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 microrganisms 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 microrganism. A more rapid effect would be an additional advantage of metabolites

57 over the living microorganism, as in the olive oil supply chain the post-harvest phase preceding the oil
58 extraction is very brief. Yeasts are other effective BCAs of post-harvest fruit diseases[25–27]. A very interesting
59 antagonistic yeast is *Candida oleophila*. which has showed a biocontrol activity against several post-harvest
60 diseases of diverse fruit crops. Strains of this yeast have been exploited as commercial bioproducts[19,28–30].
61 *Candida oleophila* strain O is the active ingredient of the bioproduct registered under the name of Nexy®.
62 Several lines of evidence indicate multiple modes of action contribute to the effectiveness of BCAs in
63 suppressing plant diseases, including the elicitation of plant immune system[31]. However, few studies have
64 addressed the transcriptomic analysis of the complex interaction pathogen/host/BCA. *Phytophthora* species
65 are known to secrete a large array of effectors during infection of the plant host. In recent decades, significant
66 progresses have been made in identifying numerous *Phytophthora* effectors, broadly categorized into two main
67 types: CRN and RxLR effectors.[32] The CRN proteins are a family of effectors that cause necrosis in the cells
68 of the host and also induce further intracellular effectors that target the host nucleus during infection. RxLR
69 effectors play a central role in pathogen–plant interactions. They are secreted proteins that manipulate the
70 host's immune responses, allowing the pathogen to establish infection[33,34]. Although several studies have
71 already elucidated the role of CRN and RxLR proteins in several *Phytophthora* spp., there is a lack of
72 knowledge about the role of these effectors in the pathosystem *P. oleae*/olive drupes.
73 Successful *Phytophthora* pathogens have evolved and adapted to a specific host range, developing a set of
74 effector proteins and phytotoxins that either directly induce plant cell death or suppress pattern-triggered
75 immunity (PTI) and trigger susceptibility of the host. Nevertheless, some pathogenesis-related proteins (PR-
76 proteins) are constitutive in some plants and represent a first barrier to the pathogen conveying a sort of innate
77 immunity. Most of the time, even such constitutive levels of PR-proteins might be triggered by the ecto-
78 symbiosis with microorganisms, such as *Trichoderma* spp., or their metabolites known to trigger both growth
79 promotional effects and plant defense mechanisms by the elicitation of salicylic acid (SA)-, ethylene (ET)-,
80 and jasmonic acid (JA)-dependent processes[22,35].
81 The aims of this study were: i. to evaluate the effectiveness of either *Trichoderma atroviride* culture filtrates
82 or *C. oleophila* strain O in suppressing rot of olive drupes inoculated with *P. oleae*; ii. to examine the
83 differential gene expression in *P. oleae* and olive drupes, treated/non treated with *C. oleophila* or *T. atroviride*-
84 culture filtrate by high throughput technologies (*i.e.* Illumina RNA sequencing).

85

86

87 **2. Results**

88 **2.1. Evaluating the effectiveness of *Candida oleophila* strain O and *Trichoderma atroviride*-culture**
89 **filtrate in controlling the rot of olive drupes incited by *Phytophthora oleae***

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

101 **2.2. Differential RNA analysis at 72 hours post inoculation with *Phytophthora oleae***

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

113 The following paragraphs describe the RNAseq analysis of olive fruit performed by analyzing binary
114 conditions (see paragraphs: comparisons 1, 2, 3, 4, 6 and 7). A cluster analysis was also carried out among all
115 the treatments for the olive genes in all the conditions analyzed (see paragraph: comparison 5). Finally, a
116 RNAseq analysis was carried out in the same samples to highlight the differential mRNA changes of *P. oleae*
117 versus the different treatments with either *C. oleophila* cell suspension or *T. atroviride*-culture filtrate; to this
118 aim, pairwise comparisons (see paragraphs: comparisons 8 and 9) or PCA plots were elaborated.

119 **2.2.1. Comparison 1: intact olive drupes (treatment ID1) vs. wounded drupes treated with sterile distilled**
120 **water (treatment ID2)**

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

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

141 represented by a family of receptors localized in the (ER) membrane. Two main types of ethylene receptors
142 are known; Ethylene response1 (ETR1) and ERS1, belong to subfamily 1c, and ETR2, EIN4, and ERS2 belong
143 to subfamily 2[43]. Other TFs are activated by ET, such as APETALA2-ethylene responsive factors (AP2-ERFs)
144 which are mediators of stress responses and developmental programs[44]. Many ET-related TFs here were DEGs
145 as the ETR TF 1B-like (LOC111378715) which belong to the AP2 DNA-binding TFs in plants such as
146 APETALA2 and EREBPs[45]. The elicitin INF-1 of *P. infestans* induced several defense responses in *Nicotiana*
147 spp., including reactive oxygen species (ROS) and ET production with consequent hypersensitive cell death
148 (HCD) and accumulation of the sesquiterpenoid phytoalexin capsidiol[46]. In this specific case, the production
149 of phytoalexins is regulated by the AP2/ERF TF, NbERF-IX-33[46].
150 Dirigent (DIR) protein 22-like (LOC111383984) was also a highly upregulated DEG, probably involved in
151 catalyzing regio- and stereoselectivity of bimolecular phenoxy radical coupling during lignin or lignan
152 biosynthesis. Dirigent proteins in plants modulate cell wall homeostasis during abiotic and biotic stress[47].
153 Another top DEG, which is typically expressed after wounding, is beta-amyrin 28-oxidase-like
154 (LOC111396592), a cytochrome CYP716A244 which is implicated in the biosynthesis of oleanane-type
155 saponin biosynthesis[48]. Beta-amyrin synthase and beta-amyrin oxidase were characterized in a yeast, where
156 they were able to form not only α-amyrin 6, but also β-amyrin 1, butyrospermol 23, and Ψ-taraxasterol 19[48].
157 Nonsterol triterpenoids of *O. europaea* are then metabolized into multioxygenated compounds and these could
158 represent the precursors of triterpene saponins[49]. Triterpenoids as well as saponins isolated from various plant
159 organs from different species are known to possess antimicrobial as well as antioxidant activities[50].
160 Among top DEGs, it is noteworthy the presence of elicitor-responsive protein 1-like (LOC111406007), which
161 encodes a protein possessing C2 domains which confer a Ca^{2+}-dependence or binding activity. Usually, such
162 C2 domains bind a wide variety of substances including bound phospholipids, inositol polyphosphates, and
163 intracellular proteins probably acting as signal transduction enzymes. The excess of calcium release during the
164 wound or infection can disrupt the calcium signaling. In *Arabidopsis*, its homologue, NP_001078296.1, is
165 annotated as a calcium-dependent lipid-binding (CaLB domain) family protein, and it acts as a novel repressor
166 of abiotic stress response by chelating the excess free Ca^{2+} [51].
167 Among the innate immunity activated defense genes/proteins which have been detected here as DEGs are
168 protease inhibitors (e.g., glu, ase, PSI, potato proteinase inhibitor I, kunitz trypsin inhibitor 2), endo-1,3-beta-

169 glucosidases, endo- and exo-chitinases, pathogenesis-related protein STH-2-like, thaumatin-like protein,
170 cysteine proteinase inhibitor B-like, and defensin-like protein (Supplementary Table 1).

171 **2.2.2. Comparison 2: *Phytophthora oleae*-inoculated wounded drupes (treatment ID3) vs. sterile distilled**
172 **water-treated wounded drupes (treatment ID2)**

173 About 2466 (854 upregulated and 1612 downregulated) genes were differentially expressed with a FDR p-
174 value of 0.001; top upregulated genes are shown in Figure 2. However, when differentially expressed genes
175 are analyzed by the DESeq2 algorithm, a base PFKM mean is also given. Both log2FoldChange and the
176 baseMean of the FPKM counts are not high enough to have a real biological significance in the differential
177 RNAseq data. Therefore, both log2FoldChange (L2FC) and PFKM (baseMean) have been further filtered by
178 a baseMean value above 2.5 (Figure 3).

179 The top differentially expressed transcripts were mRNA encoding many EXORDIUM-like proteins (e.g.
180 LOC111408846, LOC111402133, LOC111389937, etc.), presumably involved in cell proliferation.
181 EXORDIUM proteins were first identified as mediators of brassinosteroid (BR)-promoted growth in
182 *Arabidopsis*[52], but they can be negatively regulated by exogenous cytokinin[53]. EXORDIUM 2 like (EXL2,
183 Glyma15g06010) was upregulated and classified among the incompatible interaction genes (IIGs) in the case
184 of *P. sojae* infecting soya bean near isogenic lines (NILs) and associated with resistance[54].

185 Another differentially expressed gene was the BON1-associated protein 2 (LOC111391572) which is part of
186 the regulatory complex formed by the BONZAI1 (BON1):BON1-associated protein (BAP1) proteins[55].
187 BON1-associated proteins seem to exert a negative regulator role related to cell death and defense responses.
188 In plants, BON1 and BAP1 protein-protein interaction sends a negative activation signal to a suppressor of
189 npr1-1, constitutive 1 (SNC1), a nuclear R protein[56]. The product of R gene SNC1 represents a hub of different
190 signaling events coming from many different protein receptor kinases at the plasma membrane, which mediate
191 the final event leading to the activation, or not, of plant PTI or effector-triggered immunity[56].

192 Ethylene biosynthesis is also highly upregulated in the case of *P. oleae* infection, since the final enzyme
193 involved in its biosynthesis, the 1-aminocyclopropane-1-carboxylate oxidase 1-like (ACO, LOC111366119),
194 is markedly upregulated as compared to wounded fruit treated with sdw (L2FC≥ 7.6). Two other ACO isogenes
195 were detected as differentially expressed genes (e.g. LOC111370655 LF2C≥4 and LOC111370659
196 L2FC≥1.9), suggesting that this final step of ET biosynthesis occurs to a high degree during infection[57,58].

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

225 (LOC111399700). Genome remodeling via epigenetics (changes in methylation level) and transposases
226 activity are known to occur during pathogen attack and the development of plant immunity[65]. In plants,
227 acetylation of histones H3 and H4 (H3ac and H4ac), trimethylation on lysine residues, and mono-
228 ubiquitination of H2B (H2Bub1) are normally signs of heterochromatin formation with enhanced
229 transcriptional activation[66]. Transcriptional remodeling and genome rearrangement including retrotransposon
230 jumping within the chromatin play a central role in plant defense mechanisms[67,68]. Based on *de-novo* synthesis
231 of histones alone, it cannot be possible to discern their epigenetic post-translational modification, but certainly,
232 artificial treatment of plants with SA induces acetylation of histones H3 and H4[69].
233 Other differentially expressed genes are the product of the genes encoding olive E3 ubiquitin-protein ligases.
234 Some of those could be the homologue of Arabidopsis RING E3 ligase HUB1 which mediates histone mono-
235 ubiquitination with the subsequent activation of heterochromatin and probably contributes to enhancement of
236 the defense mechanism[70].
237 That genome rearrangement could be linked to the induction of plant resistance that is evident when DDE-type
238 transposases (containing a DD[E/D]-motif) are involved[71]. In this study, it is highly probable that *P. oleae*
239 induced a high expression of the protein ALP1-like (LOC111383726), which possesses a N-terminal RNase
240 H domain, a DNA binding domain and a known transposase DDE C-terminal domain. Demethylation of
241 transposable elements (TEs) makes them jump in heterochromatin genes, which, in this case, could be
242 associated with mediating a 'long-term memory', since their insertion into the promoter of these genes makes
243 them constitutively expressed. This epigenetic stress memory can be transmitted to subsequent generations
244 and involves changes in the silencing of transposable elements (TEs) by DNA methylation, histone
245 modifications, and non-coding RNAs. At late infection stages, the DNA jumping activities of functional class
246 I ('copy and paste') and class II ('cut and paste') TEs, might result in small and large mutations at the sites of
247 excision (class 2) and insertion (class 1 and 2). TE-induced insertion/excision accelerates the evolution of
248 novel defense regulatory genes and, because TEs are somewhat regulated by epigenetic mechanisms, the
249 derived defense genes will remain under stress-dependent epigenetic control and potentially able to resist to
250 biotic stresses[72].
251 Transcription factors (TFs) known to be involved during both wound response and pathogen attack are the
252 WRKY TFs[73]. These TFs contain a 60-70 amino acid protein domain composed of a conserved WRKYGQK

253 motif and a zinc-finger region (C2HC). Both domains contribute to the capability of DNA to bind with W-box
254 and/or other cis-acting elements, such as in R-genes[74] or biosynthetic genes related to specialized metabolism[75].
255 Upon *P. oleae* infection, it has been recorded an upregulated (L2FC≥ 3.4) olive WRKY TF 33
256 (LOC111405948) homologue of AtWRKY33, which functions as a positive regulator of resistance toward
257 some necrotrophic fungi[76]. However, the olive WRKY TF 18-like (LOC111377241) was even more
258 differentially expressed (L2FC≥ 4.6) and, according to the homology function with AtWRKY18, could
259 enhance certain defense signaling, boosting the transcription of PR genes and resistance against certain
260 pathogens[77]. Other upregulated WRKY TFs include WRKY TF 53 (LOC111367778. L2FC>6.1), WRKY
261 transcription factor 23 (LOC111388449, L2FC>4.7), WRKY transcription factor 70 (LOC111392938,
262 L2FC>4.3), and WRKY transcription factor 57 (LOC111373803, L2FC>1.2).
263 The main differentially upregulated R-genes were the LEAF RUST 10 DISEASE-RESISTANCE LOCUS
264 RECEPTOR-LIKE PROTEIN KINASE-like 1.1 (LOC111390435, L2FC>5.2), the protein ENHANCED
265 DISEASE RESISTANCE 2 (LOC111389201, L2FC>5), the disease resistance protein At5g63020
266 (LOC111397442, L2FC>5), receptor-like protein kinase HERK 1 (LOC111407351, L2FC>5.5;
267 LOC111407714, L2FC>2.3), the rust resistance kinase Lr10-like (LOC111411655, L2FC>3.6), the disease
268 resistance RPP13-like protein 1 (LOC111400669, L2FC>3.6), the disease resistance protein RGA1
269 (LOC111392054, L2FC>3.2), and the disease resistance RPP13-like protein 4 (LOC111379833, L2FC>2.2).
270 These plasma membrane-located receptors possess a nucleotide-binding site–leucine-rich repeat (NBS-LRR)
271 and a protein kinase C domain for intracellular signal transduction and are probably involved in the signaling
272 cascade of pathogen sensing[78]. On the other hand, upregulated F-box/LRR-repeat proteins might be associated
273 with resistance to *Phytophthora* species, since for instance, in cacao (*Theobroma cacao*), similar genes were
274 differentially upregulated in response to *P. megakarya* only in a resistant cultivar[79]. Here, upregulated F-
275 box/LRR-repeat proteins were represented by F-box/FBD/LRR-repeat protein At1g13570-like
276 (LOC111386658) and the F-box/kelch-repeat protein At1g67480-like (LOC111406244) (Supplementary
277 Table 2). Other differentially expressed TFs during *P. oleae* infection are the ethylene-responsive TFs
278 ERF104-like (LOC111386758), ERF017-like (LOC111373863), ERF017-like (LOC111373875), ERF118-
279 like (LOC111403203), ERF071-like (LOC111406367), RAP2-3-like (LOC111403560), ERF4-like
280 (LOC111395465), and ERF04 (LOC111381486). Ethylene induced immunity involves sensing and TF-

281 responsive transcription; this ET-pathway interacts both positively and negatively with the SA-pathway, in
282 relation to the type of plant and pathogen[80]. The APETALA2 (AP2)/ETHYLENE RESPONSE FACTOR
283 (ERF) transcription factor ORA59 is a major positive switch of the ET/JA-mediated defense pathway in *A.*
284 *thaliana*. In *Arabidopsis*, biosynthetic genes responsible for the formation of hydroxycinnamic acid amides
285 (HCAAs), known as phytoalexins, are regulated by ORA59 and other ET-responsive TFs[81].
286 Jasmonic acid (JA) and its precursors and derivatives, referred to as jasmonates (Jas), are important molecules
287 in the regulation of plant responses to biotic and abiotic stresses[82]. JA-dependent defense responses are
288 predominantly effective against necrotrophic pathogens and their downstream signaling pathway is typically
289 targeted by fungal effectors to prevent the expression of JA-related defense genes. Coronatine-insensitive
290 protein 1 (COI1) is an F-box protein essential for all the jasmonate responses. COI1 interacts with multiple
291 proteins to form the SCF:COI1:E3 ubiquitin ligase complex which recruits jasmonate ZIM-domain (JAZ)
292 proteins for degradation by the 26S proteasome. *Phytophthora sojae* uses an RXLR effector, Avh94, to
293 manipulate host JA signaling to promote infection hijacking-JA signaling[83]. Zhao et al. have demonstrated that
294 Avh94 interacts with soybean JAZ1 or JAZ2, which is a repressor of jasmonic acid (JA) signaling[83]. Methyl
295 jasmonate (MeJA)-triggered JAZ1 protein degradation could be inhibited by the proteasome inhibitor MG132,
296 suggesting that JAZ1 is consistently degraded by the 26S proteasome. Avh94 bind JAZ1 and protect it against
297 methyl jasmonate-induced degradation. In turn, the JAZ proteins act as repressors of JA TFs such as AtMYC2,
298 which is involved in the induction of JA biosynthetic genes. In the case of *P. oleae* infection, the coronatine-
299 insensitive protein 1-like (LOC111390662) is a differentially expressed gene and could represent a jasmonate
300 receptor. The highly differential expression of COI1 is accompanied by other upregulated differentially
301 expressed genes encoding F-box proteins: F-box protein At3g07870-like (LOC111409106), F-box protein
302 At1g61340-like (LOC111407155), and F-box protein PP2-A12-like (LOC111408420).
303 Among differentially expressed genes related to the PR proteins, in the case of *P. oleae* infection, two genes
304 are predominating, the PR proteins STH-2-like (LOC111388408, L2FC≥5) and STH-1-like (LOC111388885,
305 L2FC≥2.3). LOC111388408 was a gene upregulated in response to wounding, but it is even more upregulated
306 in response to the pathogen. Furthermore, another upregulated gene, as compared to the wound condition itself,
307 is the cysteine proteinase inhibitor B-like (LOC111390855, L2FC≥6) and the cysteine proteinase inhibitor A-
308 like (LOC111411928, L2FC>3) which could act in defense, trying to block several *P. oleae* cysteine proteases.

309 An upregulated gene related to the apoplastic immunity/defense protease was the cysteine protease RD19B
310 (LOC111394575) of the *O. europaea* homologue of RD19, an *Arabidopsis* cysteine protease that modulates
311 RESISTANT TO RALSTONIA SOLANACEARUM 1-R (RRS1-R)[84]. Other differentially expressed genes
312 related to defense response, from highest to lowest L2FC, respectively, are pollen allergen Che a 1-like
313 (LOC111394816, L2FC≥4.7), subtilisin inhibitor CLSI-I-like (LOC111374455, L2FC≥4.5), and kirola-like
314 proteins (LOC111411103, and LOC111410001). On the other hand, a defense response can be represented by
315 the upregulated expression of proteases such as aspartic proteinase Asp1-like (LOC111394357, L2FC>2.2),
316 subtilisin-like protease SBT1.6 (LOC111374885, L2FC>2.1), and SBT3.15 (LOC111386418, L2FC>1.7).
317 For most of the common differentially expressed genes detected in this comparison, a higher level of
318 upregulation was generally observed. Importantly, tetraspanin-2 (LOC111399506) and tetraspanin-8-like
319 (LOC111403728, and LOC111383284) are genes related to the wounding condition itself (Figure 1).
320 Tetraspanins are transmembrane specific proteins of extracellular vesicles (EVs) or exosomes (EXOs)[85], which
321 possess the function of cargo vesicle shipping molecules involved in plant–pathogen interaction. Exosomes
322 transport lipids, proteins (e.g. PR proteins), messenger RNA, and especially microRNAs[86]. Silencing of the
323 pathogens by miRNA is a recent trend in pathogen resistance[87]. Indeed, plant EVs inhibit fungal growth and
324 virulence by transferring their cargo content into fungal cells at the appressoria/haustoria[88]. Another role of
325 tetraspanins is in moving the EVs through infection hot spots and allowing the exosomal NADPH oxidase
326 (NOX) to release ROS molecules locally (e. g. H_2O_2 production).
327 It is also worth mentioning the differential expression of the gene encoding for the receptor-like protein kinase
328 FERONIA (FER; LOC111374777, L2FC≥5.5) (Supplementary Table 2). FER negatively regulates plant
329 immunity by inhibiting JA and coronatine (COR) signaling. It phosphorylates and destabilizes MYC2, the
330 master regulator of JA signaling, which otherwise activates SA biosynthesis[89]. FER, in response to abiotic and
331 biotic stress, acts as a sensor of cell wall integrity which could be challenged by pathogen secreted enzymes[90].
332 FER may negatively regulate plant immunity to biotrophic pathogens[91]. The plant rapid alkalinization factor
333 (RALF) are secreted peptides that were first identified through their ability to trigger a rapid increase in
334 extracellular pH [a]. Their mechanism of action is through binding to FERONIA, that once activated triggers
335 extracellular alkalinization, to favor pathogenic subtiliases (alkaline proteases) or alkaline cysteine proteases
336 and inhibits the defense response[91].

337 **2.2.3. Comparison 3: wounded olive drupes pre-treated with *Candida oleophila* and inoculated with**
338 ***Phytophthora oleae* (treatment ID5) vs. wounded olive drupes inoculated with *P. oleae* (treatment ID3).**
339 A total of 1883 (1018 upregulated and 865 downregulated) olive differentially expressed genes were detected
340 in this comparison (Supplementary Table 3). It is known that *C. oleophila* secretes various cell wall-degrading
341 enzymes, exo-β-1,3-glucanases, chitinases, and proteases, which are especially active against filamentous
342 fungi[92]. *Candida oleophila* has an *in vitro* effect on the inhibition of mycelial growth of several *Phytophthora*
343 species[93]. Due to this beneficial effect, some of the genes in *O. europaea* related to the pathogen response
344 could be downregulated, since the pre-treatment ameliorates the pathogenic index (rAUDPC = 0.21; Figure 1,
345 IDC). Thus, the downregulated genes, in this comparison, could represent the olive genes that help to
346 counteract the pathogen invasion, or which are no longer needed since the pathogenic effect of *P. oleae*
347 decreased due to *C. oleophila* pre-treatment. Among the top downregulated genes with L2FC>-21 were ALA-
348 interacting subunit 1-like (LOC111368552), the ras-related protein Rab7 (LOC111380462), and protein
349 YIF1B-like (LOC111391136), all localized into the Golgi apparatus, regulating endomembrane dynamics and
350 involved in secretory vesicle formation[94]. In the specific case here, RABs, which are the largest family of small
351 guanosine triphosphate (GTP)-binding proteins, are involved in intracellular trafficking and in autophagy or
352 plant microbe interactions, and in biotic and abiotic stress responses[94], for instance, the secretion of
353 PATHOGENESIS-RELATED 1 (PR1) protein to the apoplast. *Phytophthora capsici* RxLR effector RxLR242
354 has been found to bind RABE1-7, a ras-related protein, and inhibits vesicle trafficking[95]. This detected decrease
355 of vesicle trafficking is accompanied by the strong downregulation of the WRKY TF 18-like (LOC111377241)
356 here with L2FC>-8.5 instead of +3.5 as for *P. oleae* infection itself. Otherwise, WRKY 18, as mentioned,
357 could have further enhanced another defense signaling[75].
358 One of the possible mechanisms by which *O. europaea* counteracts *P. oleae* infection is through synthesis of
359 sesquiterpenoid phytoalexins. In potato challenged by *P. infestans*, it has been found that the infected tissue
360 produces antimicrobial sesquiterpenoid phytoalexins (*e.g.* lubimin and rishitin) which might have been
361 implicated in a resistance mechanism in some cultivars[96]. Here, there is a clear downregulation of
362 sesquiterpenoid phytoalexin biosynthesis when drupes are pre-treated with *C. oleophila* and inoculated with
363 *P. oleae* (treatment ID5) as compared to drupes inoculated with *P. oleae* (treatment ID3) (Supplementary Table
364 3). Vetispiradiene synthases or sesquiterpene cyclases and cytochrome P450 premnaspirodiene oxygenases

365 catalyze several key biosynthetic steps in the synthesis of antimicrobial (phytoalexins) sesquiterpenes[97].
366 Indeed, the expression of olive vetispiradiene synthase 2-like (LOC111371938, L2FC>-7.1), vetispiradiene
367 synthase 2-like (LOC111372151, L2FC>-7), premnaspirodiene oxygenase-like (LOC111369993, L2FC>-6.5;
368 LOC111383354, L2FC>-5.9; LOC111375139, L2FC>-5.2; LOC111374442, L2FC>-4.6), vetispiradiene
369 synthase 2-like (LOC111394812, L2FC>-6.3), vetispiradiene synthase 2-like (LOC111394298, L2FC>-5.7),
370 and (-)-isopiperitenol/(-)-carveol dehydrogenase (LOC111388383, L2FC>-5.7; LOC111388380, L2FC>-5.0)
371 clearly points to the production of sesquiterpenoid defense compounds. These enzymes are upregulated in
372 treatment with the only inoculation of *P. oleae* (ID3) (Supplementary Table 3) but also during *P. infestants*
373 infection in potato. Associated with decreased infectivity of *P. oleae* due to *C. oleophila* pre-treatment,
374 production of these phytoalexins is downregulated. However, the amount of phytoalexins produced also
375 depends on the amount of the precursor mevalonate and hence, the expression level of 3-hydroxy-3-
376 methylglutaryl CoA reductase (HMGR)[98].
377 Lignan biosynthesis is also regulated differentially from that occurring during wounding and plant defense
378 responses in this comparison. Many phytoalexins in plants are represented by complex dimeric substituted
379 lignan[99]. Dimerization of two coniferyl alcohol units produces pinoresinol, which is then reduced to
380 lariciresinol and secoisolariciresinol by pinoresinol/lariciresinol reductase (PLR), and finally into matairesinol
381 by secoisolariciresinol dehydrogenase (SIRD)[100]. Here, opposite to what was observed in olive drupes only
382 inoculated with *P. oleae* (treatment ID3) (Supplementary Table 2), the biosynthetic enzymes of the lignan
383 biosynthesis are also downregulated with *C. oleophila* co-infection: dirigent protein 22-like (LOC111374881,
384 L2FC>-3.5), dirigent protein 19-like (LOC111376314, L2FC>-3), dirigent protein 22-like (LOC111383984,
385 L2FC>-2), and the secoisolariciresinol dehydrogenase-like (LOC111412270, L2FC>-3.6; LOC111412278,
386 L2FC>-1.3) (Supplementary Table 3).
387 On the other hand, *C. oleophila* pre-treatment also triggered the upregulation of beneficial genes/proteins that
388 help to counteract the *P. oleae* infection. For instance, the PR proteins STH-21-like (LOC111388400) and
389 STH-2-like (LOC111388410) showed L2FC≥1.09 and =1.7, respectively, but baseMean (PFKM) counts of
390 4032 and 2646. This means that even a small log2-fold increase with elevated high expression counts could be
391 significant if the *p*-value and *p*-adj value are small. The lowest *p*-value and *p*-adj value of the upregulated
392 mRNA/genes during *C. oleophila* co-infection were also filtered by high baseMean and L2FC (Figure 4).

393 Many differentially expressed genes related to defense proteins are also upregulated, such as proteinase
394 inhibitor PSI-1.2-like (LOC111373900), xylanase inhibitor thaumatin-like protein 1b (LOC111367584),
395 chitinase hevamine-A-like (LOC111402962), basic endochitinase-like (LOC111379188), aquaporin PIP1-3-
396 like (LOC111408442), TIP1-1 (LOC111403122), and PIP2-2-like (LOC111372815) (Figure 4,
397 Supplementary Table 3). The involvement of the aquaporins (AQPs) is not immediate, but it has been shown
398 that they also transport H_2O_2[101]. Hydrogen peroxide acts as a ROS signaling molecule and can be produced by
399 various oxidases, superoxide and superoxide dismutase, plasma membrane NADPH oxidases, and peroxisomal
400 oxidases, by electron transport in chloroplasts and mitochondria, or by other apoplastic oxidases. In wheat
401 (*Triticum aestivum* L.), TaPIP2;10 transports the pathogen-induced apoplastic H_2O_2 into the cytoplasm of the
402 infected cell and this signal induces PTI immunity[102]. Consequently, in this comparison, the olive AQPs
403 expressed here might have a function of stimulating the H_2O_2 signaling and accelerate the PTI response.
404 Besides, the non-symbiotic hemoglobins have been implicated in the scavenging of nitric oxide signaling[103].
405 In this comparison, the possible role of the upregulation of the non-symbiotic hemoglobin 2-like
406 (LOC111376313) could be that of neutralizing the excess of NO produced during the pre-treatment
407 (Supplementary Table 3).

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

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

421 The most abundant protein was the Epl1 elicitin, which is a member of the Cerato-platanin family that contains
422 several phytotoxic proteins of about 150 AA residues. Cerato-platanin contains a cysteine-rich domain (four
423 cysteine residues that form two disulfide bonds). *Trichoderma viride* and *T. atroviride* secrete the proteins
424 Sm1 (XP_013943617.1) and Epl1 (XP_013937770.1), respectively, which elicit local and systemic disease
425 resistance in plants[107]. Deletion of epl1 in *T. atroviride* resulted in reduced systemic protection against
426 *Alternaria solani* and *Botritis cinerea*, whereas the *T. virens* sm1 KO strain was less effective in protecting
427 tomato against *Pseudomonas syringae* and *B. cinerea*. Upregulation of epl1 and sm1 led to enhanced basal
428 resistance in tomato i.e., systemic acquired resistance (SAR) and induced systemic resistance (ISR)[108].
429 Results from this study suggest that the impact of *T. atroviride*-culture filtrate pre-treatment on *P. oleae* was
430 significative, as it has been observed the lowest reduction in pathogenic index (rAUDPC = 0.19; Figure 1,
431 IDB). Furthermore, RNAseq results strongly suggest that the pre-treatment of olive drupes with this culture
432 filtrate determined a marked reduction of expression of the *P. oleae* infection marker proteins.
433 The data in Figure 5 many pairwise comparisons have been selected and filtered according to the most
434 significant upregulated differentially expressed genes (lane 7 describe the comparison 4).
435 By associating differentially expressed genes resulting from comparison 4 with results from LC-MS analysis,
436 it can be speculated that the pre-treatment of olive drupes with of *T. atroviride*-culture filtrate alerts the plant
437 tissues to pathogen attack. Indeed, one of the top upregulated gene, the HR protein-like protein 2, OeHIR-2
438 (LOC111404549) (Supplementary Table 4), is responsible for alerting the tissues[107]. In this study, two F-
439 box/LRR-repeat proteins 14 (LOC111382434) and (LOC111405202) were also upregulated, but not in
440 stoichiometric amounts as for OeHIR-2 (LOC111404549) (Supplementary Table 4). Therefore, OeHIR-2
441 should instead be responsible for the disease resistance response, perhaps triggered by *T. atroviride* culture
442 filtrate, which gave the lowest pathogenicity index in this comparison.
443 Unexpectedly, there were two possibly slightly upregulated resistance genes: LRR, leucine-rich repeat
444 receptor-like serine/threonine-protein kinase homologue of At1g06840 (LOC111371641) and MLO-like
445 protein 8 (LOC111401243) (Supplementary Table 4). Using the Arabidopsis eFP Browser[109], it is possible to
446 visualize that the expression of At1g06840 mRNA is very high 48 hpi after *Botrytis cinerea* inoculation of
447 leaves. MLO-like protein 8 (LOC111401243) is also overexpressed (Figure 5; Supplementary Table 4).In the
448 barley, the *Mlo* gene, is involved in particular mutation(s) that can cause the misfunction of the MLO protein,

449 resulting in a correlation with resistance to the fungal pathogen *Erysiphe graminis* f. sp. *hordei*[110]. It can be
450 inferred that the same mutations are also present in olive *MLO* germplasm and if those contribute to the
451 partial/total resistance to Oomycetes.

452 **2.2.5. Comparison 5: overall differential mRNA expression of olive genes**

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

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

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

476 EP3-like (LOC111393512) upregulation during the treatments with either *C. oleophila* or *T. atroviride*-culture
477 filtrate seems to be a marker for the PR-3 weapons against the oomycete.
478 Among the PR-5 highly induced by *P. oleae* infection, the pathogenesis-related protein 5-like
479 (LOC111397298) and the thaumatin-like protein (LOC111398526) were markedly differentially
480 overexpressed (Supplementary Figure 1), but the pre-treatment with either *C. oleophila* or *T. atroviride*-culture
481 filtrate slightly contributed to trigger the expression of the thaumatin-like protein (LOC111398526). The group
482 of PR-6 includes important proteinase inhibitors, such as the cysteine protease inhibitors (relevant for blocking
483 fungal cysteine proteases) and other enzyme inhibitors related to different classes of enzymes involved in the
484 defense response[112,113]. Among these PR-6, as shown in Supplementary Figure 1, the polygalacturonase
485 inhibitor LOC111382121 is the main PR-6 gene transcribed in response to fungal polygalacturonase. In this
486 study, *C. oleophila* resulted to be an effective inducer of the expression of this gene, in contrast with *T.*
487 *atroviride*-culture filtrate treatment. Neither *C. oleophila* or *T. atroviride*-culture filtrate could trigger the
488 expression of the cysteine proteinase inhibitor B-like (LOC111390855), which was only induced by *P. oleae*
489 infection itself. On the other hand, a set of inhibitors (proteinase inhibitor PSI-1.2-like LOC111390049 and
490 LOC111373900, pectinesterase inhibitor 9-like LOC111377531, and kunitz trypsin inhibitor 2-like
491 LOC111405411), including the cysteine proteinase inhibitor A-like (LOC111412304), were exclusively
492 induced by *C. oleophila*.
493 Among the subtilisin-like protease (PR-7), serine protease belonging to the peptidases_S8 subfamily, which
494 are already upregulated upon wounding alone, the highest upregulation was recorded for the gene encoding
495 the subtilisin-like protease SBT1.9 (LOC111394370) in all the treatments. Previous studies demonstrated that
496 the tomato P69 subtilase, which is a subtilisin, activates plant immunity since it can cut the pathogen-secreted
497 apoplastic "small cysteine-rich secreted protein" PC2[114]. Downstream fragments are alerting the plant
498 immunity by intracellular signaling. To evade the plant surveillance system, *Phytophthora* may have evolved
499 protease inhibitors, such as the Kazal-like, protease inhibitors (EPIs) to block PC2 extracellular processing.
500 Furthermore, *Phytophthora* may secrete cytoplasmic effectors to block the secretion of proteases (i.e.,
501 subtilisins and cysteine proteases) into the apoplast and, indirectly, block PC2-triggered immunity[115,116]. For
502 PR-8, the acidic endochitinase-like (LOC111391829) was the main upregulated PR protein in response to *P.*
503 *oleae* or *C. oleiphila* alone as compared the wound control. However, when both *P. oleae* and *C. oleophila*

504 were present then it was downregulated. Other PR-8 expressed were hevamine-A-like (LOC111402962 and
505 LOC111402963). The upregulation of PR-8 alone is hence not impressive to claim an active role in contrasting
506 the infection.
507 The PR-9 proteins are lignin-forming peroxidases that fortify the cell wall by increasing the crosslinking of
508 lignin. Results obtained here evidence that *P. oleae* infection determined the up-regulation of many PR-9
509 peroxidases (Supplementary Figure 1), but the treatment with either *C. oleophila* or *T. atroviride*-culture
510 filtrate also contributed to increase the PR-9 expression level, especially for the lignin-forming anionic
511 peroxidase-like (LOC111385717).
512 The PR-10 class is quite versatile in their biological action due to a loop that has small-chemical binding
513 capabilities and diverse role in stress signaling[117]. In this study, the upregulation of some of them (e.g.
514 pathogenesis-related protein STH-2-like - LOC111388404, LOC111388408, LOC111388411, and
515 LOC111388413) is significative in olive drupes pre-treated with either *C. oleophila* or *T. atroviride*-culture
516 filtrate as compared to olive drupes inoculated with *P. oleae* (Supplementary Figure 1). The highly expressed
517 PR-11 are the acidic mammalian chitinases-like (LOC111382479, LOC111382480, and LOC111382484), but
518 their expression is controversial, since wounding itself can trigger their higher expression and*T. atroviride*
519 culture filtrate downregulated their expression.
520 Among the PR-12 class, the defensins-like protein 1 were not expressed. Some genes encoding PR-13 and PR-
521 14 showed a slight upregulation in all the treatments.
522 The germin-like oxalate oxidases (PR-15) were also downregulated by *P. oleae* infection, even though
523 treatment with either *C. oleophila* or *T. atroviride*-culture filtrate had the effect of upregulating some of them
524 (Supplementary Figure 1).
525 Finally, the expression of PR-17 in olive drupes, which include important zinc apoplastic metalloproteinases,
526 was relevant for the LOC111379582 and LOC111391744 in case of olive drupes only inoculated with *P. oleae*
527 (treatment ID3) or drupes only pre-treated with *C. oleophila* (treatment ID7). Unfortunately, the combination
528 of the pretreatment with either *C. oleophila* or *T. atroviride*-culture filtrate and *P. oleae* infection had the effect
529 of reducing their expression (Supplementary Figure 1).
530 **2.2.6. Comparison 6: wounded olive drupes pre-treated with *Candida oleophila* cell suspension**
531 **(treatment ID7) versus wounded drupes pre-treated with sterile distilled water (treatment ID2)**

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

544 **2.2.7. Comparison 7: wounded olive drupes pre-treated with *Trichoderma atroviride*-culture filtrate**
545 **(treatment ID6) versus wounded drupes pre-treated with sterile distilled water (treatment ID2)**

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

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

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

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

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

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

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

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

658 **2.2.10. RT-qPCR validation of selected genes of *Olea europaea* and *Phytophthora oleae*.**

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

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

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

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

729 **3. Discussion**

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

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

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

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

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

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

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

899 **4. Methods**

900 **4.1. Microorganisms and plant material**

901 Microorganisms included in this study were: i. the yeast *Candida oleophila* strain O, sourced from the
902 commercial formula Nexy®, ii. the ascomycete *Trichoderma atroviride* strain TS and iii. the oomycete
903 *Phytophthora oleae* isolate VK10A. Both *T. atroviride* strain TS and *P. oleae* isolate VK10A were sourced
904 from the culture collection of the Molecular Plant Pathology Laboratory of the Department of Agriculture,
905 Food and Environment of the University of Catania (Catania, Italy) and were identified in previous studies[24,149].
906 'Coratina' olive fruits were collected from 15-year-old olive trees from an orchard in Calabria (southern Italy),
907 in the municipality of Mirto-Crosia, province of Cosenza (DATUM WGS 84, 39°36′54.5″ N 16°46′11.7″ E).
908 Drupes were used to both determine the incidence of the disease following post-harvest treatments and to study
909 the defense mechanisms triggered in the presence of the pathogen in relation to the applied treatments. Overall,
910 540 drupes at maturation index (MI)[7] 4 (MI4) were collected from five trees and stored for 3 hours inside a
911 refrigerated bag while being transported to the laboratory and stored at 8°C until used in experiments.

912 **4.2. Preparation of *P. oleae*-inoculum**

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

916 **4.3. Preparation of *T. atroviride*-culture filtrate**

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

921 **4.4. Preparation of *C. oleophila* cell suspension**

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

927 **4.5. Evaluating the Effectiveness of *C. oleophila* and *Trichoderma*-culture filtrate in Controlling Rots of**
928 **Drupes by *P. oleae***

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

944 **4.6. Assessing the experimental assay to study the Transcriptome of olive drupes and *P. oleae* in the**
945 **system Olive Drupe-*P. oleae*-*C. oleophila*/*T. atroviride*-culture filtrate**

946 Olive drupes were preliminary surface sterilized, treated with either *C. oleophila* cell suspension or *T.*
947 *atroviride*-culture filtrate and *P. oleae*-inoculated as described in 4.5. Then, drupes were incubated at 21 ±
948 1°C, with 80% relative humidity and a photoperiod of 16 h light / 8 h dark. The experimental assay included
949 the following seven treatments: (i) non-wounded drupes treated with 20 µl of sdw (ID1); (ii) wounded drupes

950 treated with 20 µl of sdw (ID2); (iii) wounded drupes inoculated with *P. oleae* (ID3); (iv) wounded drupes
951 treated with *T. atroviride*-culture filtrate and inoculated with *P. oleae* (ID4); (v) wounded drupes treated with
952 *C. oleophila* and inoculated with *P. oleae* (ID5); (vi) wounded drupes treated with *T. atroviride*-culture filtrate
953 (ID6); (vii) wounded drupes treated with *C. oleophila* (ID7). The experimental assay was designed as a
954 completely randomized block, with each treatment comprising three biological replicates, each consisting of
955 20 drupes. In order to proceed to transcriptomic analyses, at each of the above-mentioned time points, fresh
956 olive fragments from each treatment were collected and immediately frozen in liquid nitrogen and stored at -
957 80°C.

958 **4.7. RNA isolation from infected olive drupes and cDNA synthesis for RT-PCR**

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

966 **4.8. Quantitative Real Time-PCR (RT-qPCR) Analysis of Gene Expression**

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

978 XM_022993342.1) was used as an internal reference gene. For *P. oleae*, the tubulin beta chain (g8215.t2
979 (TRINITY_DN6095_c0_g1_i1) gene expression has been used to normalize the RT-qPCR data. The relative
980 expression level of each gene obtained through RT-qPCR was compared to the RNA-seq data. Expression
981 values of the $2^{-\Delta\Delta Ct}$ were represented as boxplots using Python script wit "seaborn", "pandas" and
982 "matplotlib.pyplot" libraries. Dunnett's test was used to calculate the significance (*p-value*) relative to the
983 expression level ($2^{-\Delta\Delta Ct}$) of each sample to wounded olive fruit condition by computing a Student's t-statistic
984 for each experimental conditions and time points (hpi).

985 **4.9. Total RNA preparation and Illumina mRNA sequencing**

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

1000 **4.10. *De novo* mRNA analysis**

1001 Illumina 150 PE, 30 M reads fastq[154] files were processed for removal of the adapters with Trimmomatic
1002 Version 0.39[155]. For maximizing and diversifying the mRNA expression of *Phytophthora oleae*, the Trinity *de*
1003 *novo* mRNA assembly algorithm[156] was applied to RNA samples of cultures of the isolate VK10A grown in
1004 three different substrates, including CZAPEK, MEA and PDA. Annotation of both trinity derived cDNA and
1005 AUGUSTUS predicted genes was performed against using Mercator4v6[157] analysis cross-evaluation.

1006 AUGUSTUS web prediction gave 20547 proteins of which only 12117 were containing unique scaffold
1007 represented of more than 75% as protein identity, the remaining not unique proteins represented alternate
1008 proteins or differently spliced or start (ATG) derived proteins. The same number of proteins, 20094 was found
1009 by using Trinity ORF prediction software unfiltered and after removing duplicates. Filtering the Trinity
1010 accessions for proteins capable of finding a blast hit with other Phytophthora species, then, the real number of
1011 de novo mRNA transcript was down to 13373 proteins. It needs to be considered that the Trinity prediction
1012 include partial protein/mRNA sequences due to incomplete full-length cDNA. The fasta/excel files related to
1013 AUGUSTUS and Trinity protein/CDS/mRNA prediction and annotation can be found at Zenodo
1014 (https://zenodo.org/records/11092245).

1015 **4.11. RNAseq data analysis**

1016 The raw Illumina sequence reads of fastaq format were processed as above by removing the adapters. The
1017 reference genome and gene model annotation files of *Olea europaea* var. *sylvestris* and *Phytophthora oleae*
1018 were downloaded from NCBI (*Olea europaea* var. *sylvestris* reference genome O_europaea_v1, accession
1019 number GCF_002742605.1) and *Phytophthora oleae* reference genome VK10A, from Zenodo
1020 (https://zenodo.org/records/11092245). The filtered fastaq files were aligned to the reference genome using
1021 Hisat2 v2.0.5[158]. The reads with low alignment quality values or unpaired alignments, and alignments to
1022 multiple regions of the genome were filtered out by using samtools[159] to generate duplicate checked and sorted
1023 binary BAM files of mapped reads to the genome. The number of reads and fragments per kilobase per million
1024 reads (FPKM) for each gene was calculated using Stringtie v2.2.0[160]. Differential gene expression analysis
1025 between the pathogen-inoculated and control samples at different time points was performed in R studio using
1026 the DESeq2 R package in a script (https://bioconductor.org/packages/DESeq2/) customized on the basis of the
1027 total normalized reads counts[161]. Corrections for false positives and false negatives were made using the
1028 Benjamini-Hochberg procedures[162] to generate the false discovery rate (FDR).

1029 Fold changes (L2FC, log2Ratio) were estimated from normalized gene expression levels in each sample by
1030 using the ratio of the sample Log2 of FPKM and the wounded olive fruit Log2 of FPMK as denominator. The
1031 mapped genes with an adjusted false discovery rate (FDR) ≤ 0.05 and $|\log2(\text{FoldChange})| > 1$ were considered
1032 differentially expressed. To reduce the amount of differentially expressed genes according to 0.05 and 0.001
1033 FDR, shrinkage of log2 (Fold Change) was performed using the R 'apeglm' function[163]. Differentially

1034 expressed genes (DEGs) clustering heatmaps were generated by the pheatmap and clusterProfiler R
1035 packages[164]. Multiple correlation of the variance of L2FC for all the sample was analyzed by principal
1036 components analysis (PCA).

1037 **4.12. LC-MS identification of proteins in protein filtrate**

1038 *Trichoderma atroviride* strain TS was cultured *in vitro* in a YPD liquid media supplemented with malt extract
1039 to 1%. After three days, the cultures (in triplicate) were filtered through a Whatman® qualitative filter paper,
1040 Grade 1 (WHA1001325 Sigma-Aldrich, Merck, Germany) to retain the mycelia. The filtrate was further
1041 centrifuged at 20,000 ×g for 15 minutes then sterile filtered through 0.2 µm vacuum filters. The protein
1042 preparation for proteomics including digestion by trypsin and the LC-MS conditions and identification by
1043 Waters PLGS software has been described previously[165].

1044

1045 **CRediT author statement.**

1046 **Sebastiano Conti Taguali:** Investigation, Methodology, Formal analysis, Data Curation, Writing - Original
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1053 administration.

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1056

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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

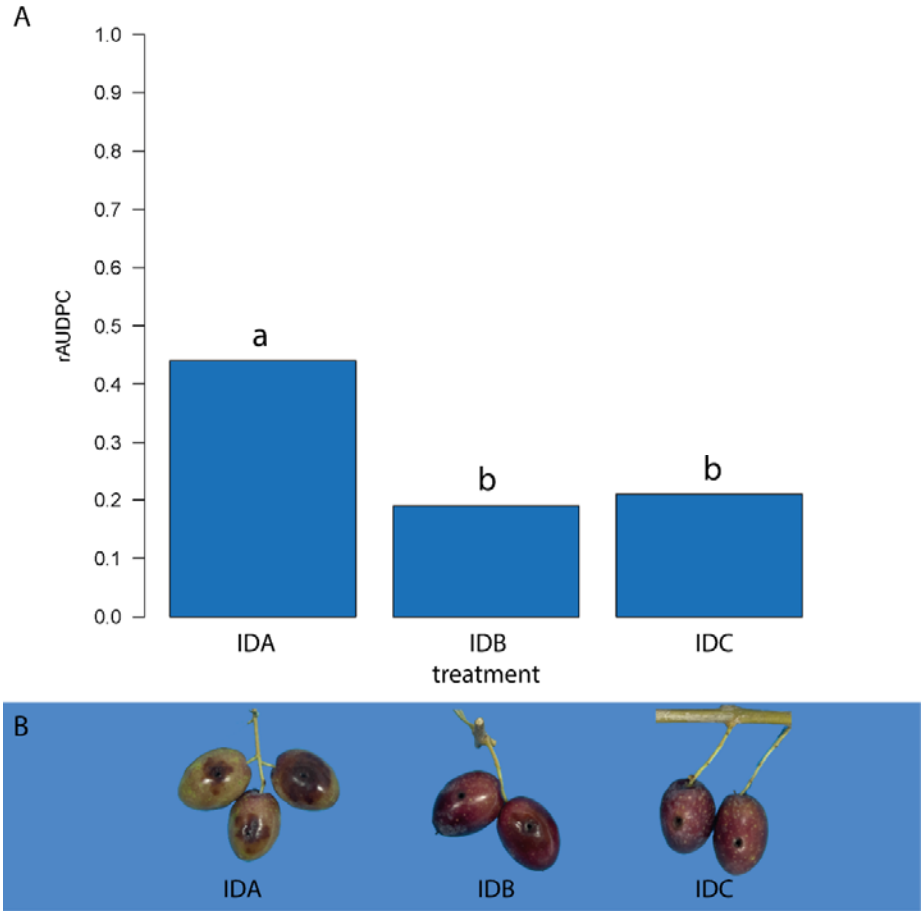
A
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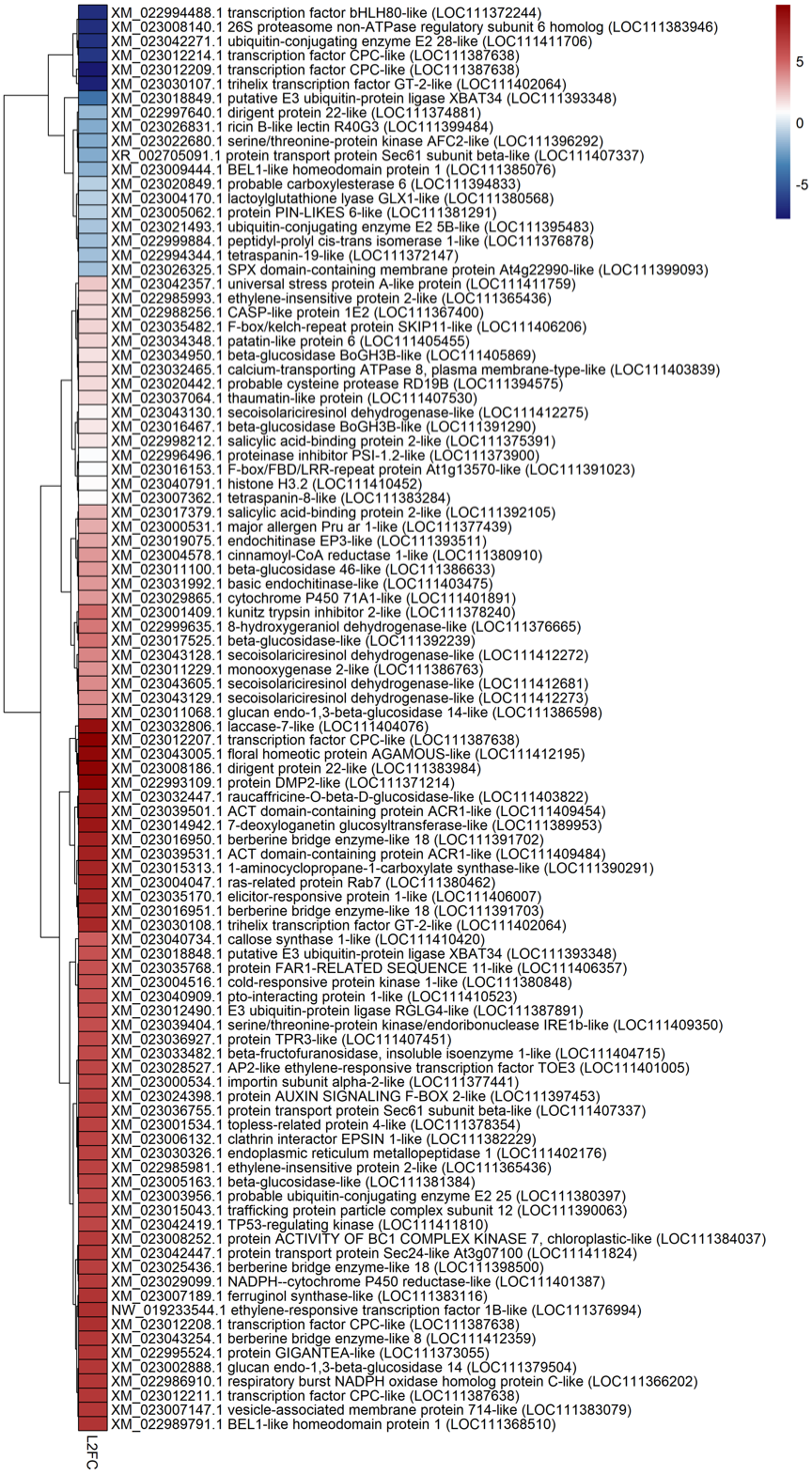
Figure 2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XM_023008140.1 26S proteasome non-ATPase regulatory subunit 6 homolog (LOC111383946)
XM_023042271.1 ubiquitin-conjugating enzyme E2 28-like (LOC111411706)
XM_023012214.1 transcription factor CPC-like (LOC111387638)
XM_023012209.1 transcription 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)
XM_022985993.1 ethylene-insensitive protein 2-like (LOC111365436)
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)
XM_023000531.1 major allergen Pru ar 1-like (LOC111377439)
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XM_023004578.1 cinnamoyl-CoA reductase 1-like (LOC111380910)
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XM_023031992.1 basic endochitinase-like (LOC111403475)
XM_023029865.1 cytochrome P450 71A1-like (LOC111401891)
XM_023001409.1 kunitz trypsin inhibitor 2-like (LOC111378240)
XM_022999635.1 8-hydroxygeraniol dehydrogenase-like (LOC111376665)
XM_023017525.1 beta-glucosidase-like (LOC111392239)
XM_023043128.1 secoisolariciresinol dehydrogenase-like (LOC111412272)
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Figure 3

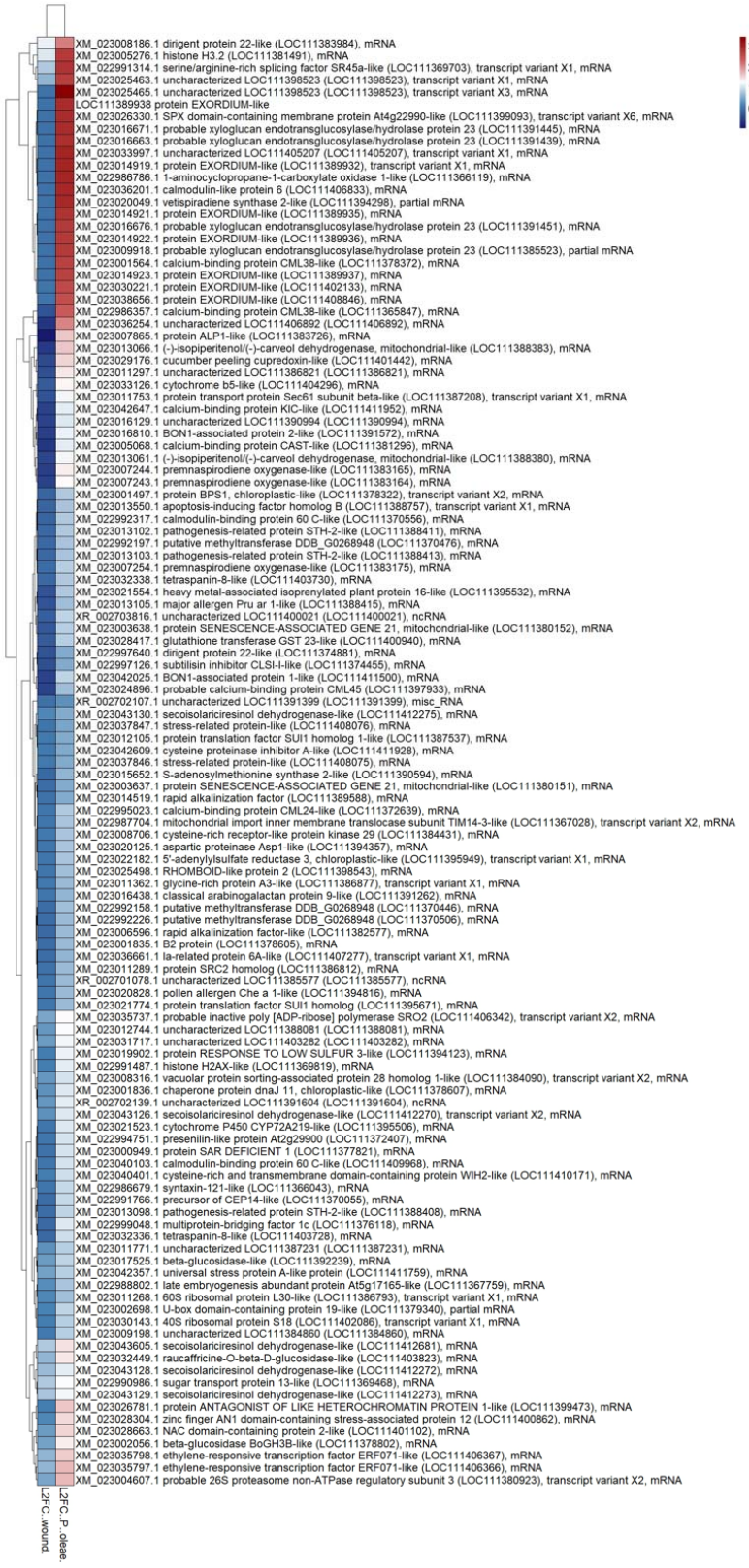
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Figure 4

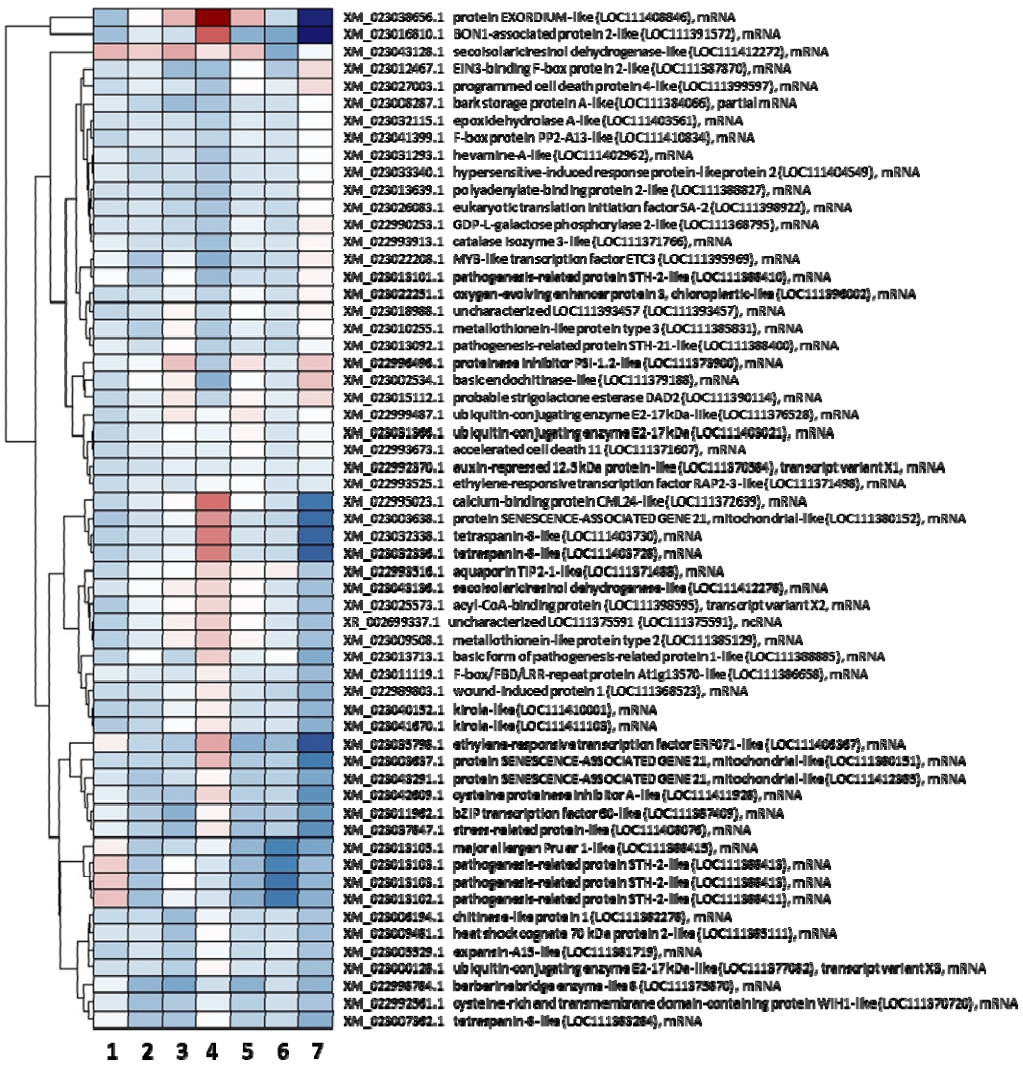
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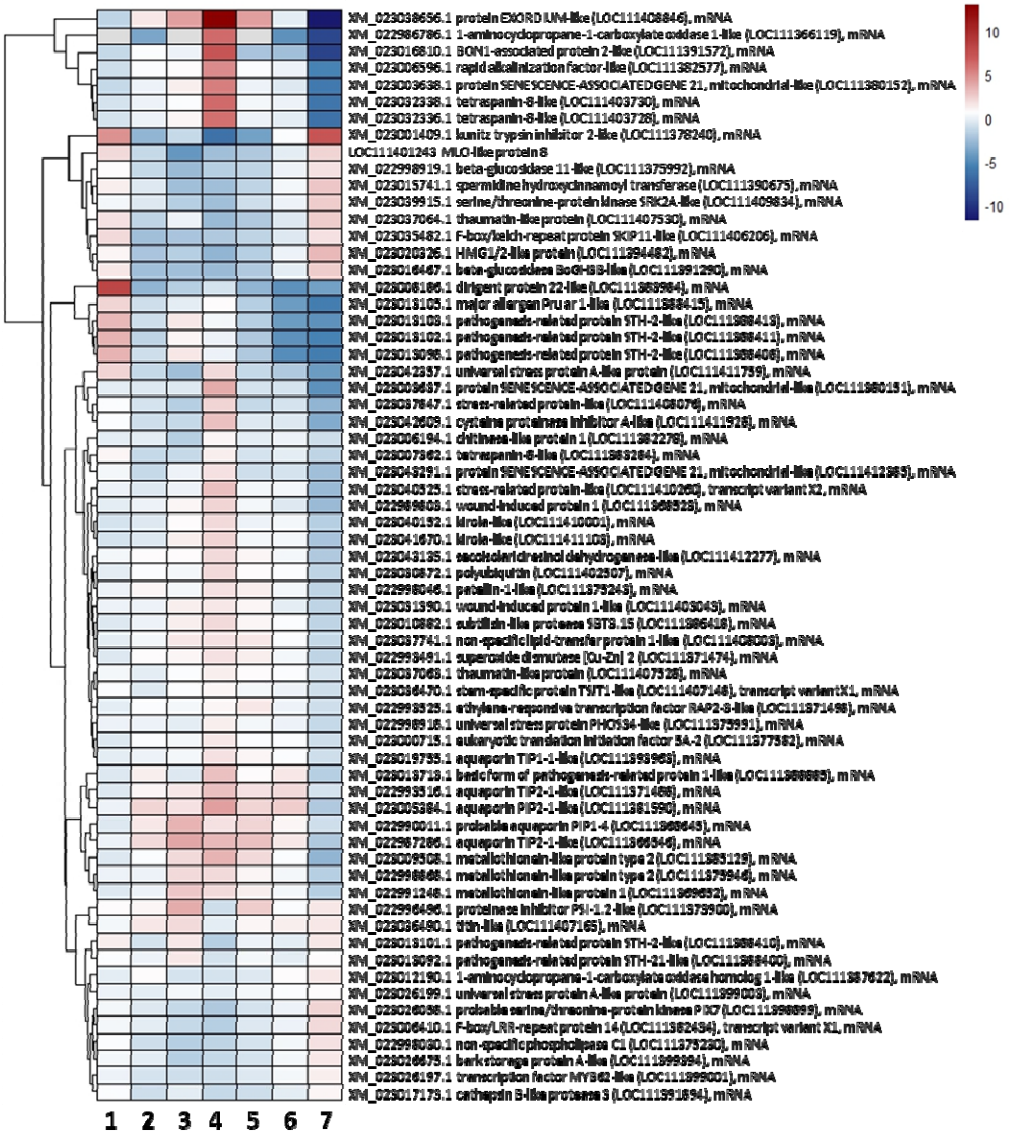
Figure 5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XM_022987286.1 aquaporin TIP2-1-like (LOC111366546), mRNA
XM_023009308.1 metallothionein-like protein type 2 (LOC111385129), mRNA
XM_022998868.1 metallothionein-like protein type 2 (LOC111375946), mRNA
XM_022991248.1 metallothionein-like protein 1 (LOC111369652), mRNA
XM_022996496.1 proteinase inhibitor PSI-1.2-like (LOC111375900), mRNA
XM_023036490.1 thin-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_023026036.1 probable serine/threonine-protein kinase PBL7 (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 berkstorage protein A-like (LOC111399394), mRNA
XM_023026197.1 transcription factor MYB62-like (LOC111399001), mRNA
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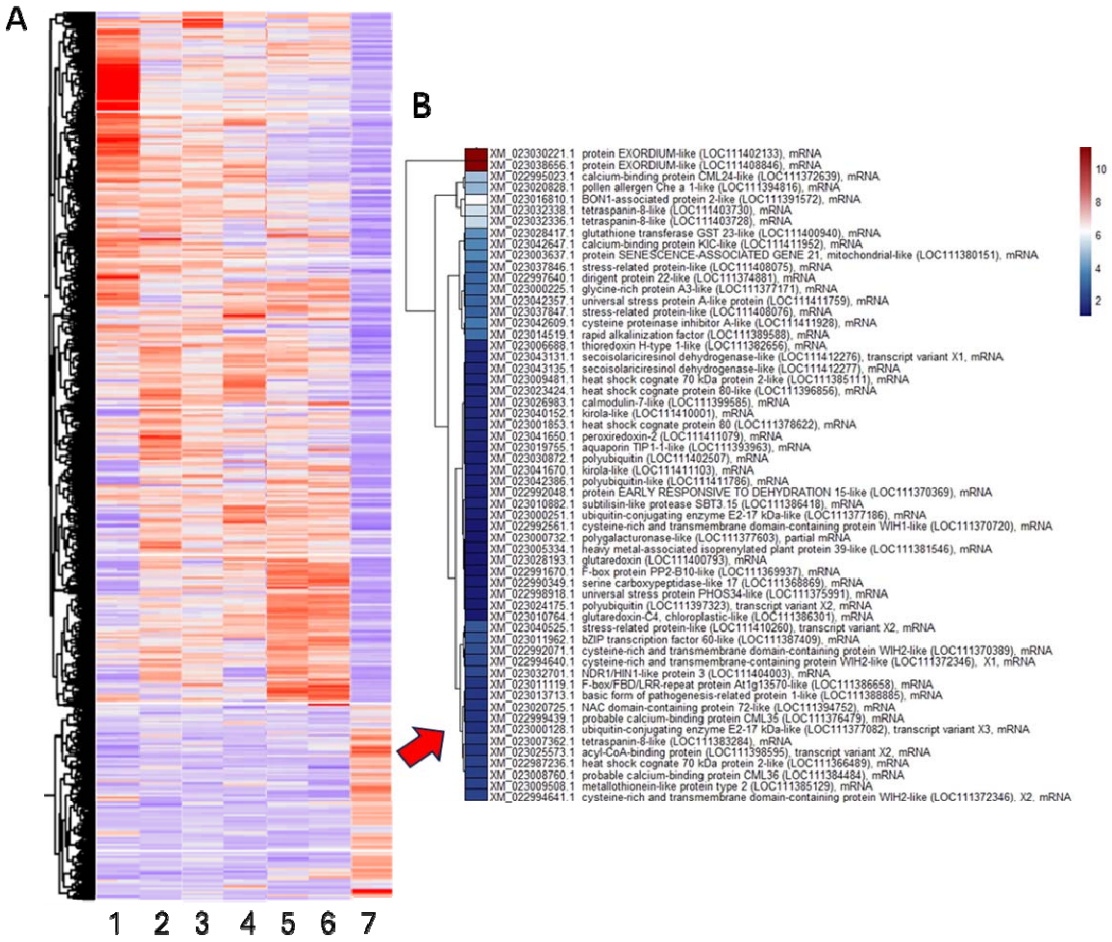
-5
-10

Figure 6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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
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XM_023028417.1 glutathione transferase GST 23-like (LOC111400940), mRNA
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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
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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_022989918.1 universal stress protein PHOS34-like (LOC111375991), mRNA
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XM_023040525.1 stress-related protein-like (LOC111410260), transcript variant X2, mRNA
XM_023011962.1 bZIP transcription factor 60-like (LOC111387409), mRNA
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8
6
4
2

Figure 7

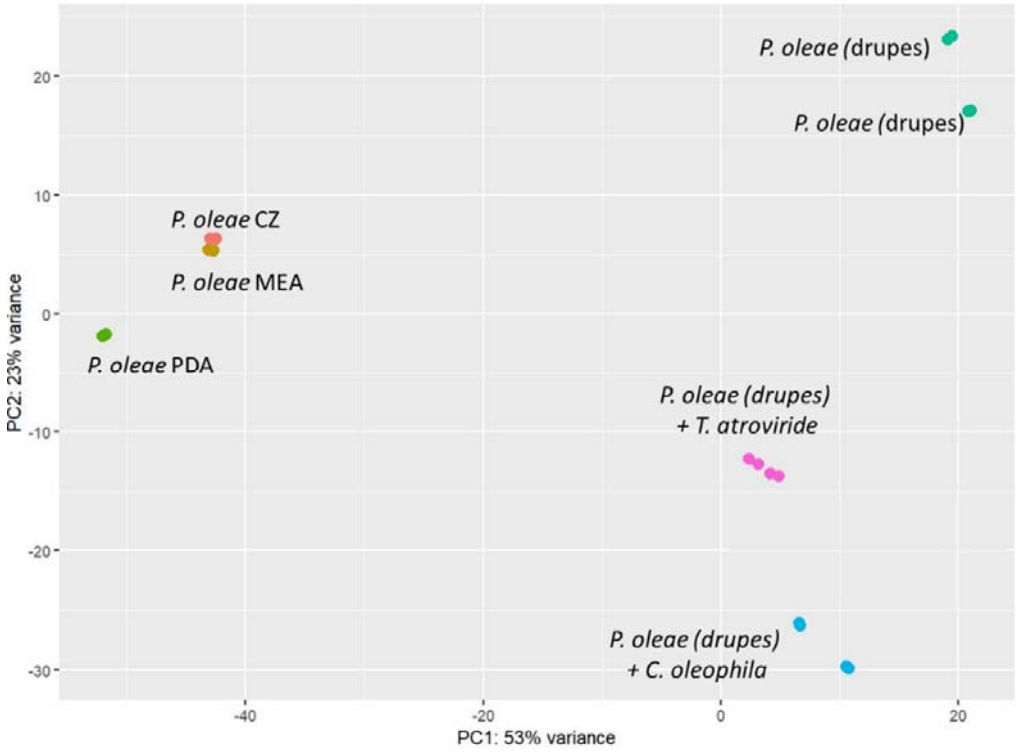


Figure 8

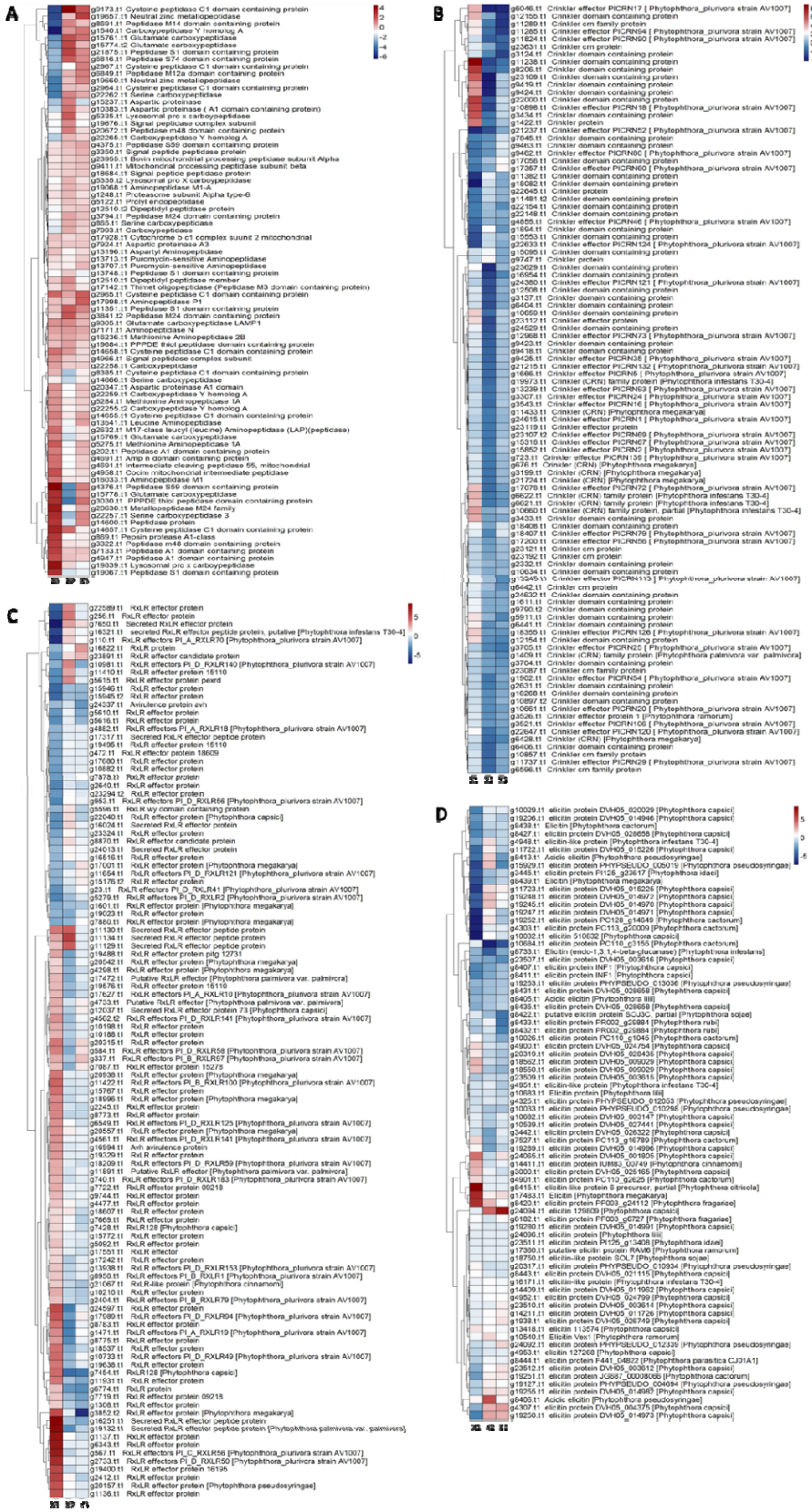
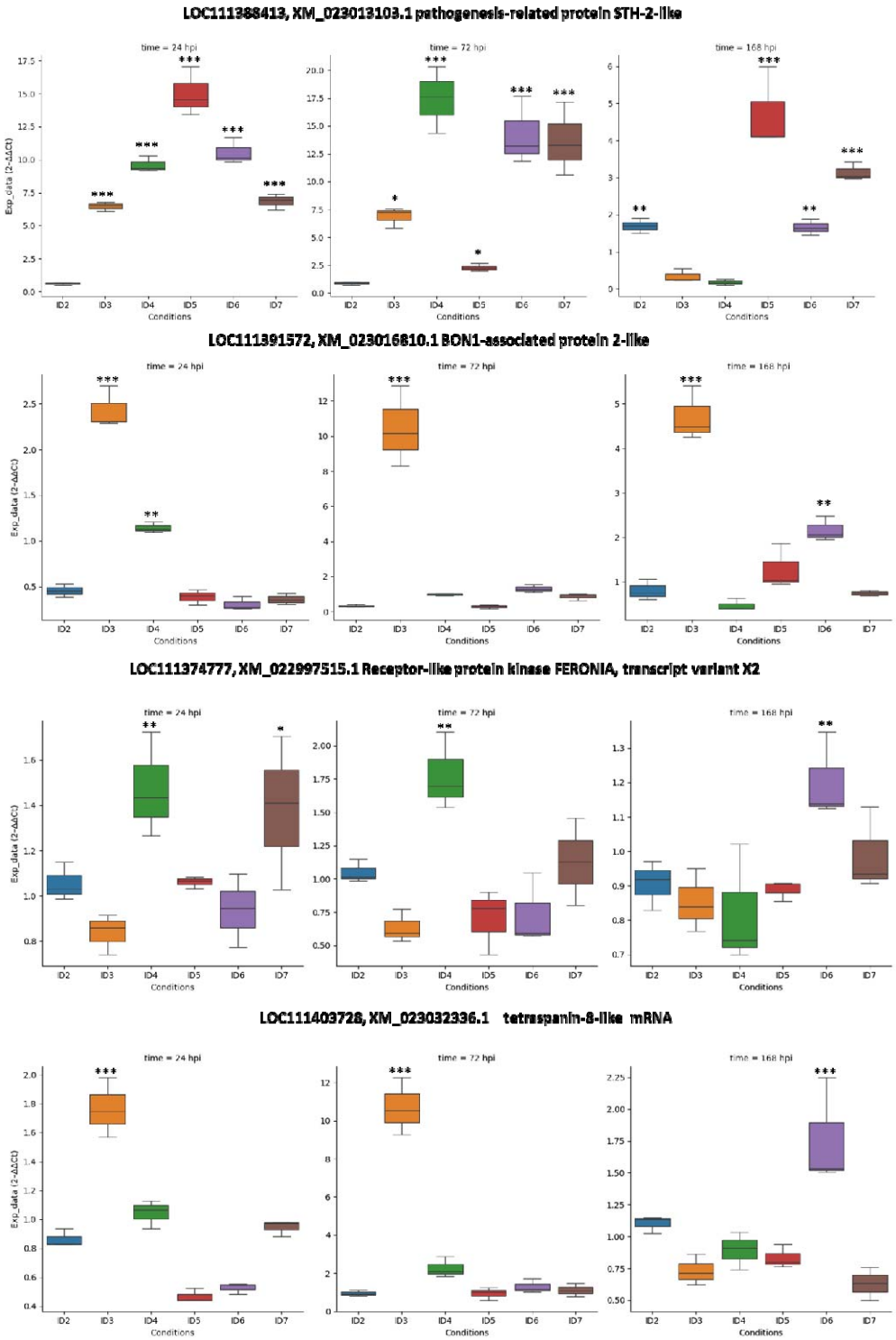


Figure 9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ID3
ID4
ID5
ID6
ID7
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LOC111374777, XM_022997515.1 Receptor-like protein kinase FERONIA, transcript variant X2
LOC111409728, XM_023032336.1 tetraspanin-8-like mRNA

Figure 10

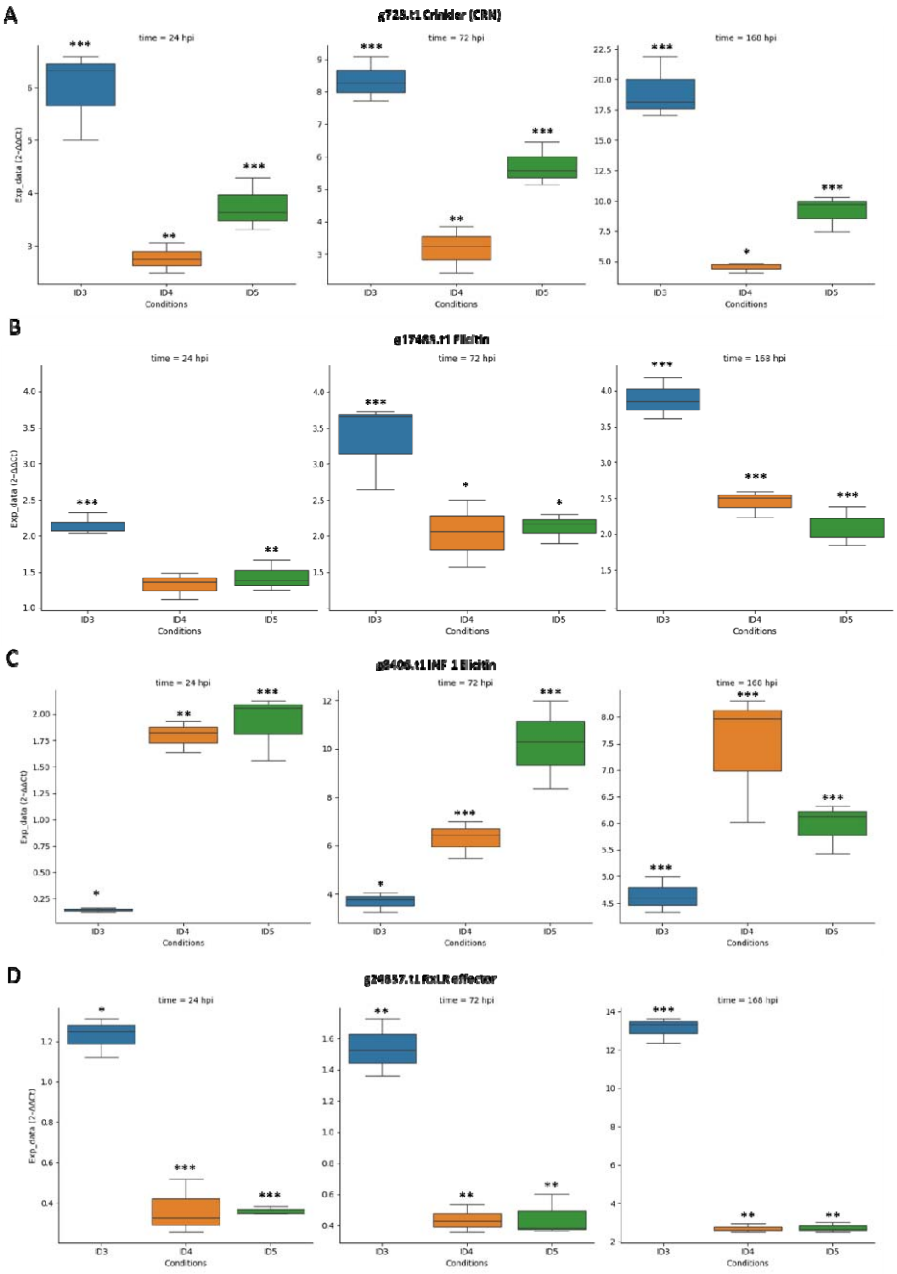
A
g723.t1 Crinkler (CRN)
time = 24 hpi
time = 72 hpi
time = 168 hpi
Exp_data (2-ΔΔCt)
ID3
ID4
ID5
Conditions
B
g17485.t1 Elicitin
C
g8406.t1 INF 1 Elicitin
D
g24857.t1 RxLR effector

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]