviride*-culture filtrate.
704 In this study, the RT-qPCR validation of some selected *P. oleae* differentially expressed encoding effectors
705 (Supplementary Table 15) at all time points considered was also carried out. As comparison, the mean value
706 of expression level of the *P. oleae* genes grown in artificial media (PDA, MEA, or Czapek) was used.
707 The Crinkler (CRN) gene g723.t1 (TRINITY_DN5906_c2_g5_i1) (Figure 10A) is homologous of the
708 Crinkler effector of *P. soja* (XP_009516255.1) and the Crinkler effector protein 5 of *P. infestans* (Q2M408.1)
709 The CRN proteins are supposed to be secreted by the oomycete into the host apoplast and, by an unknown
710 mechanism, they can penetrate and exert their toxic necrotic function against the host. Results of this study
711 strongly suggest that the presence of either *C. oleophila* (ID5) or *T. atroviride*-culture filtrate (ID4) smoothed
712 the upregulation of the Crinkler (CRN) gene g723.t1, as it is markedly upregulated in the treatment where *P.*
713 *oleae* is alone (ID3) (Figure 10A). A similar trend was recorded for the Elicitin g17483.t1 (Figure 10B). The
714 expression trend of the INF-1 elicitin encoding-genes (g8411.t1 and g8407.t1) (Figure 10C) aligns with the
715 RNAseq analysis findings at 72 hpi. In the treatment where *P. oleae* is alone (ID3), the INF-1 elicitin is down-
716 regulated at 24 hpi. Additionally, at 72 and 168 hpi, its upregulation was significantly lower than in drupes
717 that also included pre-treatment with either *C. oleophila* (ID5) or *T. atroviride*-culture filtrate (ID4). This INF-
718 1 elicitin gene trend in treatment ID3, which overall exhibited consistently lower positive regulation compared
719 to the other two treatments, might be attributed to the potential establishment of an 'infection barrier' by the
720 presence of either *C. oleophila* or *T. atroviride*-culture filtrate. Hence, to overcome this barrier, *P. oleae* might
721 have increased the synthesis of INF-1 elicitin.
722 Another host-translocated effector belonging to the RxLR family, the *P. oleae* g24837.t1
723 (TRINITY_DN8100_c0_g1_i1) (Figure 10D) homologous to HaRxLL438 (BAP69100.1) of *H. arabidopsidis*
724 Emoy2, upregulated upon infection, has also been detected as highly expressed in the case of infection with *P.*

725 *oleae* alone, but downregulated over time following treatments with either *C. oleophila* or *T. atroviride*-culture
726 filtrate.

727 **3. Discussion**

728 This study demonstrated the yeast *C. oleophila* and the culture filtrate of the filamentous fungus *T. atroviride*
729 were effective in reducing the severity of symptoms of soft rot in olive drupes infected by *P. oleae*. To the best
730 of our knowledge, this is the first report of *in planta* efficacy of these treatments, a BCA and a culture filtrate
731 of an antagonistic fungus. Results are promising, also in the light of the transcriptome analysis, indicating the
732 activation of plant immune system by the yeast and culture filtrate tested in this study. This would suggest the
733 possibility that both *C. oleophila* and *T. atroviride* culture filtrate can be effective against a broad spectrum of
734 fruit diseases caused by necrotrophic pathogens. Indeed, *C. oleophila* strain O is already available as a
735 commercial bioproduct registered for treatments of post-harvest fungal rots of several fruit crops, while the
736 practical application of *Trichoderma atroviride* metabolites as natural substances for the biocontrol of plant
737 diseases is almost unexplored. Moreover, the pathosystem *P. oleae*/olive drupe treated with either
738 *Trichoderma* culture filtrate or *C. oleophila* cell suspension proved to be an interesting model to analyze the
739 gene expression in a multiple-actor (pathogen/host plant/treatment) interaction. The transcriptome analysis of
740 this interaction provided a better understanding of the defense responses of olive to stressors, including the
741 abiotic stress provoked by wound inoculation, and the mechanisms underlying the biocidal activity of two very
742 diverse means. The components of the model were chosen and assembled in various combinations based on a
743 rationale. *Phytophthora oleae* is an emergent olive pathogen and the genus *Phytophthora* includes numerous
744 species that are responsible of serious diseases of crop, forest, ornamental and landscape plants. 'Coratina',
745 originating from the Apulia region (southern Italy), is one of the most popular Italian olive varieties for oil
746 production. It is an ancient variety thought to express many wild resistance genes and to be closer to *O.*
747 *europaea* var. *sylvestris* (taxid:158386), the wild relative of *O. europaea* var. *sativa*, in genomic sequence.
748 Hence, RNAseq analysis has been performed using the O_europaea_v1 genome assembly
749 (GCF_002742605.1). As in many studies it was demonstrated the strong efficacy of different strains of *C.*
750 *oleophila* against post-harvest fungal diseases of edible fruit crops such as apples, bananas, citrus and
751 strawberries[93,138], this BCA was selected for the treatment of olive drupes. The choice of evaluating *T.*
752 *atroviride* culture filtrate as an eco-friendly treatment was suggested by the high efficient production of

753 bioactive secondary metabolites in liquid culture with antifungal activity by other *Trichoderma* spp. [22,24,139–]
754 [141]. The elicitation of the plant immune system by liquid culture filtrate of *T. atroviride*, in this study, has
755 perhaps been triggered by the elicitin Epl1 (XP_013937770.1) Cerato-platanin, which was found the most
756 highly expressed protein by proteomics analysis (Table 1). Ep1 potential in triggering plant defense responses
757 is well known[107,108].
758 The time course of olive rot severity for each treatment, as represented by rAUDPC, is in somewhat in
759 agreement with the PCA plot (Figure 7), which shows the variance of the expression of genes involved in
760 pathogenicity as compared to the *P. oleae* growth in artificial medium (baseline or zero variance) or during
761 infection (positive variance) or infection and pre-treatment with either *C. oleophila* or *T. atroviride*-culture
762 filtrate (negative variance). In both cases, pre-treatment with *T. atroviride*-culture filtrate had the effect of
763 attenuating the virulence of *P. oleae*. *P. oleae* effectors had a marked downregulation as a consequence of pre-
764 treatment with *T. atroviride*-culture filtrate. This down regulation was probably due to a combined effect of
765 the plant tissue pre-elicited by *T. atroviride* elicitin (e.g. Epl1 Cerato-platanin) and other bioactive metabolites
766 of the culture filtrate.
767 The RNAseq analysis of olive gene differentially expressed during the infection by *P. oleae* showed the most
768 upregulated genes were EXORDIUM-like isogenes, known to inhibit cell proliferation[52] and which in soybean
769 are associated with resistance to *P. sojae*[54]. In inoculated olives the ethylene biosynthesis occurred very early
770 during the infection process and was triggered by wounding itself, as revealed by the expression of 1-
771 aminocyclopropane-1-carboxylate synthase-like genes (ACC synthases; LOC111390291, LOC111392037 and
772 LOC111370655) (Figure 2; Supplementary Table 1). In olive drupes only inoculated with *P. oleae*, 1-
773 aminocyclopropane-1-carboxylate oxidase 1-like (ACO, LOC111366119) was also highly upregulated (Figure
774 3; Supplementary Table 2) and represented the last step in ET biosynthesis. Infection by *P. oleae* upregulated
775 significantly also Ca^{2+} signaling, more than wounding itself as shown by the upregulation of protein SRC2
776 homolog (LOC111386812; Figure 3), which is the target of the main *P. oleae* elicitin INF-1 (g8411.t1 and
777 g8407.t1).
778 The most upregulated gene in olive fruit during *P. oleae* infection, the BON1-associated protein 2
779 (LOC111391572), function as a negative regulator of cell death and defense responses in association with
780 BAP1 and negatively regulating the R-protein "suppressor of npr1-1, constitutive 1" (SNC1)[56,57]. Infection by

781 *P. oleae* led to suppression of the natural immunity response by regulating BON1/SNC1 at the site of infection,
782 but perhaps was not sufficient to inhibit the production of salicylic acid and pipecolic acid by maintaining the
783 upregulated expression of SAR DEFICIENT 1-like (LOC111402349 and LOC111377821), initiating the
784 systemic acquired resistance (SAR) in distal parts of the plant[60,62]. As already reminded, SA and Pip trigger
785 the activation of immune-related genes, such as many pathogenesis-related protein genes (PRs), including
786 pathogenesis-related proteins STH-21-like, kirola-like proteins, hevamine-A-like, thaumatin-like proteins,
787 cysteine proteinase inhibitors A/B-like, kunitz trypsin inhibitor 2-like, chitinases, endoglucanases, and β-1,3-
788 glucanases. These are upregulated during *P. oleae* infection (Figure 3; Supplementary Table 2) and
789 significantly even more following treatment with *C. oleophila* (Table 1; Supplementary Table 3) and *T.*
790 *atroviride*-culture filtrate (Supplementary Table 4).
791 The receptor kinase FERONIA is downregulated by *P. oleae* infection and upregulated by the presence of *C.*
792 *oleophila* and *T. atroviride* extracts as also validated over time by qPCR(Figure 9). During cell elongation,
793 RALF23 plant peptides, when cleaved by the site-1 protease (S1P, a serine protease of the family of proprotein
794 convertase subtilisin/kexins), can create a complex with FER and the binding activate it kinase activity
795 resulting in phosphorylation of plasma membrane H^+-ATPase 2 at serine-899, causing inhibition of proton
796 transport and in general an arrest of root growth[142]. The FER activation suppresses also flg22-induced reactive
797 oxygen species (ROS) burst and immune responses. This suppression is achieved by destabilizing the complex
798 formation of EFR and FLS2 with their co-receptor BAK1, illustrating how RALF-FER signaling can modulate
799 immune signaling pathways[143]. Pathogens, such as fungi, have evolved strategies to manipulate plant signaling
800 pathways to facilitate infection. The discovery of potential RALF peptides in fungal and bacterial genomes
801 suggests that pathogens might exploit RALF-FER signaling to manipulate plant immune responses and
802 facilitate infection. Some fungal pathogens (e. g. *Fusarium oxysporum*) produce peptides that mimic plant
803 signaling molecules, known as RALFs-like peptides, which can interact with FER by activating it and hence
804 promote infection. These pathogen-derived RALF mimics can hijack the FER signaling pathway, leading to
805 increased virulence and successful colonization of the plant host. The interaction between RALF peptides and
806 FER triggers a signaling cascade that can inhibit cell growth, as seen in the inhibition of primary root growth
807 in *Arabidopsis* upon binding of RALF1 to FER[144]. For instance, *F. oxysporum f. sp. lycopersici* RALF-like
808 peptides inhibited the growth of tomato seedlings and elicited responses in tomato and *Nicotiana benthamiana*

809 typical of endogenous plant RALF peptides (reactive oxygen species burst, induced alkalinization and
810 mitogen-activated protein kinase activation)[145]. To make the hypothesis that FER is upregulated due to its
811 inhibition by *P. oleae* effectors, we searched into a group of small proteins, top differentially expressed, in
812 case of *P. oleae* infection (Supplementary Table 9) and aligned their protein sequence to known RALF
813 peptides from *O. europaea* and/or fungal RALF-like peptide homologues (Supplementary Figure 2). The
814 alignment revealed a conservation of critical cysteine residues and FER binding motifs even if those were more
815 similar to the *Colletotrichum higginsianum* RALF-like protein (CCF44719) rather than *F. graminearum*
816 (FG05_30327). *P. oleae* putative RALF-like homologues, differentially expressed, were: g3545.t1, g8557.t1
817 and g5663.t1. The hypothesis here is that *P. oleae* putative RALF-like proteins/peptides might activate
818 FERONIA, and hence *P. oleae* could block the JA, COR and SA signaling, facilitating its pathogenesis. We
819 need, of course, to confirm by other experiments whether the putative bioinformatics identified *P. oleae* RALF-
820 like proteins here (Supplementary Figure 2) really interact with FERONIA.
821 Many R-genes were upregulated during *P. oleae* infection (Figure 3; Supplementary Table 2), probably acting
822 in conjunction with WRKY TFs and F-box/LRR-repeat proteins to express such PRs. During 72hpi of *T.*
823 *atroviride*-culture filtrate pre-treatment followed by *P. oleae* infection, the R-genes were particularly
824 upregulated: LRR receptor-like serine/threonine-protein kinase At1g06840 (LOC111371641), LEAF RUST
825 10 DISEASE-RESISTANCE LOCUS RECEPTOR-LIKE PROTEIN KINASE-like 1.1 (LOC111390435),
826 MLO-like protein 2 (LOC111370597), rust resistance kinase Lr10-like (LOC111411655), pto-interacting
827 protein 1-like (LOC111410523), WRKY transcription factor 20 (LOC111391535), and the LRR receptor-like
828 serine/threonine-protein kinase At1g06840 (LOC111393227). Calcium (Ca^{2+}) signaling plays a pivotal role in
829 plant immunity, functioning as a key secondary messenger in response to various stimuli, including pathogen
830 attack. In plants, Ca^{2+} signaling is central to both pattern-triggered immunity (PTI) and effector-triggered
831 immunity (ETI), with the generation of characteristic cytoplasmic Ca^{2+} elevations being common to both[146].
832 The interaction between FERONIA and MLO-like proteins (e.g. NORTIA, NTA) is known in pollen tube
833 reception and fungal invasion causing a release of intracellular Ca^{2+} and related signaling[91]. Here, FERONIA
834 and MLO-like protein2 might have interacted and triggered the same Ca^{2+} release and signaling followed by
835 upregulation of many Ca^{2+} binding proteins for further signaling and immunity activation. On another hand,
836 *T. atroviride*-culture filtrate pre-treatment triggered the upregulated of other R-genes: the LRR receptor-like

837 serine/threonine-protein kinase At1g06840 (LOC111371641), LRR receptor-like serine/threonine-protein
838 kinase At1g06840 (LOC111393227), and the MLO-like protein 8 (LOC111401243). Besides, BON1-
839 associated protein 2 (LOC111391572) was downregulated following pre-treatment with either *C. oleophila* or
840 *T. atroviride*-culture filtrate (Supplementary Tables 3, and 4).
841 Necrotrophic pathogens and beneficial antagonist microorganisms induce or prime ET- and JA-dependent
842 signaling pathways. For example, the necrotrophic pathogen *Botrytis cinerea* causes a rapid activation of ET
843 biosynthesis in *Arabidopsis* by the action of Mitogen Activated Protein Kinase3 (MAPK3) and MAPK6 that
844 phosphorylate the ET biosynthesis proteins 1-Aminocyclopropane-1-Carboxilic Acid (ACC) Synthase 2
845 (ACS2) and ACS6[147]. In the case of *P. oleae* infection alone, ethylene biosynthesis was highly upregulated
846 since it was found that the final step involving biosynthetic enzyme 1-aminocyclopropane-1-carboxylate
847 oxidase 1-like (ACO, LOC111366119), was very highly upregulated as compared to wounded fruit (L2FC≥
848 7.6; Figure 3; Supplementary Table 2). Besides the boost to ET biosynthesis, it was observed a significant
849 increase in JA biosynthesis and an upregulation of many ET responsive genes. The Apetala 2 (AP2)/Ethylene
850 Response Factor (ERF) seems to be the driver of the ET/JA-mediated defense pathway. Its homologue in olive
851 is the ethylene-responsive TF 1B-like (ERF-1B) of which many isogenes are present in the genome. Upon *P.*
852 *oleae* infection, many ERF-1B like factors were upregulated (Supplementary Table 2). As described above,
853 JA binds to the JA receptor Coronatine Insensitive 1 (COI1, LOC111390662 in *O. europeae*), which results in
854 the degradation of JAZ repressor proteins by the 26S proteasome and subsequent release of activating TFs. ET
855 binds to the receptor ETR1, and this in turn stabilizes the EIN3/EIL1 TFs which bind to the promoters of both
856 JA and ET responsive/defense genes, activating the ERF branch of the JA defense pathway[148].
857 On the other hand, to link *P. oleae* effectors with known *Phytophthora* avirulent genes, for instance, we could
858 not find a homologue of *P. sojae* RxLR effector avh94 in the *P. oleae* genome/proteome. RxLR effector avh94
859 was shown to interact with soybean JAZ1/2, which is a repressor of JA signaling[83]. However, by genome
860 sequencing and annotation, *P. oleae* was shown to possess more than 350 effectors (RxLR, crinklers, elicitins,
861 etc.) whose function is unknown and whose identity has only been confirmed by annotation pipelines.
862 Here we have performed simultaneously a dual RNA-seq, with a total RNA extraction, of both host (*O.*
863 *europaea*) and pathogen (*P. oleae*), with or without treatment with either *C. oleophila* or *T. atroviride*-culture
864 filtrate. The outcome of the *P. oleae* gene expression on the host has revealed a expression pattern that was

865 modulated by either *C. oleophila* or *T. atroviride*-culture. For instance, the cluster analysis for top DEGs of
866 elicitins (Figure 8D) revealed that g8415.t1, g17483.t1, and g8420.t1 were upregulated upon *P. oleae* infection
867 and downregulated by the effect of either *C. oleophila* or *T. atroviride*-culture filtrate (Figure 8D). The trend
868 of expression for the elicitin g17483.t1 as monitored by RT-qPCR confirmed that biocontrol agents constantly
869 over time downregulated such important elicitin (Figure 8B). Opposite trend of expression was observed for
870 the elicitins g24094.t1, g4307.t1 and g19250.t1, which were upregulated upon treatment with either *C.*
871 *oleophila* or *T. atroviride*-culture filtrate and which role is unknown. The same opposite trend was observed
872 at 72 hpi for the INF-1 elicitins (g8407.t1 and g8411.t1), known inducer of HR (Figure 8D).
873 Our cluster analysis on *P. oleae* effectors (Figure 8) revealed a group of Crinkler highly differentially expressed
874 during the infection with *P. oleae* alone (e. g. g11238.t1, g8206.t1, g23109.t1, g9419.t1, g9424.t1, g22000.t1,
875 g10898.t1, g3434.t1 and g1422.t1) but downregulated by the treatment with either *C. oleophila* or *T.*
876 *atroviride*-culture filtrate (Figure 8B). RNAseq revealed the same trend for Crinkler g723.t1 and RT-qPCR
877 confirmed downregulation of this gene overtime as a consequence of the treatment with the BCAs (Figure
878 10A).
879 The same trend was observed for the RxLR effectors. In detail, the differentially expressed genes upregulated
880 upon infection with *P. oleae* alone and downregulated by the treatment with either *C. oleophila* or *T.*
881 *atroviride*-culture filtrate, having the highest L2FC, were g3852.t1, g16251.t1, g19132.t1, g1137.t1, g6343.t1,
882 g567.t1, g2733.t1, g19400.t1, g24837.t1, g2412.t1, g20157.t1, and g1136.t1 respectively. For the RxLR
883 effector g24837.t1 this trend of qPCR expression remained unchanged over time (Figure 10C).
884 Results obtained here strongly support the hypothesis that the molecular counteraction exerted by either *C.*
885 *oleophila* or *T. atroviride*-culture filtrate applied one day before *P. oleae* inoculation, reduced significantly the
886 pathogenicity of *P. oleae* by lowering the most effective effectors/exoenzymes by a still unclear mechanism.
887 However, despite its action was long lasting (up to 168 hpi), it did not halt but only slowed the invasion of
888 olive drupes by *P. oleae*. Further research will be needed to increase the effectiveness and extend further the
889 duration of the inhibitory activity exerted by agents of biological control.
890 In conclusion, this study unveiled the transcriptomic events of the two- and three-actors interactions of *P. oleae*
891 with olive fruits and each of the two evaluated biocontrol agents. Genome sequencing of *P. oleae* has strongly
892 contributed to the study of its transcriptomics and description of the annotated pathogenesis-related genes. The

893 olive cultivar 'Coratina' can be regarded as a susceptible host, despite several R-genes described here were
894 upregulated in response to the infection (compatible host), and the two biocontrol agents only temporarily
895 restricted the growth of the pathogen inside the drupes. However, over time (up to 168 hpi) these treatments
896 were not able to stop *P. oleae* infection progression

897 **4. Methods**

898 **4.1. Microorganisms and plant material**

899 Microorganisms included in this study were: the ex-type of the yeast *C. oleophila* Montrocher strain O, ii.
900 the ascomycete *T. atroviride* strain TS and iii. the oomycete *P. oleae* isolate VK10A. C. *oleophila* strain O, *T.*
901 *atroviride* strain TS and *P. oleae* isolate VK10A were sourced from the culture collection of the Molecular
902 Plant Pathology Laboratory of the Department of Agriculture, Food and Environment of the University of
903 Catania (Catania, Italy) and were identified in previous studies[24,149].

904 'Coratina' olive fruits were collected from 15-year-old olive trees from an orchard in Calabria (southern Italy),
905 in the municipality of Mirto-Crosia, province of Cosenza (DATUM WGS 84, 39°36′54.5″ N 16°46′11.7″ E).
906 Drupes were used to both determine the incidence of the disease following post-harvest treatments and to study
907 the defense mechanisms triggered in the presence of the pathogen in relation to the applied treatments. Overall,
908 540 drupes at maturation index (MI)[7] 4 (MI4) were collected from five trees and stored for 3 hours inside a
909 refrigerated bag while being transported to the laboratory and stored at 8°C until used in experiments.

910 **4.2. Preparation of *P. oleae*-inoculum**

911 *Phytophthora oleae* isolate VK10A was preliminary grown on V8-Agar (V8A) at 20 ± 2°C. Then, inoculum
912 used in following tests was represented by culture fragments (size 2 mm) cut from the growing edge of seven-
913 day-old cultures.

914 **4.3. Preparation of *T. atroviride*-culture filtrate**

915 *Trichoderma atroviride* was grown in the dark in a 500 mL Erlenmeyer flask containing 200 mL of potato
916 dextrose broth (PDB) at pH 7.0 under constant stirring in an orbital shaker for 30 days (28 ± 2 °C, 140 rpm)[24].
917 After incubation, the supernatant was collected, filtered through a 13-mm/0.22-µm nylon syringe filter and
918 used as treatment for olive drupes in following tests.

919 **4.4. Preparation of *C. oleophila* cell suspension**

920 *C. oleophila* was grown in 500 ml shake flask cultures containing nutrient yeast broth (NYDB) at 24°C for 48
921 hours. Cells of *C. oleophila* were collected by centrifugation at 5000×g for 10 minutes at 4°C; then, collected
922 cells were suspended in sterile distilled water to reach the concentration to 1×10^8 CFU/ml (a hemacytometer
923 was used). Obtained *C. oleophila* cell suspension was used as treatment for olive drupes in following tests.

924 **4.5. Evaluating the Effectiveness of *C. oleophila* and *Trichoderma*-culture filtrate in Controlling Rots of**
925 **Drupes by *P. oleae***

926 Olive drupes were preliminarily subjected to surface-sterilization by immersion in a 0.5 % NaClO for 30s,
927 rinsing in sterile distilled water (sdw) and air drying for 24 hours. Once dried, olive drupes were punctured
928 once with a sterile needle in an equatorial position and a 20 µl droplet of *C. oleophila* cell suspension, *T.*
929 *atroviride*-culture filtrate or sdw (control) was pipetted onto the wound. After 24 h of incubation at 21 ± 1°C,
930 the wound of each drupe was inoculated with a 2 mm agar plug collected from a V8A culture of *P. oleae*
931 isolate VK10A. After inoculation, drupes were incubated at 21 ± 1°C, with 80% relative humidity and a
932 photoperiod of 16 h light / 8 h dark. Severity of *Phytophthora* rots in olive drupes was assessed at 24, 72, and
933 168 hours post pathogen inoculation (hpi) and rated based on the following empirical scale: (0) no symptoms;
934 (1) 0-25% of fruit rot around inoculation point; (2) 26-50% fruit rot; (3) 50-75 % of the fruit covered by
935 mycelium; (4) 100 % fruit covered from mycelium. These values were used to calculate the rAUDPC according
936 to Riolo and other authors[7,150,151]. Overall, the experimental assay included the following three treatments: i.
937 *P. oleae*-inoculated olive drupes treated with sdw (control, IDA); ii. *P. oleae*-inoculated olive drupes treated
938 with *T. atroviride*-culture filtrate (IDB); iii. *P. oleae*-inoculated olive drupes treated with *C. olephila* cell
939 suspension (IDC). The experimental assay was designed as a completely randomized block, with each
940 treatment comprising three biological replicates, each consisting of 20 drupes.

941 **4.6. Assessing the experimental assay to study the Transcriptome of olive drupes and *P. oleae* in the**
942 **system Olive Drupe-*P. oleae*-*C. oleophila*/*T. atroviride*-culture filtrate**

943 Olive drupes were preliminary surface sterilized, treated with either *C. oleophila* cell suspension or *T.*
944 *atroviride*-culture filtrate and *P. oleae*-inoculated as described in 4.5. Then, drupes were incubated at 21 ±
945 1°C, with 80% relative humidity and a photoperiod of 16 h light / 8 h dark. The experimental assay included
946 the following seven treatments: (i) non-wounded drupes treated with 20 µl of sdw (ID1); (ii) wounded drupes
947 treated with 20 µl of sdw (ID2); (iii) wounded drupes inoculated with *P. oleae* (ID3); (iv) wounded drupes

948 treated with *T. atroviride*-culture filtrate and inoculated with *P. oleae* (ID4); (v) wounded drupes treated with
949 *C. oleophila* and inoculated with *P. oleae* (ID5); (vi) wounded drupes treated with *T. atroviride*-culture filtrate
950 (ID6); (vii) wounded drupes treated with *C. oleophila* (ID7). The experimental assay was designed as a
951 completely randomized block, with each treatment comprising three biological replicates, each consisting of
952 20 drupes. In order to proceed to transcriptomic analyses, at each of the above-mentioned time points, fresh
953 olive fragments from each treatment were collected and immediately frozen in liquid nitrogen and stored at -
954 80°C.

955 **4.7. RNA isolation from infected olive drupes and cDNA synthesis for RT-PCR**

956 Total RNA was extracted using RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Drupes from each
957 treatment (100 mg) were ground to a fine powder with liquid nitrogen, following the manufacturer's protocol
958 and treated with TURBO DNA-free Kit (ThermoFisher Scientific, Italy). The RNA concentration was then
959 adjusted to 200 ng/µl, and its quality was verified by performing denaturing RNA electrophoresis gel in TAE
960 agarose[152]. First-strand cDNA was synthesized with an oligo d(T)21 primer and reverse transcription was
961 performed using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA,
962 United States) according to the manufacturer's instructions.

963 **4.8. Quantitative Real Time-PCR (RT-qPCR) Analysis of Gene Expression**

964 The RT-qPCR for validating expression levels of selected genes of olive drupes and *P. oleae* (Supplementary
965 Table 13) was performed using the QuantGene 9600 system (Bioer Technology, Hangzhou, China). Reactions
966 were performed in a total volume of 20 µl with 10 ng of cDNA, 1 µl of 10 µM of each primer, and 10 µl of
967 PowerUp™ SYBR™ Green Master Mix (2X, Applied Biosystems™, Foster City, CA, United States). For
968 each treatment, three biological replicates were analysed, with triplicate RT-qPCR experiments per replicate.
969 Thermocycling conditions included 2 min at 50°C for UDG activation, 2 min at 95°C (Dual-Lock™ DNA
970 polymerase), followed by 40 cycles of two steps: 95°C for 15 s (denaturation) and 60°C (annealing/extension)
971 for 1 min. Expression levels of selected genes were calculated using the $2^{-\Delta\Delta Ct}$ method[153], where $\Delta\Delta Ct = (Ct$
972 of target gene − Ct of reference gene$)_{sample}$ − (Ct of target gene − Ct of reference gene$)_{calibrator}$ and Ct is the
973 threshold cycle of each transcript, defined as the point at which the amount of amplified target reaches a fixed
974 threshold above the background fluorescence. *O. europaea* tubulin alpha-3 chain (LOC111371391,
975 XM_022993342.1) was used as an internal reference gene. For *P. oleae*, the tubulin beta chain (g8215.t2

976 (TRINITY_DN6095_c0_g1_i1) gene expression has been used to normalize the RT-qPCR data. The relative
977 expression level of each gene obtained through RT-qPCR was compared to the RNA-seq data. Expression
978 values of the $2^{-\Delta\Delta Ct}$ were represented as boxplots using Python script wit "seaborn", "pandas" and
979 "matplotlib.pyplot" libraries. Dunnett's test was used to calculate the significance (*p-value*) relative to the
980 expression level ($2^{-\Delta\Delta Ct}$) of each sample to wounded olive fruit condition by computing a Student's t-statistic
981 for each experimental conditions and time points (hpi).

982 **4.9. Total RNA preparation and Illumina mRNA sequencing**

983 Withdrawal of plant-fungal-oomycete material was carried out around the wounded area from the non-infected
984 and infected drupes. The material was pulverized in liquid nitrogen and stored at -80°C until RNA extraction.
985 Total RNA extraction was performed using the Direct-zol RNA Miniprep kit (Zymo Research, Nordic BioSite,
986 Sweden) with a modified protocol. Frozen samples were extracted with 1.5 ml of TRI Reagent (Zymo
987 Research, Nordic BioSite, Sweden) using ZR BashingBead Lysis (0.5 mm Y_2O_3 Stabilized Zirconia Beads;
988 Zymo Research, Nordic BioSite, Sweden) and the FastPrep-24™ 5G bead beating grinder and lysis system
989 (MP Biomedicals, Fischer Scientific, Denmark). After homogenizing, samples were spin at 21000 ×g for 1
990 minute and then, phenol:chloroform:isoamyl alcohol 25:24:1 (Sigma-Aldrich, Merck, Germany) extraction
991 was performed. Further steps were performed according to the Direct-zol RNA Miniprep kit. Eluted total RNA
992 quality check was performed using a 2100 Bioanalyzer system (Agilent, Denmark). Two biological samples
993 were extracted in technical triplicate and samples with RNA Integrity Number (RIN) above 8 were selected
994 for RNAseq. Samples were sequenced at Novogene (Cambridge, UK) with Illumina hiseq 4000 and 150 pair
995 end read length (PE), at 30 million of read depths. Data quality control revealed a Q30 [(Base count of Phred
996 value > 30) / (Total base count)] average of 93% for a total of 98% of clean reads.

997 **4.10. *De novo* mRNA analysis**

998 Illumina 150 PE, 30 M reads fastq[154] files were processed for removal of the adapters with Trimmomatic
999 Version 0.39[155]. For maximizing and diversifying the mRNA expression of *Phytophthora oleae*, the Trinity *de*
1000 *novo* mRNA assembly algorithm[156] was applied to RNA samples of cultures of the isolate VK10A grown in
1001 three different substrates, including CZAPEK, MEA and PDA. Annotation of both trinity derived cDNA and
1002 AUGUSTUS predicted genes was performed against using Mercator4v6[157] analysis cross-evaluation.
1003 AUGUSTUS web prediction gave 20547 proteins of which only 12117 were containing unique scaffold

1004 represented of more than 75% as protein identity, the remaining not unique proteins represented alternate
1005 proteins or differently spliced or start (ATG) derived proteins. The same number of proteins, 20094 was found
1006 by using Trinity ORF prediction software unfiltered and after removing duplicates. Filtering the Trinity
1007 accessions for proteins capable of finding a blast hit with other Phytophthora species, then, the real number of
1008 de novo mRNA transcript was down to 13373 proteins. It needs to be considered that the Trinity prediction
1009 include partial protein/mRNA sequences due to incomplete full-length cDNA. The fasta/excel files related to
1010 AUGUSTUS and Trinity protein/CDS/mRNA prediction and annotation can be found at Zenodo
1011 (https://zenodo.org/records/11092245).

1012 **4.11. RNAseq data analysis**

1013 The raw Illumina sequence reads of fastaq format were processed as above by removing the adapters. The
1014 reference genome and gene model annotation files of *O. europaea* var. *sylvestris* were downloaded from NCBI
1015 (*Olea europaea* var. *sylvestris* reference genome O_europaea_v1, accession number GCF_002742605.1) and
1016 *P. oleae* reference genome VK10A, from Zenodo (https://zenodo.org/records/11092245). The filtered fastaq
1017 files were aligned to the reference genome using Hisat2 v2.0.5[158]. The reads with low alignment quality values
1018 or unpaired alignments, and alignments to multiple regions of the genome were filtered out by using
1019 samtools[159] to generate duplicate checked and sorted binary BAM files of mapped reads to the genome. The
1020 number of reads and fragments per kilobase per million reads (FPKM) for each gene was calculated using
1021 Stringtie v2.2.0[160]. Differential gene expression analysis between the pathogen-inoculated and control samples
1022 at different time points was performed in R studio using the DESeq2 R package in a script
1023 (https://bioconductor.org/packages/DESeq2/) customized on the basis of the total normalized reads counts[161].
1024 Corrections for false positives and false negatives were made using the Benjamini-Hochberg procedures[162] to
1025 generate the false discovery rate (FDR).

1026 Fold changes (L2FC, log2Ratio) were estimated from normalized gene expression levels in each sample by
1027 using the ratio of the sample Log2 of FPKM and the wounded olive fruit Log2 of FPMK as denominator. The
1028 mapped genes with an adjusted false discovery rate (FDR) ≤ 0.05 and $|\log2(\text{FoldChange})| > 1$ were considered
1029 differentially expressed, from L2FC < 0.5 were considered downregulated. To reduce the amount of
1030 differentially expressed genes according to 0.05 and 0.001 FDR, shrinkage of log2 (Fold Change) was
1031 performed using the R 'apeglm' function[163]. Differentially expressed genes (DEGs) clustering heatmaps were

1032 generated by the pheatmap and clusterProfiler R packages[164]. Multiple correlation of the variance of L2FC for
1033 all the sample was analyzed by principal components analysis (PCA).

1034 **4.12. LC-MS identification of proteins in protein filtrate**

1035 *T. atroviride* strain TS was cultured *in vitro* in a YPD liquid media supplemented with malt extract to 1%.
1036 After three days, the cultures (in triplicate) were filtered through a Whatman® qualitative filter paper, Grade
1037 1 (WHA1001325 Sigma-Aldrich, Merck, Germany) to retain the mycelia. The filtrate was further centrifuged
1038 at 20,000 ×g for 15 minutes then sterile filtered through 0.2 µm vacuum filters. The protein preparation for
1039 proteomics including digestion by trypsin and the LC-MS conditions and identification by Waters PLGS
1040 software has been described previously[165].

1041

1042 **CRediT author statement.**

1043 **Sebastiano Conti Taguali:** Investigation, Methodology, Formal analysis, Data Curation, Writing - Original
1044 Draft, Writing - Review & Editing

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1050 administration.

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1052 Editing, Resources, Supervision, Funding acquisition, Project administration.

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Data Availability Statement

Raw data for this study will be available to researchers upon reasonable request to the corresponding authors.

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Figure(s) Legend

Figure 1. Mean values of the relative area under the disease progress curve (rAUDPC) in olive drupes inoculated with *Phytophthora oleae* and treated with sterile distilled water (control) (IDA), *Candila oleophila* suspension (IDB) or *Trichoderma* atroviride-culture filtrate (IDC). Panel A, rAUDPC values of treatments IDA, IDB and IDC calculated on the basis of % rot severity value recorded at 24, 72 and 168 hours after inoculation (hpi). Values sharing the same letter do not statistically differ according to Tukey's honestly significant difference (HSD) test ($p \leq 0.05$). Panel B, Drupes at 72 hpi showing the different size of necrotic lesion: (IDA) wounded drupes inoculated with *P. oleae*; (IDB) wounded drupes inoculated with *P. oleae* and treated with culture filtrate of *T. atroviride*; (IDC) wounded drupes inoculated with *P. oleae* and *C. oleophila*.

Figure 2. Selection of top up/down-regulated genes present at 72h post mechanical wounding in mature olive cv. Coratina fruits versus intact drupes. The DESeq2 algorithm has been used to calculate the statistics and the differentially expressed genes were sorted according to the log2FoldChange (L2FC). Filtering by FDR with p-value of 0.01 has been applied. Heatmap colors with similar LF2C have been clustered. The complete set of up- down-regulated genes is present in **Supplementary Table 1**.

Figure 3. Heatmap of filtered mRNA DEGs present at 72 h post *P. oleae* inoculation of wounded drupes vs. non-inoculated wounded drupes. The wounded L2FC has been calculated between wounded vs not wounded drupes. The DESeq2 algorithm has been used to calculate the statistics and the DEGs were sorted and clustered according to *P. oleae* infection triggering the highest olive mRNAs (positive values L2FC). Filtering by FDR with p-value of 0.001 has been applied. Heatmap shows filtered mRNA as FPKM > 50 as baseMean. The complete set of up- and down-regulated genes is shown in **Supplementary Table 2**.

Figure 4. Cluster heatmap analysis of the top mRNAs related to DEGs of *O. europaea* detected 72 hpi from the significative upregulated genes during *C. oleophila* co-infection with *P. oleae* vs. infection with *P. oleae* alone (lane 7). Filtering after DESeq2 analysis was applied to the DEGs in Supplementary Table 3 by sorting for the lowest *p*-value/*p*-adj-value and highest baseMean or L2FC. Lane 1, L2FC of wounded drupes vs. intact drupes; lane 2, L2FC of wounded drupes with *T. atroviride* extract vs. wounded drupes; lane 3, L2FC of wounded drupes plus *C. oleophila* vs. wounded drupes; lane 4, L2FC of wounded drupes plus *P. oleae* vs. wounded drupes; lane 5, L2FC of wounded drupes plus *C. oleophila* and *P. oleae*; lane 6, L2FC of wounded drupes plus *T. atroviride*

extract plus *P. oleae* vs. wounded drupes; lane 7, L2FC of wounded drupes plus *C. oleophila* and *P. oleae* vs. wounded drupes and *P. oleae*.

Figure 5. Cluster heatmap analysis of the top mRNAs related to DEGs genes of *O. europaea* detected during 72 hpi from the significant upregulated genes during *T. atroviride culture filtrate* pre-treatment plus *P. oleae* vs infection with *P. oleae* alone (lane 7). The DESeq2 data have been filtered first by lowest pvalue/padj value and then highest baseMean and L2FC from Supplementary Table 4. Lane 1: L2FC of wounded olives vs intact ones; lane 2: L2FC of wounded plus *T. atroviride* extract vs wounded; lane 3: L2FC of wounded plus *C. oleophila* vs wounded; lane 4: L2FC of wounded plus *P. oleae* vs wounded; lane 5: L2FC of wounded plus *C. oleophila* and *P. oleae*; lane 6: L2FC of wounded plus *T. atroviride extract* plus *P. oleae* vs wounded; lane 7: L2FC of wounded plus *T. atroviride* culture filtrate pre-inoculation plus *P. oleae* vs infection with *P. oleae* alone.

Figure 6. Cluster heatmap analyses of the differential RNAseq related to olive genes among all treatments at 72 hpi. This heatmap has been constructed assuming three groups of samples (all with technical duplicates and some of them with more biological repeats). Panel A (left) cluster analysis in which the wounded drupe treatment LF2C has been normalized to "not wound" ones, while the rest of conditions has been normalized to the "wound" condition. The data has been normalized with L2FC being = +/-4 by Z score. Legend: 1) intact olive drupes (ID1); 2) wounded drupes (ID2); 3) wounded drupes pre-treated with *C. oleophila* cells (ID7); 4) wounded drupes pre-treated with *T. atroviride* culture filtrate (ID6); 5) wounded drupes pre-treated with *C. oleophila* cells and inoculated with *P. oleae* (ID5); 6) wounded drupes pre-treated with *T. atroviride* culture filtrate and inoculated with *P. oleae* (ID4); 7) wounded drupes inoculated with *P. oleae* (ID3). Panel B (right) list of FPKM base mean filtered (>100 counts) upregulated and L2Fc sorted genes in the *P. oleae* alone condition (the total 739 genes are listed in Supplementary Table 5).

Figure 7. PCA plot including all the *P. oleae* samples alone or with either *C. oleophila* or *T. atroviride*-culture filtrate from 72hpi DESEq2 data. The horizontal axis (PC1) represents the distance from *P. oleae* grown in artificial media (CZ, MEA, or PDA) proportional to the variance of pathogenicity (rAUDPC) starting from the coordinates 0, 0. Negative PC1 variance means no infection. The closer the horizontal distance from 0, 0, the less is the degree of infectivity for the cases where infection is ongoing. The vertical variance of this PCA describes the variation of genes involved in severity of the disease as compared to original characteristics (no infection) of

P. oleae grown in artificial media (CZ, MEA, or PDA). The distance/variance in PC2 between *P. oleae* infecting the drupe alone has a positive value while the distance/variance in PC2 of *P. oleae* infecting the drupe plus either *C. oleophila* or *T. atroviride*-culture filtrate is proportional to the reduction of infectivity detected in each mixed condition (*P. oleae* + either *C. oleophila* or *T. atroviride*-culture filtrate variance) where was observed a negative variance in PC2.

Figure 8. Cluster heatmap analyses of *P. oleae* peptidases, Crinkler, RxLR effectors and elicitins differentially expressed in three conditions tested. X1= L2FC of *P. oleae* grown on olive drupes vs. *P. oleae* grown on artificial media; X2= L2FC of *P. oleae* grown on drupes plus *C. oleophila* vs. *P. oleae* grown on drupes; X3= L2FC of *P. oleae* grown on drupes plus *T. atroviride* filtrate vs. *P. oleae* grown on drupes.

Figure 9. RT-qPCR validation of chosen DEGs for *Olea europaea* at three time points and under different conditions. Asterisks represents statistically different samples according to Dunnett's test (∗ = $p < 0.05$, ∗∗ = $p < 0.01$, and ∗∗∗ = $p < 0.001$), as compared to unwounded fruits. The normalization has been performed according to the tubulin alpha-3 chain (LOC111371391, XM_022993342.1). The RT-qPCR primers are shown in Supplementary Table 15. The bars represent the standard deviation (SD). Conditions legend: ID2, wound; ID3, wound + *P. oleae*; ID4, wound + *T. atroviride* + *P. oleae*; ID5, wound + *C. oleophila* + *P. oleae*; ID6, wound + *T. atroviride*; ID7, wound + *C. oleophila*.

Figure 10. RT-qPCR validation of chosen DEGs for *P. oleae* during the three time points and different conditions. Asterisks represent statistically different responses as compared to the expression in artificial media, according to Dunnett's test (∗ = $p < 0.05$, ∗∗ = $p < 0.01$, and ∗∗∗ = $p < 0.001$). The normalization has been performed according to the tubulin beta chain g8215.t2 (TRINITY_DN6095_c0_g1_i1). The RT-qPCR primers are shown in Supplementary Table 15. The bars represent the standard deviation (SD).

Figure 1

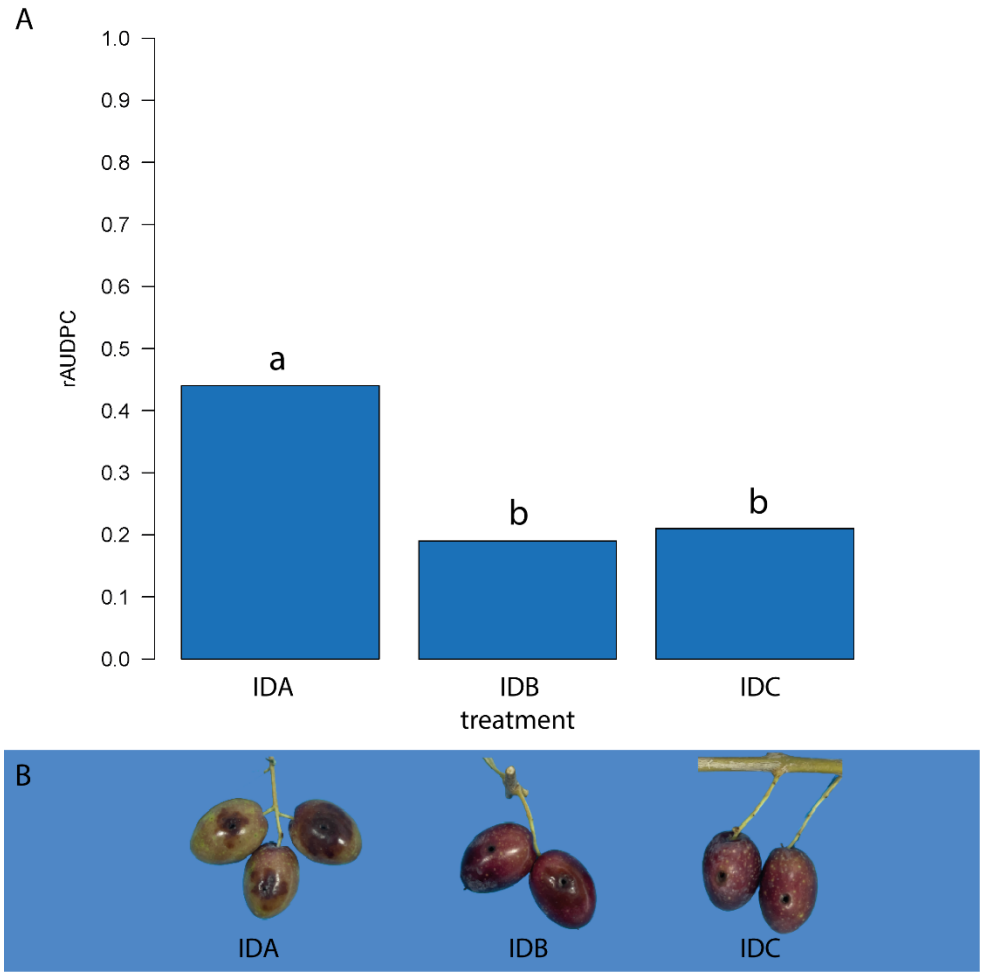
A
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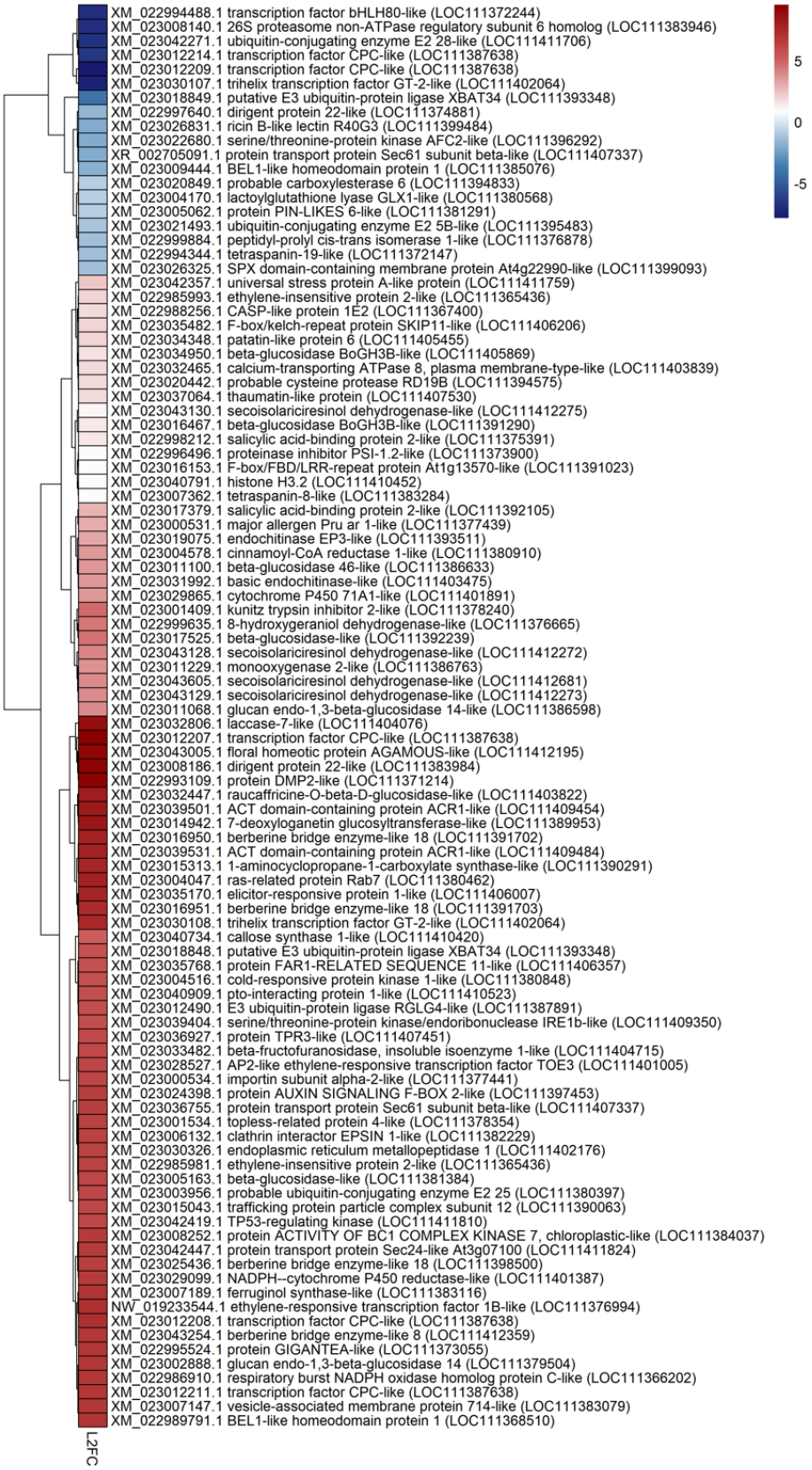
Figure 2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XM_023008140.1 26S proteasome non-ATPase regulatory subunit 6 homolog (LOC111383946)
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XM_023012214.1 transcription factor CPC-like (LOC111387638)
XM_023012209.1 transcription factor CPC-like (LOC111387638)
XM_023030107.1 trihelix transcription factor GT-2-like (LOC111402064)
XM_023018849.1 putative E3 ubiquitin-protein ligase XBAT34 (LOC111393348)
XM_022997640.1 dirigent protein 22-like (LOC111374881)
XM_023026831.1 ricin B-like lectin R40G3 (LOC111399484)
XM_023022680.1 serine/threonine-protein kinase AFC2-like (LOC111396292)
XR_002705091.1 protein transport protein Sec61 subunit beta-like (LOC111407337)
XM_023009444.1 BEL1-like homeodomain protein 1 (LOC111385076)
XM_023020849.1 probable carboxylesterase 6 (LOC111394833)
XM_023004170.1 lactoylglutathione lyase GLX1-like (LOC111380568)
XM_023005062.1 protein PIN-LIKES 6-like (LOC111381291)
XM_023021493.1 ubiquitin-conjugating enzyme E2 5B-like (LOC111395483)
XM_022999884.1 peptidyl-prolyl cis-trans isomerase 1-like (LOC111376878)
XM_022994344.1 tetraspanin-19-like (LOC111372147)
XM_023026325.1 SPX domain-containing membrane protein At4g22990-like (LOC111399093)
XM_023042357.1 universal stress protein A-like protein (LOC111411759)
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XM_022988256.1 CASP-like protein 1E2 (LOC111367400)
XM_023035482.1 F-box/kelch-repeat protein SKIP11-like (LOC111406206)
XM_023034348.1 patatin-like protein 6 (LOC111405455)
XM_023034950.1 beta-glucosidase BoGH3B-like (LOC111405869)
XM_023032465.1 calcium-transporting ATPase 8, plasma membrane-type-like (LOC111403839)
XM_023020442.1 probable cysteine protease RD19B (LOC111394575)
XM_023037064.1 thaumatin-like protein (LOC111407530)
XM_023043130.1 secoisolariciresinol dehydrogenase-like (LOC111412275)
XM_023016467.1 beta-glucosidase BoGH3B-like (LOC111391290)
XM_022998212.1 salicylic acid-binding protein 2-like (LOC111375391)
XM_022996496.1 proteinase inhibitor PSI-1.2-like (LOC111373900)
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XM_023040791.1 histone H3.2 (LOC111410452)
XM_023007362.1 tetraspanin-8-like (LOC111383284)
XM_023017379.1 salicylic acid-binding protein 2-like (LOC111392105)
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XM_022999635.1 8-hydroxygeraniol dehydrogenase-like (LOC111376665)
XM_023017525.1 beta-glucosidase-like (LOC111392239)
XM_023043128.1 secoisolariciresinol dehydrogenase-like (LOC111412272)
XM_023011229.1 monooxygenase 2-like (LOC111386763)
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Figure 3

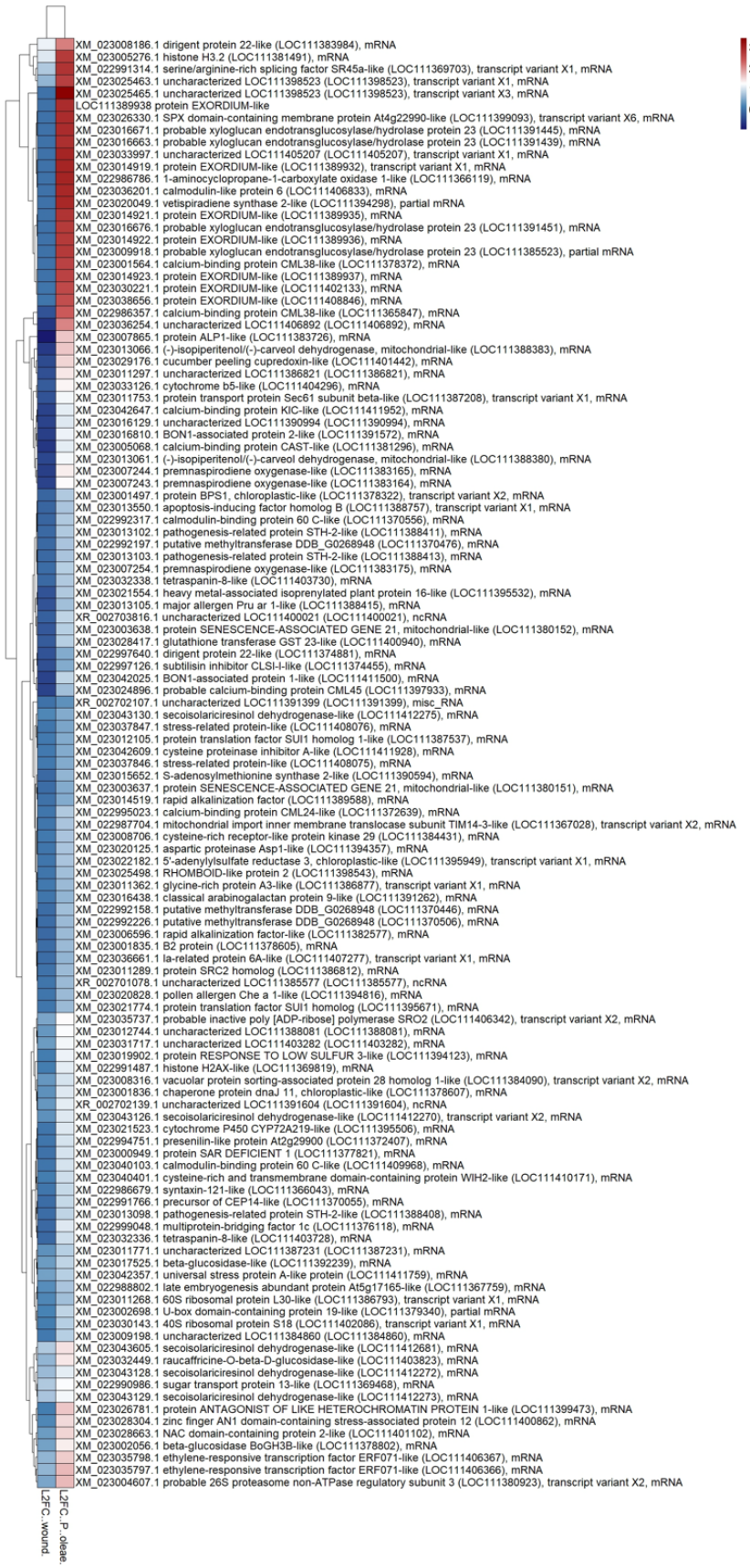
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Figure 4

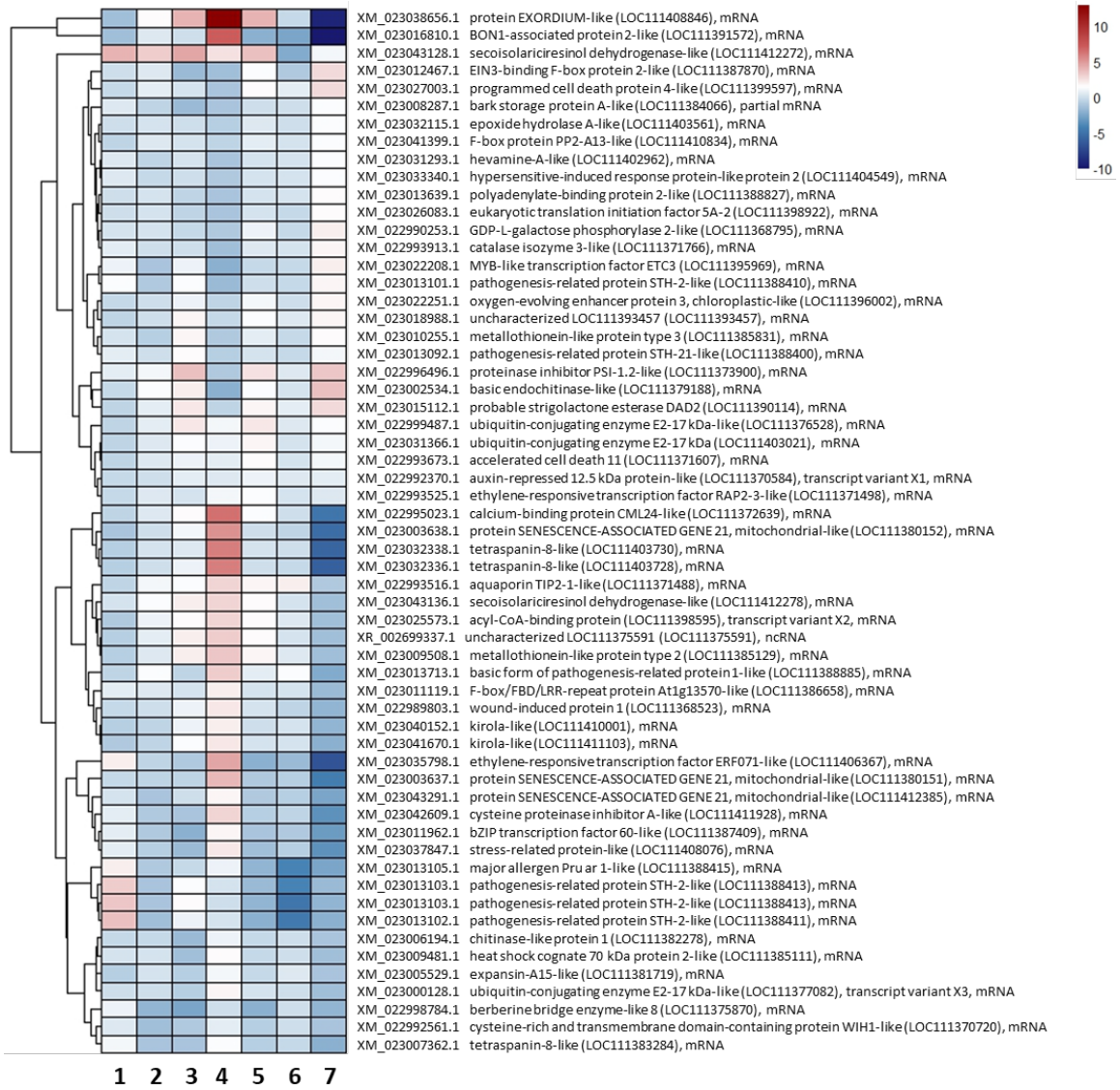
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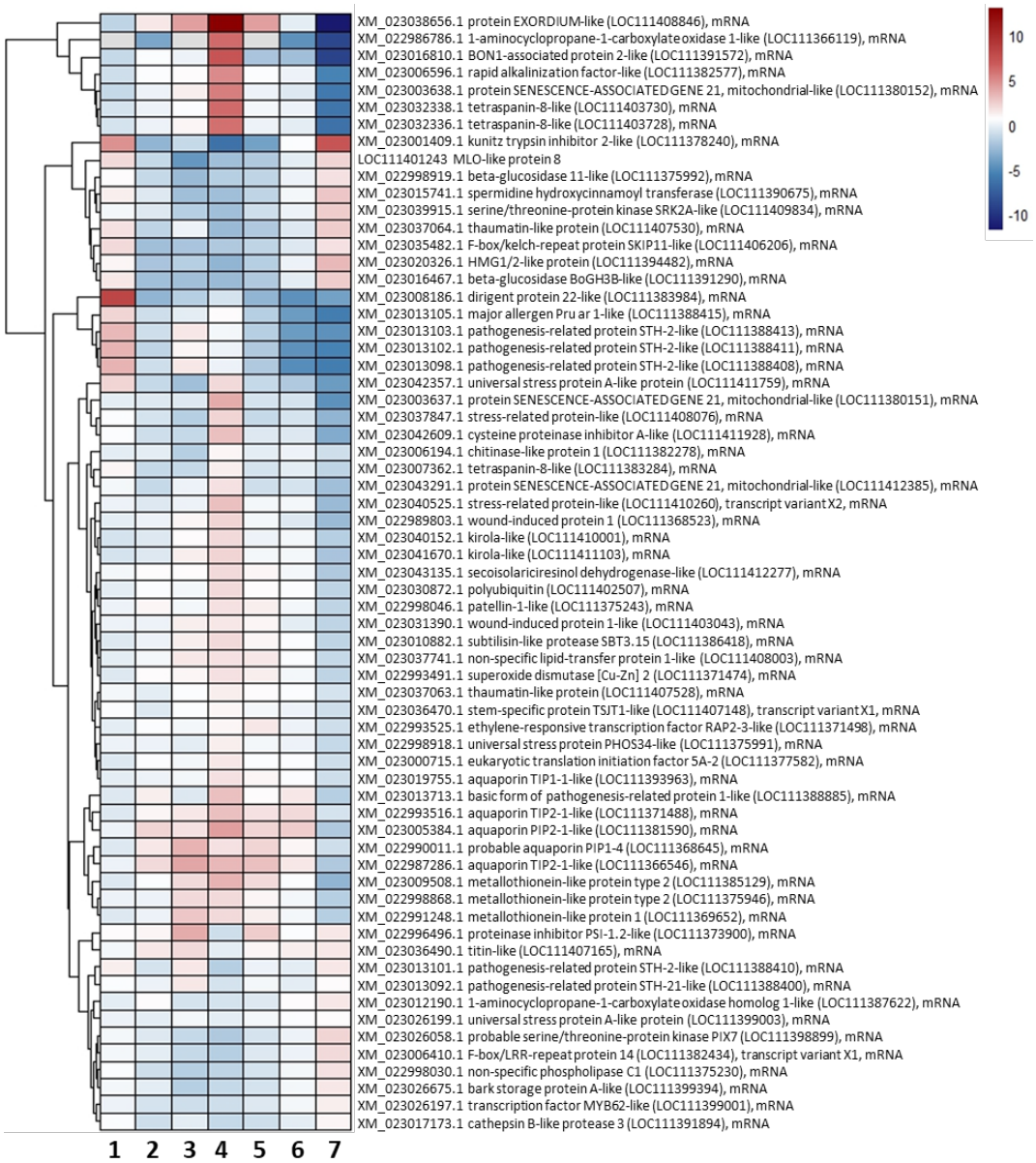
Figure 5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XM_023009508.1 metallothionein-like protein type 2 (LOC111385129), mRNA
XM_022998868.1 metallothionein-like protein type 2 (LOC111375946), mRNA
XM_022991248.1 metallothionein-like protein 1 (LOC111369652), mRNA
XM_022996496.1 proteinase inhibitor PSI-1.2-like (LOC111373900), mRNA
XM_023036490.1 titin-like (LOC111407165), mRNA
XM_023013101.1 pathogenesis-related protein STH-2-like (LOC111388410), mRNA
XM_023013092.1 pathogenesis-related protein STH-21-like (LOC111388400), mRNA
XM_023012190.1 1-aminocyclopropane-1-carboxylate oxidase homolog 1-like (LOC111387622), mRNA
XM_023026199.1 universal stress protein A-like protein (LOC111399003), mRNA
XM_023026058.1 probable serine/threonine-protein kinase PIX7 (LOC111398899), mRNA
XM_023006410.1 F-box/LRR-repeat protein 14 (LOC111382434), transcript variant X1, mRNA
XM_022998030.1 non-specific phospholipase C1 (LOC111375230), mRNA
XM_023026675.1 bark storage protein A-like (LOC111399394), mRNA
XM_023026197.1 transcription factor MYB62-like (LOC111399001), mRNA
XM_023017173.1 cathepsin B-like protease 3 (LOC111391894), mRNA
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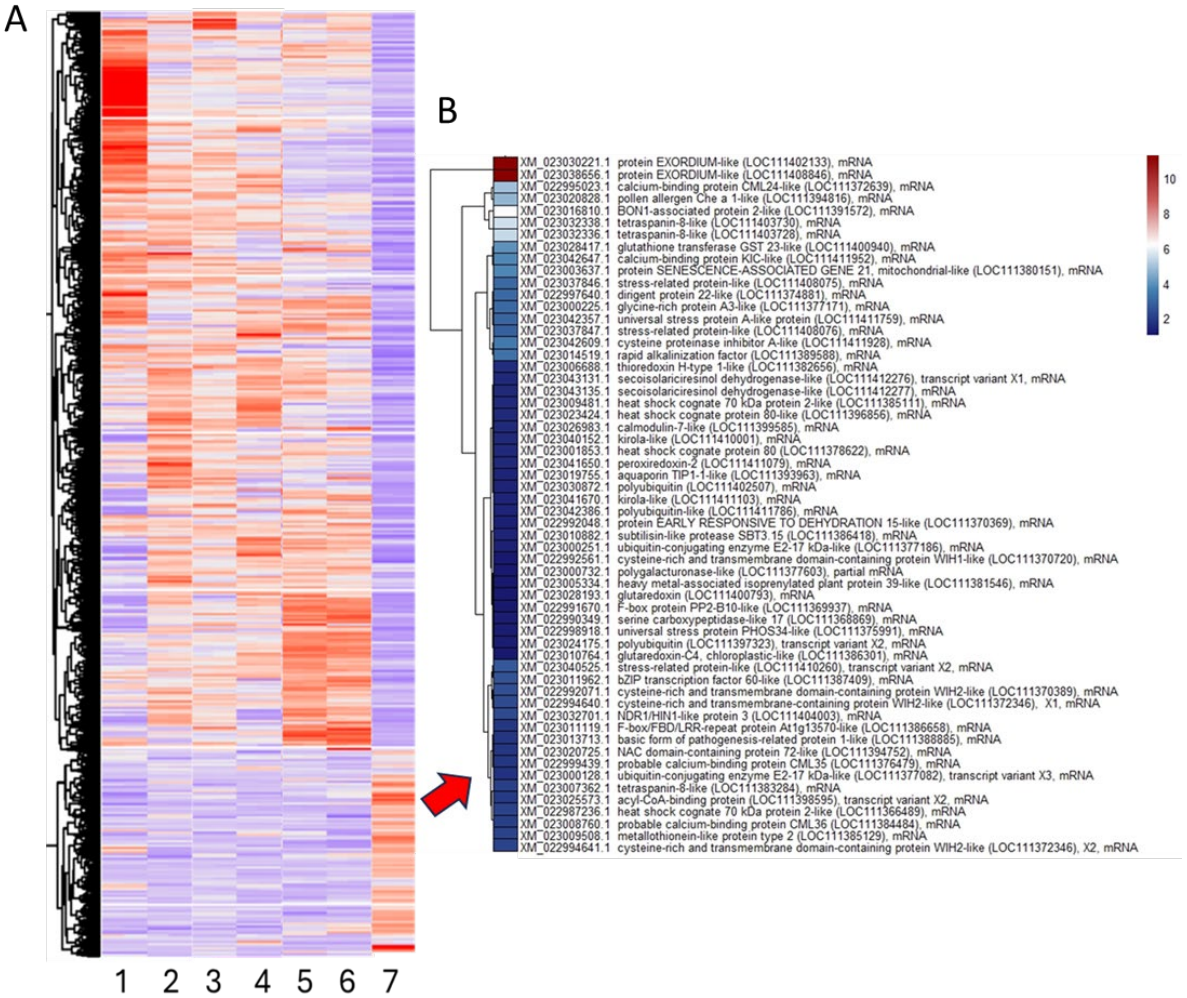
0
-5
-10

Figure 6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B
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XM_023038656.1 protein EXORDIUM-like (LOC111408846), mRNA
XM_022995023.1 calcium-binding protein CML24-like (LOC111372639), mRNA
XM_023020828.1 pollen allergen Che a 1-like (LOC111394816), mRNA
XM_023016810.1 BON1-associated protein 2-like (LOC111391572), mRNA
XM_023032338.1 tetraspanin-8-like (LOC111403730), mRNA
XM_023032336.1 tetraspanin-8-like (LOC111403728), mRNA
XM_023028417.1 glutathione transferase GST 23-like (LOC111400940), mRNA
XM_023042647.1 calcium-binding protein KIC-like (LOC111411952), mRNA
XM_023003637.1 protein SENESCENCE-ASSOCIATED GENE 21, mitochondrial-like (LOC111380151), mRNA
XM_023037846.1 stress-related protein-like (LOC111408075), mRNA
XM_022997640.1 dirigent protein 22-like (LOC111374881), mRNA
XM_023000225.1 glycine-rich protein A3-like (LOC111377171), mRNA
XM_023042357.1 universal stress protein A-like protein (LOC111411759), mRNA
XM_023037847.1 stress-related protein-like (LOC111408076), mRNA
XM_023042609.1 cysteine proteinase inhibitor A-like (LOC111411928), mRNA
XM_023014519.1 rapid alkalinization factor (LOC111389588), mRNA
XM_023006688.1 thioredoxin H-type 1-like (LOC111382656), mRNA
XM_023043131.1 secoisolariciresinol dehydrogenase-like (LOC111412276), transcript variant X1, mRNA
XM_023043135.1 secoisolariciresinol dehydrogenase-like (LOC111412277), mRNA
XM_023009481.1 heat shock cognate 70 kDa protein 2-like (LOC111385111), mRNA
XM_023023424.1 heat shock cognate protein 80-like (LOC111396856), mRNA
XM_023026983.1 calmodulin-7-like (LOC111399585), mRNA
XM_023040152.1 kirola-like (LOC111410001), mRNA
XM_023001853.1 heat shock cognate protein 80 (LOC111378622), mRNA
XM_023041650.1 peroxiredoxin-2 (LOC111411079), mRNA
XM_023019755.1 aquaporin TIP1-1-like (LOC111393963), mRNA
XM_023030872.1 polyubiquitin (LOC111402507), mRNA
XM_023041670.1 kirola-like (LOC111411103), mRNA
XM_023042386.1 polyubiquitin-like (LOC111411786), mRNA
XM_022992048.1 protein EARLY RESPONSIVE TO DEHYDRATION 15-like (LOC111370369), mRNA
XM_023010882.1 subtilisin-like protease SBT3.15 (LOC111386418), mRNA
XM_023000251.1 ubiquitin-conjugating enzyme E2-17 kDa-like (LOC111377186), mRNA
XM_022992561.1 cysteine-rich and transmembrane domain-containing protein WIH1-like (LOC111370720), mRNA
XM_023000732.1 polygalacturonase-like (LOC111377603), partial mRNA
XM_023005334.1 heavy metal-associated isoprenylated plant protein 39-like (LOC111381546), mRNA
XM_023028193.1 glutaredoxin (LOC111400793), mRNA
XM_022991670.1 F-box protein PP2-B10-like (LOC111369937), mRNA
XM_022990349.1 serine carboxypeptidase-like 17 (LOC111368869), mRNA
XM_022998918.1 universal stress protein PHOS34-like (LOC111375991), mRNA
XM_023024175.1 polyubiquitin (LOC111397323), transcript variant X2, mRNA
XM_023010764.1 glutaredoxin-C4, chloroplastic-like (LOC111386301), mRNA
XM_023040525.1 stress-related protein-like (LOC111410260), transcript variant X2, mRNA
XM_023011962.1 bZIP transcription factor 60-like (LOC111387409), mRNA
XM_022992071.1 cysteine-rich and transmembrane domain-containing protein WIH2-like (LOC111370389), mRNA
XM_022994640.1 cysteine-rich and transmembrane-containing protein WIH2-like (LOC111372346), X1, mRNA
XM_023032701.1 NDR1/HIN1-like protein 3 (LOC111404003), mRNA
XM_023011119.1 F-box/FBD/LRR-repeat protein At1g13570-like (LOC111386658), mRNA
XM_023013713.1 basic form of pathogenesis-related protein 1-like (LOC111388885), mRNA
XM_023020725.1 NAC domain-containing protein 72-like (LOC111394752), mRNA
XM_022999439.1 probable calcium-binding protein CML35 (LOC111376479), mRNA
XM_023000128.1 ubiquitin-conjugating enzyme E2-17 kDa-like (LOC111377082), transcript variant X3, mRNA
XM_023007362.1 tetraspanin-8-like (LOC111383284), mRNA
XM_023025573.1 acyl-CoA-binding protein (LOC111398595), transcript variant X2, mRNA
XM_022987236.1 heat shock cognate 70 kDa protein 2-like (LOC111366489), mRNA
XM_023008760.1 probable calcium-binding protein CML36 (LOC111384484), mRNA
XM_023009508.1 metallothionein-like protein type 2 (LOC111385129), mRNA
XM_022994641.1 cysteine-rich and transmembrane domain-containing protein WIH2-like (LOC111372346), X2, mRNA
10
8
6
4
2

Figure 7

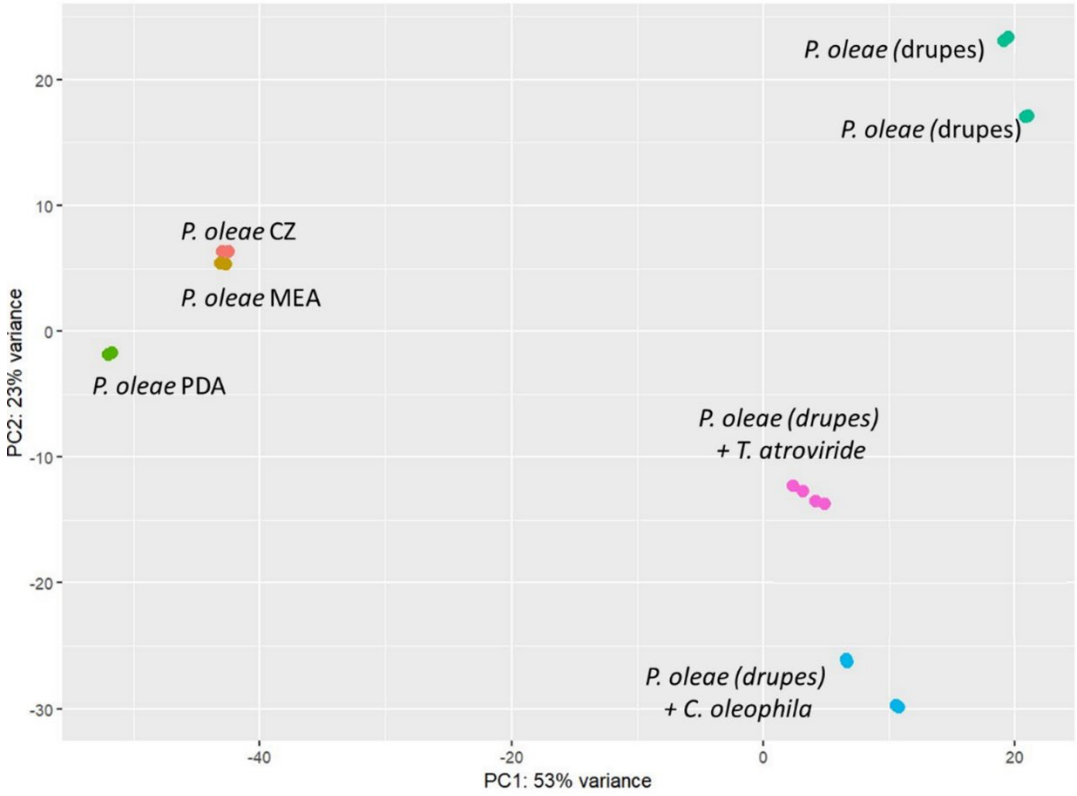

P. oleae (drupes)
P. oleae (drupes)
P. oleae CZ
P. oleae MEA
P. oleae PDA
P. oleae (drupes)
+ T. atroviride
P. oleae (drupes)
+ C. oleophila
20
10
0
-10
-20
-30
-40
-20
0
20
PC2: 23% variance
PC1: 53% variance

Figure 8

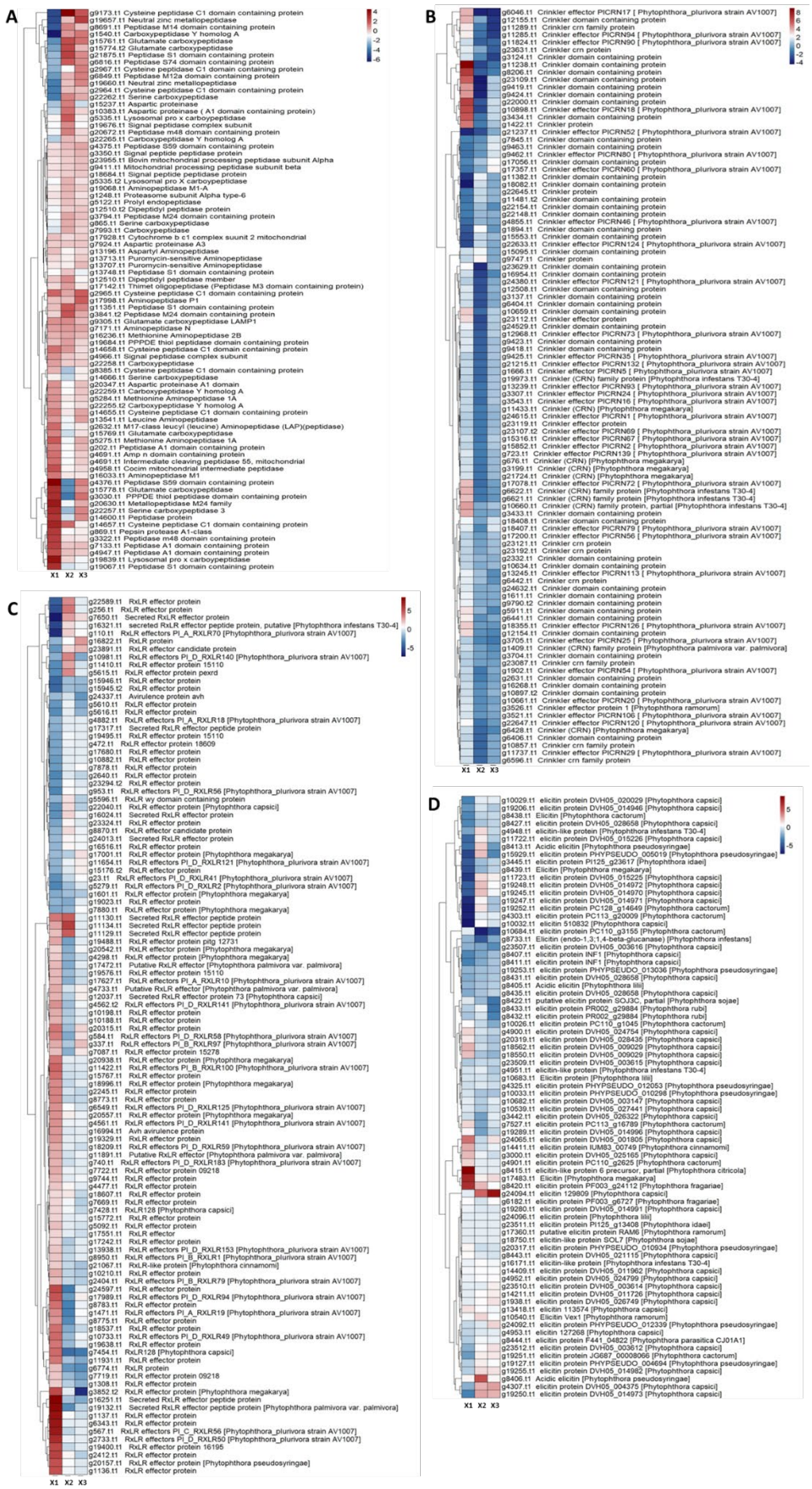
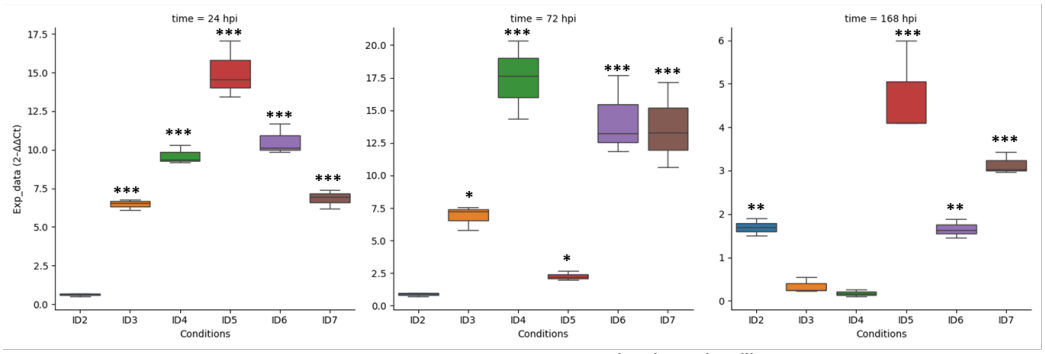
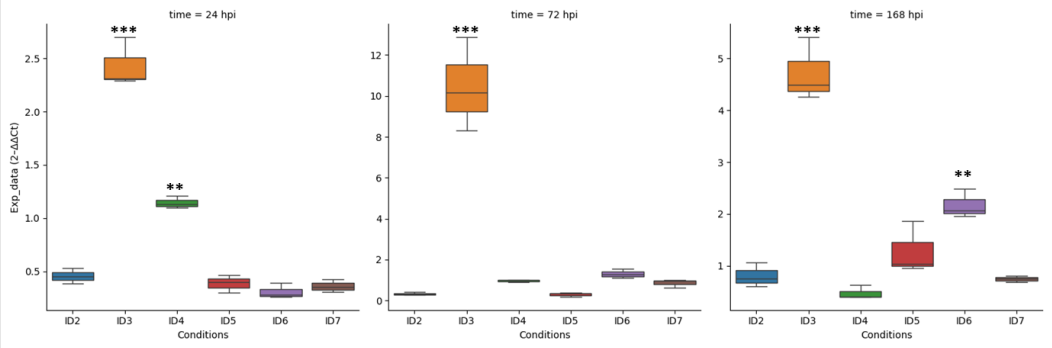


Figure 9

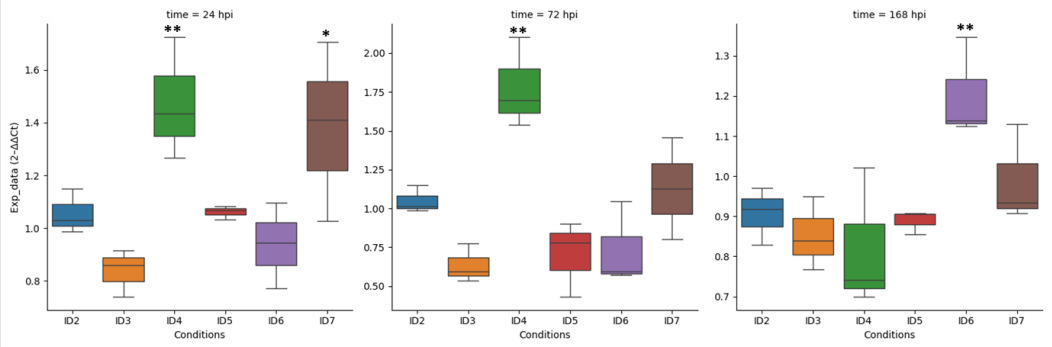
LOC111388413, XM_023013103.1 pathogenesis-related protein STH-2-like



LOC111391572, XM_023016810.1 BON1-associated protein 2-like



LOC111374777, XM_022997515.1 Receptor-like protein kinase FERONIA, transcript variant X2



LOC111403728, XM_023032336.1 tetraspanin-8-like mRNA

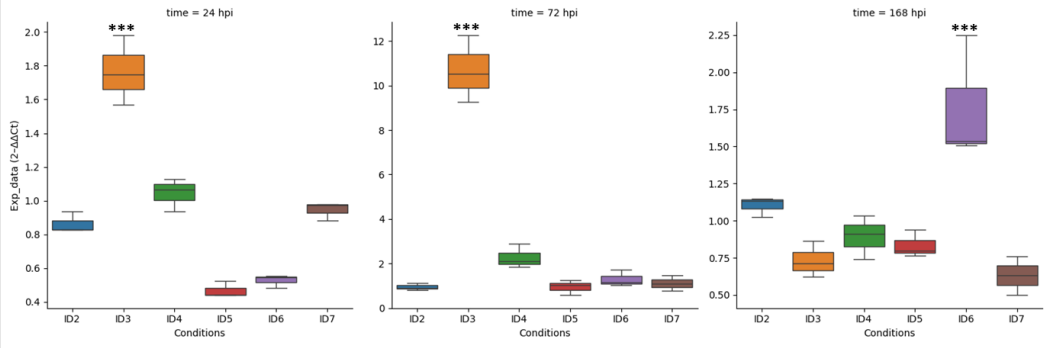


Figure 10

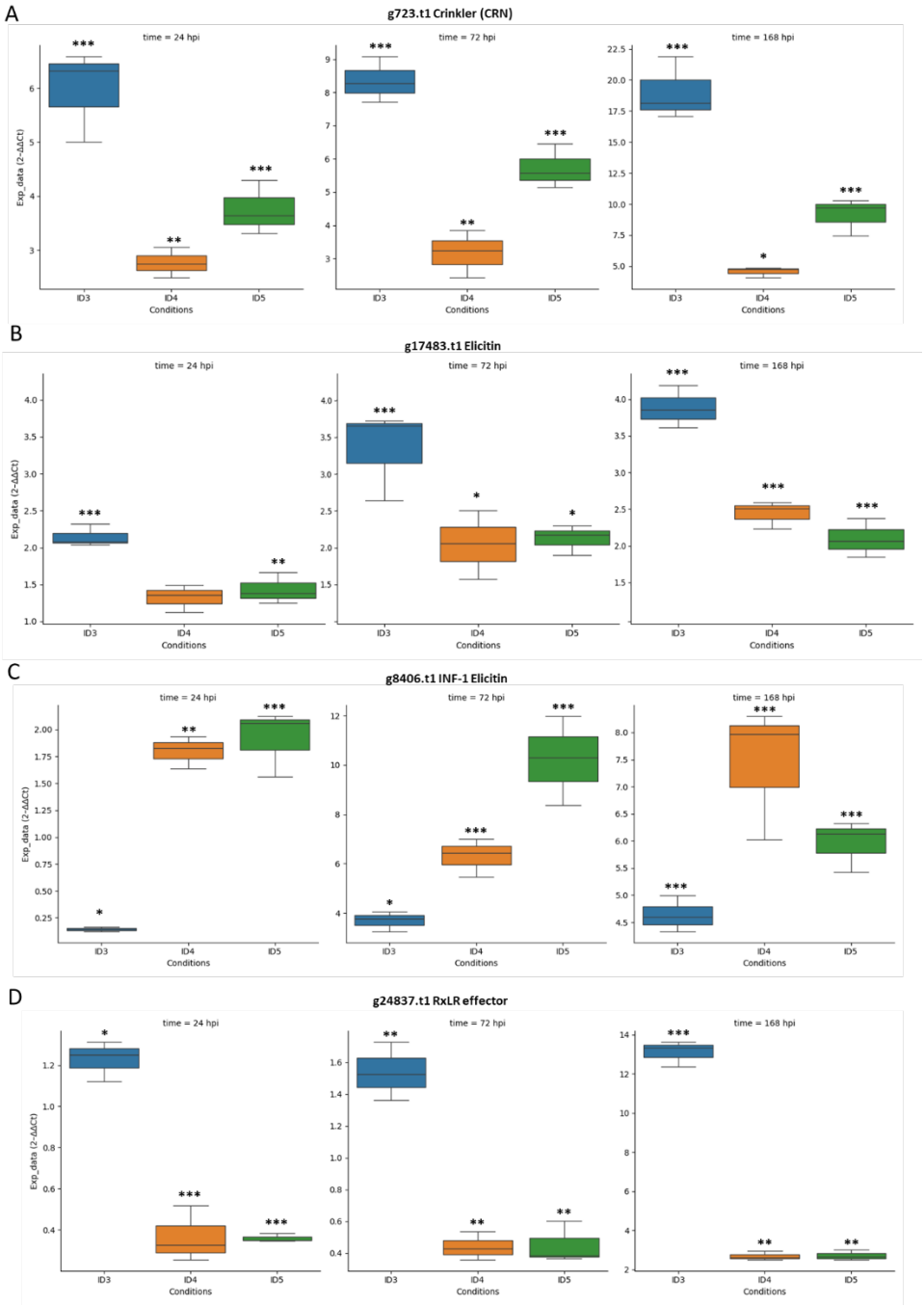

A
g723.t1 Crinkler (CRN)
time = 24 hpi
time = 72 hpi
time = 168 hpi
Exp_data (2-ΔΔCt)
ID3
ID4
ID5
Conditions
B
g17483.t1 Elicitin
C
g8406.t1 INF-1 Elicitin
D
g24837.t1 RxLR effector

Table 1. Proteins with the highest concentration in *Trichoderma atroviride* strain TS-culture filtrate. Identification of the tryptic peptides was made by LC-MS and runs were analyzed by the software Protein Lynx Global Server (PLGS v3.0, Waters, Milford, USA).

Accession	Description	mW (Da)	PLGS Score	Peptides	Theoretical Peptides	Protein Coverage (%)	RMS Mass Error (ppm)
XP_013937770.1	Epl1 Cerato-platanin (eliciting plant response like protein)	14537	3428.453	10	8	44.9275	2.664
XP_013947697.1	Fungal specific cysteine rich protein TRIATDRAFT 297515	14227	2068.27	8	6	21.3333	2.6779
XP_013947323.1	SSCRP homologue to *Alternaria alternata* allergen 1 (TRIATDRAFT 297835)	21243	929.0758	2	7	7.0707	2.8815
XP_013944816.1	FAD-binding containing oxidase TRIATDRAFT 299189	65421	588.5992	17	37	31.9079	2.7963
XP_013947876.1	Calmodulin-like TRIATDRAFT 297616	16971	429.0748	8	24	48.3221	2.9071
XP_013947715.1	Glycoside hydrolase family 72 protein	57251	336.4076	8	28	19.3015	2.3381
XP_013944241.1	Heterokaryon incompatibility protein (HET) TRIATDRAFT 292246	41924	316.7303	8	32	15.2406	2.7148
XP_013938290.1	Collagen triple helix repeat-containing protein TRIATDRAFT 89284	14635	264.8594	7	20	23.2558	2.6052
XP_013938761.1	Coiled-coil domain-containing protein TRIATDRAFT 29005	17709	215.9985	8	15	21.7391	1.7383

Table 2. Selected upregulated mRNA related to *P. oleae* effectors differentially expressed into olive drupes during 72h of infection as compared to the mRNA expression during the growth in PDA (FDR =0.001). The gene expression has been filtered by the highest baseMean FPKM counts from Supplementary Table 11.

gene_id	baseMean	log2FoldChange	nr_accession	description
g8411.t1	11029.8423	-3.4545	AFY98083.1	elicitin protein INF1 [Phytophthora capsici]
g8407.t1	8980.9793	-3.1698	AFY98083.1	elicitin protein INF1 [Phytophthora capsici]
g8435.t1	1273.8463	-2.0990	KAG1694610.1	Acidic elicitin DVH05_028658 [Phytophthora capsici]
g8431.t1	884.7458	-2.1785	KAG1694610.1	Acidic elicitin DVH05_028658 [Phytophthora capsici]
g15852.t1	665.5780	1.0264	KAG7377058.1	Crinkler domain containing protein PHYPSEUDO_012238 [Phytophthora pseudosyringae]
g723.t1	521.1116	1.5093	XP_009516255.1	Crinkler domain containing protein PHYSODRAFT_250419 [Phytophthora sojae]
g17200.t1	492.0255	0.9111	KAG2775728.1	Crinkler (CRN) protein Pcac1_g13827 [Phytophthora cactorum]
g9420.t1	444.7386	0.7532	ETM53299.1	Crinkler effector protein L914_03213 [Phytophthora parasitica]
g8427.t1	427.0925	-3.2409	KAG1694610.1	Acidic elicitin DVH05_028658 [Phytophthora capsici]
g9425.t1	407.7032	0.8020	ETL34626.1	Crinkler effector protein L916_13176 [Phytophthora parasitica]
g16268.t1	364.1512	1.2954	XP_009523239.1	Crinkler domain containing protein PHYSODRAFT_328631 [Phytophthora sojae]
g23121.t1	345.5128	1.0980	KAG2759001.1	Crinkler (CRN) protein Pcac1_g28927 [Phytophthora cactorum]
g23192.t1	332.1388	1.0418	KAG2759001.1	Crinkler (CRN) protein Pcac1_g28927 [Phytophthora cactorum]
g24529.t1	270.2418	1.8162	GMF21132.1	Crinkler (CRN) protein [Phytophthora lilii]
g202.t1	259.0405	0.7907	KAG1704798.1	Apoplastic effector (Aspartic Peptidase A1) DVH05_004825 [Phytophthora capsici]
g4561.t1	251.1319	3.1599	GMF31732.1	Protease DDE [Phytophthora fragariaefolia]
g12968.t1	211.1898	1.3185	ETP25357.1	Crinkler effector protein F441_01749 [Phytophthora parasitica CJ01A1]
g23731.t1	208.4466	-0.9947	KAG1702536.1	RxLR effector protein DVH05_009486 [Phytophthora capsici]
g17483.t1	207.6982	6.7996	OWZ24715.1	Elicitin [Phytophthora megakarya]
g8438.t1	196.5607	-3.2032	KAF1780997.1	Elicitin [Phytophthora cactorum]
g585.t1	178.7704	1.4733	KAG1697742.1	Ubiquitin-dependent protease (DA1) DVH05_015699 [Phytophthora capsici]
g11723.t1	163.7110	-6.3604	KAG1698237.1	Elicitin protein DVH05_015225 [Phytophthora capsici]
g8405.t1	145.8855	-1.8859	GMF34482.1	Acidic elicitin [Phytophthora lilii]
g21188.t1	144.3538	-2.7376	KAG1697993.1	Elicitor transglutaminase protein DVH05_015477 [Phytophthora capsici]
g3030.t1	134.9453	2.5023	KAG1692710.1	PPPDE thiol peptidase DVH05_025188 [Phytophthora capsici]
g14300.t1	126.2690	0.7730	KAG3233470.1	Crinkler domain containing protein PI124_g21451 [Phytophthora idaei]
g23107.t2	119.6059	0.8056	ETL34626.1	Crinkler effector protein L916_13176, partial [Phytophthora parasitica]
g23119.t1	113.2387	0.8713	ETP00001.1	Crinkler effector protein F441_22572, partial [Phytophthora parasitica CJ01A1]
g6404.t1	110.4554	1.1941	KAG3126550.1	Crinkler domain containing protein PC128_g27212 [Phytophthora cactorum]
g3543.t1	108.9492	1.1206	XP_009525765.1	Crinkler domain containing protein PHYSODRAFT_285836 [Phytophthora sojae]
g1666.t1	98.3432	1.0580	KAG3050369.1	Crinkler domain containing protein PI125_g26450 [Phytophthora idaei]
g11549.t1	91.7458	1.0846	ETI44683.1	Cystatin cysteine protease inhibitor EPIC [Phytophthora parasitica P1569]
g1938.t1	87.6575	9.8643	KAG1691739.1	Elicitin protein DVH05_026749 [Phytophthora capsici]
g19255.t1	85.2815	-1.0839	KAG1698440.1	Elicitin protein DVH05_014982 [Phytophthora capsici]
g20938.t1	79.1749	3.7683	OWZ17931.1	RxLR effector protein [Phytophthora megakarya]
g24013.t1	78.5695	-1.7169	KAG1690006.1	Secreted RxLR effector protein DVH05_001833 [Phytophthora capsici]
g2412.t1	78.0523	5.7525	XP_009517714.1	RxLR effector protein PHYSODRAFT_470423, partial [Phytophthora sojae]
g21215.t1	75.0605	1.1873	XP_009525765.1	Crinkler domain containing protein PHYSODRAFT_285836 [Phytophthora sojae]
g3199.t1	69.5818	1.4011	OWZ21632.1	Crinkler (CRN) [Phytophthora megakarya]
g21724.t1	68.8456	1.3974	OWZ21632.1	Crinkler (CRN) [Phytophthora megakarya]
g9756.t1	67.2585	1.4343	KAF1781372.1	Serine protease (peptidase S1) [Phytophthora cactorum]
g16483.t1	63.3187	0.7658	KAG1699076.1	RxLR effector protein DVH05_014446 [Phytophthora capsici]
g9111.t1	62.2498	1.0313	KAG6613212.1	RxLR-like protein [Phytophthora cinnamomi]
g9424.t1	59.2075	4.2622	QST87862.1	Crinkler CRN125_11 protein, partial [Phytophthora capsici]
g21067.t1	58.7755	1.7041	KAG6613212.1	RxLR-like protein [Phytophthora cinnamomi]
g9419.t1	58.3041	4.3746	QST87862.1	Crinkler CRN125_11 protein, partial [Phytophthora capsici]
g676.t1	56.3898	1.2711	OWZ21632.1	Crinkler (CRN) [Phytophthora megakarya]
g24615.t1	55.9194	0.8335	OWZ21632.1	Crinkler (CRN) [Phytophthora megakarya]
g20317.t1	54.3757	0.7280	KAG7377828.1	Elicitin protein PHYPSEUDO_010934 [Phytophthora pseudosyringae]
g11433.t1	54.0197	1.0609	OWZ21632.1	Crinkler (CRN) [Phytophthora megakarya]
g14655.t1	53.7483	0.9118	KAG1684410.1	Cysteine peptidase C1 DVH05_011155 [Phytophthora capsici]
g20315.t1	52.3149	3.8751	XP_009517714.1	RxLR effector protein PHYSODRAFT_470423, partial [Phytophthora sojae]
g19973.t1	50.6314	1.0442	XP_002997237.1	Crinkler (CRN) family protein [Phytophthora infestans T30-4]
g13239.t1	48.1890	0.7862	KAG3105523.1	Crinkler domain containing protein PI125_g13422 [Phytophthora idaei]
g5831.t1	45.8712	0.6652	KAG1713044.1	RxLR protein DVH05_000772 [Phytophthora capsici]
g19206.t1	42.3917	-2.7982	KAG1698404.1	Elicitin protein DVH05_014946 [Phytophthora capsici]
g4307.t1	41.8255	-1.9681	KAG1705445.1	Elicitin protein DVH05_004375 [Phytophthora capsici]
g8773.t1	37.0533	3.4176	KAG7380604.1	RxLR effector protein PHYPSEUDO_006998 [Phytophthora pseudosyringae]
g16251.t1	31.9605	8.4079	KAG1693317.1	Secreted RxLR effector peptide proteinDVH05_023781 [Phytophthora capsici]
g20157.t1	22.6564	5.2388	A0A8T1VW52	RxLR effector protein [Phytophthora pseudosyringae]
g8439.t1	21.8038	-6.5190	OWZ01960.1	Elicitin [Phytophthora megakarya]
g13418.t1	20.3765	1.2881	AIU99520.1	Elicitin 113574 [Phytophthora capsici]
g10032.t1	16.6270	-7.5518	AIU99526.1	Elicitin 510832 [Phytophthora capsici]
g11722.t1	15.8853	-4.8215	KAG1698238.1	Elicitin protein DVH05_015226 [Phytophthora capsici]
g14657.t1	14.4881	1.9889	KAG1684411.1	Cysteine peptidase C1 DVH05_011156 [Phytophthora capsici]
g16516.t1	11.8968	-1.8912	POM73746.1	Avr1b-1 Avirulence-like protein (RxLR protein) [Phytophthora palmivora var. palmivora]
g22048.t1	10.1113	-6.8316	KAG2767785.1	Elicitor transglutaminase protein Pcac1_g20938 [Phytophthora cactorum]
g19132.t1	8.0089	6.4152	POM65797.1	Secreted RxLR effector peptide protein [Phytophthora palmivora var. palmivora]
g19245.t1	6.8210	-5.7677	KAG1698428.1	Elicitin protein ral13e DVH05_014970 [Phytophthora capsici]
g19253.t1	5.5695	-1.9078	KAG7376623.1	Elicitin protein PHYPSEUDO_013036 [Phytophthora pseudosyringae]
g11296.t1	3.3055	3.6026	AUD40032.1	Necrosis inducing protein (Nep1-like Pc118551) [Phytophthora capsici]
g22046.t1	1.9340	-4.4446	KAG1705011.1	Elicitor transglutaminase protein DVH05_005036 [Phytophthora capsici]