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Phenotypic and transcriptomic analyses reveal major differences between apple and pear scab nonhost resistance

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1 **Title**

2 **Phenotypic and transcriptomic analyses reveal major differences between apple and pear scab**
3 **nonhost resistance**

4

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16

17 **Abstract**

18 **Background.** Nonhost resistance is the outcome of most plant/pathogen interactions, but it has
19 rarely been described in Rosaceous fruit species. Apple (*Malus x domestica* Borkh.) is a nonhost for
20 *Venturia pyrina*, the scab species attacking European pear (*Pyrus communis* L.). Reciprocally, *P.*
21 *communis* is a nonhost for *Venturia inaequalis*, the scab species attacking apple. The major objective
22 of our study was to compare the scab nonhost resistance in apple and in European pear, at the
23 phenotypic and transcriptomic levels.

24 **Results.** Macro- and microscopic observations after reciprocal scab inoculations indicated that, after
25 a similar germination step, nonhost apple/*V. pyrina* interaction remained nearly symptomless,

26 whereas hypersensitive reactions were observed during nonhost pear/*V. inaequalis* interaction.
27 Comparative transcriptomic analyses of apple and pear nonhost interactions with *V. pyrina* and *V.*
28 *inaequalis*, respectively, revealed considerable differences. Very few differentially expressed genes
29 were detected during apple/*V. pyrina* interaction, which is consistent with a symptomless type I
30 nonhost resistance. On the contrary, numerous genes were differentially expressed during pear/*V.*
31 *inaequalis* interaction, as expected in a type II nonhost resistance involving visible hypersensitive
32 reaction. Pre-invasive defense, such as stomatal closure, was detected, as well as several post-
33 invasive defense mechanisms (apoplastic reactive oxygen species accumulation, phytoalexin
34 production and alterations of the epidermis composition). In addition, a comparative analysis
35 between pear scab host and nonhost interactions indicated that, although specificities were
36 observed, two major defense lines were shared in these resistances: cell wall and cuticle
37 modifications and phenylpropanoid pathway induction.

38 **Conclusion.** This first deciphering of the molecular mechanisms underlying a nonhost scab resistance
39 in pear offers new possibilities for the genetic engineering of sustainable scab resistance in this
40 species.

41

42 **Keywords:** apple, pear, nonhost resistance, transcriptomics

43

44 **Background**

45 Apple (*Malus domestica* Borkh.) and European pear (*Pyrus communis* L.) are two closely related
46 species belonging to the *Rosaceae* family. Reclassification of the *Rosaceae* placed both *Pyrus* and
47 *Malus* genera in the subfamily *Spiraeoideae*, tribe *Pyreae* and subtribe *Pyrinae*, this subtribe
48 corresponding to the long-recognized subfamily *Maloideae* [1]. Efforts to resolve relationships within
49 this subtribe have frequently failed, and Campbell et al [2] concluded that the genera of this subtribe
50 *Pyreae* have not diverged greatly genetically. The recent sequencing of the pear genome [3] allowed
51 a precise comparison with the apple genome [4] and led to the estimation of a divergence time

52 between the two genera of \approx 5.4 – 21.5 million years ago. Furthermore, apple and pear genomes
53 share similar chromosome number ($n=17$), structure and organization.

54 Scab disease, caused by *Venturia* spp., affects several rosaceous fruit tree species. These
55 hemibiotrophic pathogens can infect only a limited host-range during their parasitic stage, but they
56 can overwinter as saprophytes in the leaf litter of a larger range of plant species [5]. Scab disease is
57 caused by *V. inaequalis* on apple, by *V. pyrina* (formerly named *V. pirina* [6]) on European pear, and
58 by *V. nashicola* on Japanese (*P. pyrifolia* Nakai) and Chinese (*P. ussuriensis* Maxim) pears. Cross
59 inoculations of *Venturia* spp. on different rosaceous fruit trees indicates that these pathogens are
60 highly host specific, probably indicating a close co-evolution of these pathogens with their hosts [7].

61 A plant species unable to be successfully infected by all isolates of a pathogen species is considered
62 as a nonhost for this pathogen. Nonhost interactions of *Venturia* spp. on apple and pear have rarely
63 been described. Microscopic observations have been made on *P. communis* / *V. nashicola* [8] as well
64 as *M. domestica* / *V. pirina* and *P. communis* / *V. inaequalis* [5, 9]. In all cases, conidia germinated
65 and produced appressoria and runner hyphae, but failed to establish a network of stroma. No
66 macroscopic symptoms were visible.

67 Because of its durability, nonhost resistance has attracted numerous studies over the last decade,
68 which have uncovered its multiple and complex defense components. The underlying mechanisms of
69 nonhost resistance comprise pre-invasion resistance with preformed or induced cell-wall defenses,
70 metabolic defense with phytoanticipin or phytoalexin accumulation, pattern-triggered immunity (PTI)
71 as well as elicitor-triggered immunity (ETI) and various signaling pathways [10]. To our knowledge,
72 the molecular bases of scab nonhost resistance of apple and pear have never been investigated. The
73 objectives of our study were 1) to precisely describe nonhost resistance symptoms in *M. domestica* /
74 *V. pyrina* and *P. communis* / *V. inaequalis* interactions 2) to analyze the underlying molecular
75 mechanisms of both nonhost interactions through a transcriptomic study 3) to compare the
76 mechanism of host [12] and nonhost scab resistance in apple and European pear.

77

78 **Results and discussion**

79 Variable symptoms of nonhost resistance

80 Nonhost interactions were observed in a test performed on ‘Gala’ and ‘Conference’, all inoculated by
 81 a *V. pyrina* strain (VP102) and a *V. inaequalis* strain (VI EUB05). At the macroscopic level, a total
 82 absence of sporulation was observed on all nonhost interactions (Table 1). The apple ‘Gala’ remained
 83 completely symptomless after *V. pyrina* inoculations (Fig. 1C). This is similar to the observation of
 84 Chevalier et al [9] after inoculation of ‘Gala’ with another *V. pyrina* strain. On the contrary, pear
 85 plants inoculated with *V. inaequalis* presented frequent pin points symptoms (Fig. 1A) and occasional
 86 chlorotic lesions (Fig. 1B). Chlorotic lesions had already been observed by Chevalier et al [9] after
 87 inoculation of the pear ‘Pierre Corneille’ with the *V. inaequalis* strain EUB04, but pin points had never
 88 been reported in this nonhost interaction. According to our observations, apple nonhost resistance
 89 could be classified as type I and pear as type II according to Mysore and Ryu [11] definition based on
 90 the absence/presence of visible HR reaction.

91

92 **Table 1: Scab qualitative note of pear and apple lines inoculated with *V. pyrina* and *V. inaequalis*.** .

Percentage of plants in the different classes of symptoms, 42 days after inoculation				
Class of symptoms	<i>V. pyrina</i> strain VP102		<i>V. inaequalis</i> strain EUB05	
	‘Conference’	‘Gala’	‘Conference’	‘Gala’
0	0	100	90	0
1	0	0	5	0
2	0	0	5	0
3a	0	0	0	0
3b	0	0	0	0
4	100	0	0	100

93 Class 0: absence of symptoms

94 Class 1: hypersensitivity (pin points)

95 Class 2: resistance (chlorotic lesions, slight necrosis, crinkled aspect)

96 Class 3a: weak resistance (necrotic or chlorotic lesions with occasional very light sporulation)

97 Class 3b: weak susceptibility (clearly sporulating chlorotic or necrotic lesions)

98 Class 4: susceptibility (sporulation only)

99

100 At the microscopic level, three days after inoculation, there was no clear difference between host
 101 and nonhost interactions: the conidia of *V. inaequalis* and *V. pyrina* germinated equally on both hosts
 102 forming one or two appressoria (Fig. 1 D and F). However, 14 days after inoculation, there was a clear
 103 reaction of the plant cells in contact with the appressoria (accumulation of red autofluorescent
 104 compounds and enlargement of these cells), which could indicate very small scale hypersensitive
 105 reactions (HR) reactions (Fig. 1 E and G) in both plant species. No formation of subcuticular stroma
 106 and no conidiogenesis were observed in the nonhost interactions, contrary to the host-resistance
 107 reactions [12]. These observations are similar to the collapsed cells described by Chevalier et al [9] in
 108 apple and pear nonhost reactions, and to the rare HR-like reactions observed by Stehmann et al [5]
 109 on apple inoculated by *V. pyrina*.

110 Our results indicate that the leaf surface morphology of apple and pear is equally compatible with *V.*
 111 *pyrina* and *V. inaequalis* conidia germination, without specific inhibition at this stage. Recognition
 112 probably occurs only at the appressorium site, leading to the cellular reactions observed. These
 113 reactions were limited to a few cells without visible symptoms in apple / *V. pyrina* interaction, but
 114 extended and produced macroscopic symptoms in pear / *V. inaequalis* interaction.

115
 116 Different patterns of global gene expression in nonhost resistance in pear versus apple
 117 Differentially expressed genes (DEGs) were analyzed by comparing transcript abundance in leaves
 118 between T0 and 24 hours post inoculation (hpi) and between T0 and 72hpi, in the nonhost
 119 interactions ‘Gala’ / *V. pyrina* VP102 and ‘Conference’ / *V. inaequalis* EUB05. In total, 60 DEGs in
 120 apple and 1857 DEGs in pear were identified, which amounts to 0.19 % of all apple genes on the
 121 apple AryANE v2.0 microarray, and 4.23 % of all pear genes on the Pyrus v1.0 microarray (Table 2).

122 **Table 2. Number of DEGs identified during apple and pear nonhost response to *V. pyrina* and *V.***
 123 ***inaequalis***

	‘Gala’ / VP102		‘Conference’ / EUB05	
	24hpi	72hpi	24hpi	72hpi
Total # of DEGs*	49	11	1570	364

DEGs in % of all genes on the microarray**	0.16	0.03	3.58	0.83
% upregulated DEGs	67.3	36.4	74.5	25.5
% of downregulated DEGs	32.7	63.6	25.5	74.5
% of DEGs without TAIR name	27.1	30.4	0.70	1.09

124 *: DEGs numbers were calculated using the p-adj values ≤ 0.01 as selection threshold

125 **: 31311 genes on the apple Ariane V2 microarray, 43906 genes on the pear V1 microarray

126

127 The very small number of DEGs detected in the apple nonhost interaction at 24 or 72hpi is in
128 agreement with the total absence of macroscopic symptoms observed during this interaction.

129 However, at the microscopic level, small HR-like reactions were detected in the apple / *V. pyrina*
130 interaction. Because these reactions involve only a few cells in the leaves, the changes in gene
131 expression are probably below the threshold of DEG detection applied in this experiment.

132 On the contrary, the number of DEGs detected during the pear / *V. inaequalis* interaction is in the
133 same order of magnitude as the number of DEGs detected during pear host resistance to *V. pyrina*
134 (see [12]). This is in agreement with the frequent observation of macroscopic symptoms of resistance
135 (chlorotic lesions or pin points) in this interaction. Among the 1857 pear DEGs, 80.2 % were only
136 detected at 24hpi and 15.4 % only at 72hpi, whereas 4.2 % were upregulated or down regulated
137 similarly at both time points of the kinetics. Among all the pear DEGs observed at 24 and 72hpi, the
138 proportion of up-regulated DEGs was higher (68.8 %) than the proportion of downregulated DEGs
139 (31.2 %). Using MapMan to map the DEGs TAIR names, we observed that the main functional
140 categories represented in this set of DEGs were similar to those observed during pear host resistance
141 to *V. pyrina* (see [12]): protein, RNA, signaling, transport and cell cycle (Fig. 2).

142 To basically validate the transcriptomic data, 12 DEGS with varied ratios (between -1.9 and 2.9) have
143 been tested in QPCR (Table S1), on the two biological repeats used for transcriptomic analyses.

144 Considering the weak number of DEGs found for apple in this study, we only tested two of them in
145 QPCR. As seen in Table 2 for pear, at 24hpi, a majority of DEGs are up-regulated and at 72hpi, a
146 majority of DEG are down-regulated. QPCR was then performed essentially on DEGs with positive

147 ratios at 24hpi and negative ratios at 72hpi (Table S1). The QPCR results confirmed the induced or
148 repressed status of all tested DEGs.

149

150 Weak involvement of hormone signaling pathways classically associated to resistance

151 Pear DEGs were found that indicate that the jasmonic acid (JA) pathway was repressed. The JA
152 biosynthesis and metabolic conversions were reviewed by Wasternack et al [13]. In our data, at
153 24hpi, the first step of JA biosynthesis, that is the conversion of linoleic acid in 12-oxo-phytodienoic
154 acid (OPDA), is compromised given the repression of six about seven lipoxygenases (LOX) (three
155 LOX1, two LOX2 and two LOX5), the last one being induced (Fig. 3). OPDA produced in the chloroplast
156 is then transported to the peroxisome for subsequent conversion to JA via the action of OPR3 (12-
157 oxo-phytodienoic acid reductase) and β -oxidation enzymes (reviewed in [14] and in [13]). In pear,
158 three β -oxidation enzymes were found activated more or less rapidly: ACX4 (24hpi), MFP2 (72hpi)
159 and the thioesterase homolog to *At2g29590* (72hpi), which suggests that constitutive OPDA stocks
160 were turned into JA. But the early and long-lasting induction of *JMT* and *ST2A* genes is in favor of a
161 rapid conversion of JA in inactive compounds, *JMT* induction being reinforced by *BBD1* repression
162 (24hpi). *BBD1* is actually known as a negative regulator of *JMT* [15].

163 The defense response depending on JA was also clearly repressed in pear (Fig. 3). The transcription
164 activator MYC2 of JA-induced genes is known to be repressed by its interaction with JAZ proteins
165 (reviewed in [13]), and two *JAZ1* and one *JAZ3* coding genes were found activated at 24hpi in pear.
166 UBP12 is known as a stabilizer of MYC2 [16]. In our data, *UBP12* was found repressed at 72hpi, which
167 reinforces the inactivation of MYC2. WRKY33 is known as an activator of the JA defense pathway [17]
168 and WRK70 [18] or AS1 (or MYB91; [19]) as inhibitors, and among JA-responsive proteins, the
169 pathogenesis-related PR3, PR4 and PR12 act downstream MYC2 activation [20]. In our data,
170 accordingly with the repression of the activator WRKY33 and the activation of the inhibitors WRK70
171 and AS1, some JA-responsive genes were also found repressed, such as the chitinase coding genes
172 *PR4* (also called *HEL*) and *ATEP3*. Furthermore, no DEGs were found for PR3 and PR12 functions. To

173 conclude, in the nonhost interaction between pear and *V. inaequalis*, some JA seems to be produced,
174 but rapidly converted in inactive compounds and the subsequent defense response is clearly
175 repressed.

176 Pear DEGs were found that seems to indicate that the salicylic acid (SA) pathway was slightly
177 engaged and rapidly repressed (Fig. 3). *WRKY70* was induced at 72hpi in our data. This transcription
178 factor is known as a negative regulator of SA biosynthesis but a positive regulator of SA-mediated
179 defense genes in Arabidopsis ([21]; [22]; [23]), among them *PR2*, *PR5* but not *PR1* [24]. *WRKY33*
180 which is known as a negative regulator of SA-responsive genes [25], was also repressed at 72hpi in
181 our data. *PR2* and 5 are well-known anti-fungal proteins ([26]; [27]; [28]). At 24hpi a *PR2*, two *PR2-*
182 *like*, a *PR5* and a *PR5-like* coding genes were found induced in our work, another *PR2-like* and two
183 others *PR5-like* being repressed. The differential expression was maintained at 72hpi for only two of
184 the activated ones. Furthermore no DEG was found for the *PR1* function but three *PR1-like* genes
185 were found repressed: *ATPRB1* and genes homolog to *At5g57625* and *At4g33720*. *ATPRB1* was
186 already reported as repressed by SA treatment [29]. In our data, the *WRKY70* transcription factor was
187 induced later than the induced *PR* genes so we could imagine that induced *PR* genes were activated
188 by another precocious regulation, such as an oxidative burst (see below), rather than by *WRKY70*.
189 Furthermore, *WRKY70* induction seems not sufficient to enable a long lasting induction of these
190 defense genes.

191 SA accumulation was also rather mixed. *CBP60a* [30], *ACA11* [31], *EICBP.B* (or *CATMA1*; [32]), all
192 three coding calcium-sensor proteins, are known as negative regulators of SA accumulation and
193 biosynthesis, as well as the light signaling factor *FAR1* [33] or the SA glucosyltransferase *UGT74F1*
194 which convert SA in inactive SA 2-O-beta-D-glucoside or the glucose ester of SA [34]. On the contrary
195 *EDS1*, *PAD4* (reviewed in [35]) and *MKS1* [36] are known as positive regulators of SA accumulation. In
196 our data, the repression of *CBP60a*, *ACA11* and *EICBP.B* genes sustained a SA biosynthesis and
197 accumulation. In addition, *EDS1* activation allowed to consider a positive feedback loop likely to
198 potentiate SA action via *EDS1* cytosolic homodimers, even though *PAD4* was repressed. But, as well

199 as *WRKY70* induction, the repression of the MAPK *MKS1* and the activation of the light signaling
200 factor *FAR1* (2 times) or the SA glucosyltransferase *UGT74F1* were in favor of less free SA. Concerning
201 SAR, *MES1* is known as required in healthy systemic tissues of infected plants to release the active SA
202 from methyl-SA, which serves as a long-distance signal for systemic acquired resistance (SAR) [35]
203 and *ACBP6* may be involved in the generation of SAR inducing signal(s) [37]. In our data, SAR seemed
204 compromised given the repression of *ACBP6* at 24hpi and *MES1* at 72hpi. To conclude, in the nonhost
205 interaction between pear and *V. inaequalis*, the SA pathway could be engaged but transiently and
206 presumably reduced to the few infection sites and not spread by SAR in healthy systemic tissues.

207

208 Calcium influx and reactive oxygen species (ROS) production act as secondary messengers
209 and lead to stomatal closure

210 Early responses of plants upon pathogen perception include calcium influx and ROS production,
211 which both act as secondary messengers ([38], reviewed in [10]). Three pear DEGs were found that
212 indicate early increased cytosolic calcium level. The CSC (Calcium permeable Stress-gated cation
213 Channel) ERD4 (found two times) and the two glutamate receptors GLR3.4 and GLR2.7, are known as
214 calcium permeable channels ([39]; [40]). They were induced at 24hpi in our data. An increased
215 cytosolic calcium level can lead to a pre-invasive defense response by stomatal closure and promote
216 the post-invasive defense response ROS accumulation [41].

217 Calcium influx has been reported to promote stomatal closure through the regulation of potassium
218 flux and the activation of anion channels in guard cells (reviewed in [10]). The stomata closure is
219 known to be induced via the inhibition of inward potassium currents which is achieved via activation
220 of calcium dependent protein kinases (CDPK) such as CPK13 and CPK8/CDPK19 ([42]; [43]); but also
221 via activation of CBL1 of the CBL1-CIPK5 complex, which activates the GORK potassium outward
222 channel [44]. CPK13, CPK8/CDPK19 and CBL1 were all activated at 24 hpi in our data.

223 A NADPH oxidase *RBOHB* (respiratory burst oxidase homologs, RBOH) is early and long-lasting
224 induced in the pear/*V. inaequalis* nonhost interaction suggesting a rapid and maintained apoplastic

225 ROS production. Indeed, the apoplastic ROS are mainly produced by plasma membrane localized
226 NADPH oxidases, cell wall peroxidases and amine oxidases [45]. In addition, posttranslational
227 regulation of RBOH is required for its activation and ROS production. Calcium, phosphatidic acid, and
228 direct interactors such as Rac1 GTPase and RACK1 (Receptor for Activated C-Kinase 1) have been
229 reported to be positive regulators of RBOHs (reviewed in [46]). For example, the Rac-like/ROP
230 GTPase ARAC3 is known to interact with a RBOH to promote ROS production [47]. In our data,
231 RBOHB activity was also supported by the presence of positive regulators such as Rac-like/ROP
232 GTPase. The three Rac-like/ROP GTPase *ARAC1*, *ARAC3* and the homolog of *At4g03100* were induced
233 at 24hpi. CDPKs such as *CPK1* are also known to activate RBOHs in response to increased cytosolic
234 calcium level [48]. But repression of *CPK1* in our data seems to indicate that this way of activation did
235 not function.

236 In response to abscisic acid (ABA) or microbe-associated molecular pattern (MAMP) immunity,
237 stomatal closure is known to be regulated by apoplastic ROS production (reviewed in [49]) and
238 cysteine-rich receptor-like kinases (CRK) are also known to be elements between ROS production and
239 downstream signaling leading to stomatal closure, sometimes activated (*CRK10*), sometimes
240 inhibited (*CRK2* and *CRK29*; [50]). Three DEGs coding for CRK were found in our data and the
241 repression of *CRK2* and *CRK29* (found two times) was consistent with the stomata closure previously
242 found, but the repression of *CRK10* (found two times) was not. Beyond closure, inhibition of stomatal
243 development could be seen as an extreme defense. *YODA* (found two times) and *MPK6* (found two
244 times) MAPKs belong to a pathway involved in the negative regulation of stomata development [51].
245 These two genes were early induced in our data.

246 To conclude, in pear/*V. inaequalis* nonhost interaction, a calcium influx leads to the development of
247 the stomatal closure pre-invasive defense, but also promotes a post-invasive defense: apoplastic ROS
248 accumulation. Apoplastic ROS, acting themselves as messengers, come to strengthen the stomatal
249 closure (Fig. 4).

250

251 Transcription factors and sphingolipids maintain HR under control

252 ROS are known to mediate cellular signaling associated with defense-related gene expression,
253 hypersensitive response (HR) i. e. the programmed cell death (PCD) at the site of infection during a
254 pathogen attack, and phytoalexin production [52]. *Arabidopsis thaliana* RCD1 regulator has been
255 proposed to positively regulate cell death in response to apoplastic ROS by protein-protein
256 interactions with transcription factors (reviewed in [53]) and WRKY70 and SGT1b were identified as
257 cell death positive regulators functioning downstream of RCD1 [53]. *RCD1* and *WRKY70* genes were
258 found induced in our data, at 24hpi and 72hpi respectively.

259 In *Arabidopsis*, the F-box protein CPR1, in association with the Skp1-Cullin-F-box (SCF) ubiquitin ligase
260 complex, targets for degradation NLR (nucleotide-binding domain and leucine-rich repeats containing
261 proteins) resistance protein such as SNC1, RPM1 or RPS2, to prevent overaccumulation and
262 autoimmunity (reviewed in [54]). A *Skp1-like* (*ASK19*; 72hpi) gene and *CPR1* (24hpi) gene were found
263 induced in our data. A gene coding for RPM1 function was also found repressed at 24hpi. These
264 results are in favor of the hypothesis that NLR receptors do not take part in the HR development
265 observed in the *pear/V. inaequalis* nonhost interaction (Fig. 1A). In addition, the induction of an
266 *AtSerpin1* gene homolog at 24hpi (found two times) in our data is consistent with that hypothesis.
267 Indeed, AtMC1 is a pro-death caspase-like protein required for full HR mediated by intracellular NB-
268 LRR immune receptor proteins such as RPP4 and RPM1 [55] and *AtSerpin1* is a protease inhibitor
269 which block AtMC1 self-processing and inhibit AtMC1-mediated cell death [56].

270 The differential expression of two others components of the proteasome pathway is in favor of an HR
271 development: the induction of the *RIN3* ubiquitin E3 ligase (24hpi) and the repression of the *BRG3*
272 ubiquitin E3 ligase (24hpi). Indeed, RIN3 is known as positive regulator of RPM1 dependent HR [57].
273 And BRG3 is known as a negative regulator of HR in plant/necrotrophic pathogen interactions [58].

274 Sphingolipids are involved in the control of PCD, either as structural components of membranes but
275 also as initiators in the cell death regulatory pathway. According to Huby et al [59], free ceramides
276 and long chain/sphingoid base components (LCBs) are able to trigger cell death, via ROS production,

277 whereas their phosphorylated counterparts, ceramide phosphates and long chain base phosphate
278 components (LCB-Ps) promote cell survival. The induction of PCD by LCB is based on the activation of
279 protein kinases, among them MPK6 [60]. As already mentioned, *MPK6* was found early induced in
280 our data and we found numerous DEGs in the nonhost interaction between pear and *V. inaequalis*
281 that indicate the presence of free ceramides and LCB, which possibly participate to the HR
282 development. Free LCB presence is demonstrated by the activations of *SBH1* (24hpi), *SLD1* (24hpi)
283 and another sphingolipid $\Delta 8$ long-chain base desaturase homolog to *At2g46210* (24hpi; found two
284 times), and their relative conversion in ceramides is demonstrated by the differential expressions of
285 the ceramide synthases *LOH2* (repressed at 24hpi) and *LOH3/LAG13* (induced at 24 and 72hpi). LCB
286 non-conversion in phosphorylated counterparts is shown by the *AtLCBK1* repression (72hpi) and free
287 ceramides maintenance is attested by their non-conversion in glycosylated ones given the repression
288 of a glucosyl ceramide synthase homolog to *At2g19880* (24hpi).

289 The differential expression of numerous known regulators of HR in our data is again consistent with
290 the HR phenotype observed. The mechanosensor *MSL10* and the calmodulin-activated Ca^{2+} pump
291 (autoinhibited Ca^{2+} -ATPase [ACA]) *ACA11* were found engaged: at 24hpi *MSL10* was induced and
292 *ACA11* was repressed. *MSL10* is known as a positive regulator of cell death [61] and *ACA11* is known
293 as a negative regulator of SA-dependent programmed cell death [31]. Their modulation is linked with
294 the noticed calcium influx discussed above ([31]; [62]). The participation of the SA pathway in the
295 development of the hypersensitive response could also be supported by the repression of *EDR1* (at
296 72hpi). Indeed, the MAPKKK *EDR1* is known as a negative regulator of the SA-dependent HR
297 (reviewed in [63]).

298 Three other regulators of HR were found modulated in our data. The transcription factor *AS1*
299 (*MYB91*) was found induced at 24hpi. It is known as a positive regulator of HR and implicated in JA
300 pathway (reviewed in [18]). The transcription factor *WRKY40* was found repressed at 72hpi. It is
301 known as a negative regulator of HR [64] and implicated in PTI [65]. Another negative regulator of HR
302 is the lipid-binding domains containing protein *VAD1* [66]. It was found repressed at 72hpi.

303 The behavior of two others genes in our data seems to indicate that the developed HR was contained
304 and not carried away due to too much intracellular ROS production and damages. The function
305 *UGT73B3* and *CAT2* were thus activated (24hpi). *UGT73B3* and *CAT2* are known as restrictors of HR
306 expansion via their action in ROS scavenging (*CAT2*; [67]) or in detoxification of ROS-reactive
307 secondary metabolites (*UGT73B3*; [68]).

308 To conclude, in pear/*V. inaequalis* nonhost interaction, HR was spread out, in link with the calcium
309 influx, but especially following apoplastic ROS production and ROS production via free sphingolipids
310 accumulation and not via NLR receptors. Furthermore, the behavior of not less than height regulators
311 indicate that the developed HR is under control (Fig. 4).

312

313 Cell wall carbohydrates content and cuticle composition are altered

314

315 The first obstacle encountered by host as well as nonhost pathogens attempting to colonize plant
316 tissues is the plant cell wall, which is often covered with a cuticle. Preinvasive penetration barrier, as
317 a preformed physical barrier, or as the onset place of defensive signaling pathways, is considered an
318 important factor, especially in nonhost resistance in which non adapted pathogens normally fail to
319 penetrate nonhost plant cells when blocked by the cell wall ([10]; [41]). Plant cell wall alterations, of
320 the carbohydrates or the phenolic components, either by impairing or overexpressing cell wall-
321 related genes, have been demonstrated to have a significant impact on disease resistance and/or on
322 abiotic stresses (reviewed in [69] and [70]).

323 We found numerous genes related to the cell wall with a modified expression during nonhost
324 interaction between pear and *V. inaequalis*, among them about thirty related to the biosynthesis or
325 the modification of carbohydrates. These genes are presented in table 3, except those related to the
326 lignin and other phenolic compounds, which will be discussed later. We saw in particular several
327 genes related to cellulose (8) and even more genes related to pectin (14) but no genes related to
328 callose.

329 Concerning these particular carbohydrate components, the model proposed by Bacete et al [69] is as
 330 follows. Firstly, alterations in cellulose biosynthesis from primary or secondary cell wall trigger
 331 specific defensive responses, such as those mediated by the hormones JA, ET or abscisic acid (ABA),
 332 activate biosynthesis of antimicrobial compounds, but also might attenuate pattern triggered
 333 immunity (PTI) responses. Secondly, alterations of cell wall pectins, either in their overall content,
 334 their degree of acetylation or methylation, activate specific defensive responses, such as those
 335 regulated by JA or SA, and trigger PTI responses, probably mediated by damage-associated molecular
 336 patterns like oligogalacturonides. Thus, even though our results do not completely support a role of
 337 these genes, we think that the modified expression of cell wall related genes during nonhost
 338 interaction between pear and *V. inaequalis* is meaningful.

339

340

341 **Table 3: Main DEGs related to cell wall carbohydrates synthesis/modification detected during non-**
 342 **host interaction pear/*V. inaequalis*.**

343

	Gene	Action	Expression*
Primary cell wall			
Cellulose	<i>CSLA2</i>	synthesis	I
	<i>PNT1</i>	synthesis	I
	<i>COBL2</i>	deposition (GPI-anchored protein)	R
	<i>AtGH9A4</i>	catabolism	I
	<i>XTR7</i>	loosening	I
Hemi-cellulose (xyloglucan)	<i>At5g15490</i>	synthesis	I
Pectin	<i>At3g42180</i>	synthesis	I
	<i>At4g01220</i>	synthesis	I
	<i>GHMP kinase</i>	synthesis	I
	<i>RHM1</i>	synthesis	R
	<i>PME</i>		
	<i>At2g45220</i>	methylesterification	I
	<i>PME</i>		
	<i>At2g46930</i>	methylesterification	I
	<i>PME</i>		
	<i>At3g05910</i>	methylesterification	R
	<i>PME</i>		
	<i>At1g02810</i>	methylesterification	R
	<i>PME44</i>	methylesterification	R

	<i>PG At3g16850</i>	depolymerisation	I
	<i>PG At3g59850</i>	depolymerisation	I
	<i>PG At4g13710</i>	depolymerisation	I
	<i>PG At3g62110</i>	depolymerisation	R
	<i>IDA</i>	degradation	R
Arabinogalactan protein	<i>AGP11</i>	–	I
	<i>AGP1</i>	–	R
Secondary cell wall			
Cellulose	<i>CESA09</i>	synthesis	I
	<i>CESA10</i>	synthesis	I
	<i>CSLG1</i>	synthesis	I
Hemi-cellulose (xylan)	<i>FRA8</i>	synthesis	I
Undetermined			
Expansin	<i>EXP15</i>	loosening	I
	<i>EXPB3</i>	loosening	I
Hemi-cellulose	<i>ATFUC1</i>	modification	I
	<i>XTH33</i>	growth and assembling	R

344 *I: induced, R: repressed

345

346 Concerning the cuticle layer, most cuticles are composed largely of cutin, an insoluble polyester of
347 primarily long-chain hydroxy fatty acids. This lipophilic cutin framework is associated with
348 hydrophobic compounds collectively referred to as waxes. The cuticle is also thought to contain
349 varying amounts of intermingled cell wall polysaccharides and sometimes also a fraction termed
350 cutan (reviewed in [71]). Cutin monomers are synthesized by the modification of plastid-derived 16C
351 and 18C fatty acids in the endoplasmic reticulum (ER), yielding variously oxygenated fatty acid–
352 glycerol esters referred to as monoacylglycerols, which polymerize upon arrival at the growing cuticle
353 (Fig. 5, reviewed in [71]).

354 C16 and C18 fatty acids are also important precursors of cuticular wax synthesis (Fig. 5). Upon
355 transport to the ER, the C16 and C18 fatty acids are extended to form very-long-chain fatty acids
356 (VCLFAs; C>20), and this extension is carried out by the fatty acid elongase (FAE) complex located on
357 the ER membrane. The very-long-chain FAs are then converted into the varied cuticular waxes
358 (primary alcohols, aldehydes, alkanes, secondary alcohols, ketones) by many ways (reviewed in [72]).
359 Interestingly, we found three genes upregulated 24hpi belonging to the FAS (fatty acid synthase)
360 chloroplastic complex implicated in the production of the C16 precursor (Fig. 5): *ACCD*, *FabG* and

361 *MOD1* (found two times). ACCD encodes the carboxytransferase beta subunit of the Acetyl-CoA
362 carboxylase complex which catalyzes the first committed step in fatty acid synthesis: the
363 carboxylation of acetyl-CoA to produce malonyl-CoA. FabG and MOD1 are respectively a β -ketoacyl
364 ACP-reductase and an enoyl-ACP-reductase which catalyze respectively the conversion of
365 acetoacetyl-ACP into β -hydroxyacyl-ACP and the second reductive step from enoyl-ACP to butyryl-
366 ACP (reviewed in [72]).

367 In the ER, the four functions we found related to waxes biosynthesis in our data were repressed at
368 24hpi: *KCS4* (found two times), *CER1* and *CER3*, or 72hpi: *ECR/CER10*. *KCS4* and *ECR/CER10* belong to
369 the FAE complex ([73]; [74]). The last two genes are implicated in aldehydes (*CER1*) and alkanes
370 (*CER1 and 3*) generation (reviewed in [72]). On the contrary, the eight genes we found connected to
371 cutin biosynthesis were induced at 24hpi except a gene homolog to *At5g14450*, which was induced
372 at 72hpi. One of them is a glycerol-3-phosphate acyltransferase (GPAT) coding gene: GPAT8, which
373 catalyzes the transfer of a fatty acid from coenzyme A (CoA) to glycerol-3-phosphate (Fig. 4; reviewed
374 in [71]). GPAT8 function in cutin formation has been functionally confirmed in association with
375 GPAT4 [75]. The seven others genes code GDSL-lipases enzyme (*At1g28600*, *At1g28660*, *At1g54790*,
376 *At3g16370*, *At3g48460*, *AtCUS4: At4g28780*, *At5g14450*), some of which have been shown to
377 function as cutin synthase (Fig. 4; [76]; reviewed in [71]) and polymerize monoacylglycerols.

378 We also found induced respectively at 24 and 72hpi two genes involved in waxes and cutin
379 biosynthesis positive regulation: *MYB16* and *SHN1*. The SHN genes (*SHN1–SHN3*), a set of three
380 largely redundant APETALA 2 family transcription factors from *A. thaliana*, are regulators of floral
381 cutin and epidermal cell morphology. SHN1 is regulated by the MYB family transcription factor
382 MYB106, which, along with its paralog MYB16, controls many aspects of cuticle and epidermis
383 formation in *A. thaliana* (reviewed in [77] and [71]).

384 Cutin and cuticular waxes play an important role in plant-insect and plant-microbe interactions.
385 Numerous Arabidopsis mutants in cutin and waxes biosynthetic or transport genes, such as Acyl-CoA
386 binding proteins (ACBP), show varying degrees of cuticle impairment, alterations in cutin and/or wax

387 composition, and defects in SAR (reviewed in [72]). We found *ACBP6* repressed at 24hpi. That
388 repression is not inconsistent with the previously described amplification of cutin biosynthesis and
389 polymerization, given that *acbp6* KO mutation is not associated with a defect in that pathway [37].
390 That repression is also consistent with the SAR repression observed above as the *acbp6* KO mutant
391 show compromised SAR [37].

392 To conclude, our analysis of nonhost pear/*V. inaequalis* interaction identified an alteration of the
393 cuticle composition with more cutin and less waxes synthesis. The increase in cutin polymerization
394 could lead to a thickening of the cuticular layer to prevent fungus penetration via its appressoria.

395

396 Secondary metabolism leads to G unit lignin polymerization and simple coumarin or
397 hydrocinnamic acid amine phytoalexins synthesis

398 As distinguished from primary metabolism, plant secondary metabolism refers to pathways and small
399 molecule products of metabolism that are non-essential for the survival of the organism. But they are
400 key components for plants to interact with the environment in the adaptation to both biotic and
401 abiotic stress conditions. Plant secondary metabolites are usually classified according to their
402 chemical structure. Several groups of large molecules, including phenolic acids and flavonoids,
403 terpenoids and steroids, and alkaloids have been implicated in the activation and reinforcement of
404 defense mechanisms in plants (reviewed in [78]).

405 Terpenoids and steroids, or isoprenoids, are components of both the primary and secondary
406 metabolisms in cells, and mono-, tri-, sesqui- and polyterpenes are considered as secondary
407 metabolites (reviewed in [79]). Our results on pear identified seven DEGs and five DEGs belonging to
408 the chloroplastic methylerythritol phosphate (MEP) and to the cytosolic mevalonic acid (MVA)
409 pathway of isoprenoids production respectively (Table 4), which results, among others compounds,
410 in tri- and sesquiterpenes secondary metabolites. The majority of these genes contribute to produce
411 primary metabolites according to Tetali [79]. Except *SMT2*, that we found induced at 24hpi, there is
412 no report concerning a putative implication of others genes in plant biotic resistance. *SMT2* is

413 involved in sterols production and *smt2* mutation was reported to compromise bacterial resistance in
 414 *Nicotiana benthamiana* [80]. The hypothesis is that sterols regulate plant innate immunity against
 415 bacterial host and nonhost infections by regulating nutrient efflux into the apoplast. *V. inaequalis* is
 416 an hemi biotrophic pathogens which colonizes only the apoplast compartment since the beginning of
 417 the interaction. *SMT2* strong relative induction in our data could indicate that a similar mechanism of
 418 nutrient efflux regulation via sterols could take place to limit the fungus growth in pear nonhost
 419 resistance against *V. inaequalis*.

420

421

422 **Table 4: Main DEGs involved in biosynthetic pathways for terpenes and isoprenoids during pear/*V.***
 423 ***inaequalis* non-host interaction.**

	Gene	Function	Expression*
Cytosolic MVA (mevalonic acid) pathway enzymes	<i>HMGS</i>	catalyze the second step of the pathway	R
	<i>HMGR1</i>	catalyze the third step of the pathway	R
	<i>SMT2</i>	sterols production	I
	<i>FLDH</i>	sesquiterpenes production	R
	<i>SQE2</i>	triterpenes production	I
Chloroplastic MEP (methylerythritol phosphate) pathway enzymes	<i>DXR</i>	catalyzes the second step of the pathway	I
	<i>GG reductase</i>	chlorophylls production	R
	<i>VTE4</i>	tochopherols production	I
	<i>KAO1</i>	gibberellins production	R
	<i>PDS2</i>	plastoquinones production	I
	<i>LYC</i>	carotenoids production	I
	<i>PGGT1</i>	covalent attachment of a prenyl group to a protein	I

424

425 In our data, the other DEGs that were linked to the secondary metabolism belong to the
 426 phenylpropanoid pathway production (Fig. 6). Among them we found four genes belonging to the
 427 flavonoid production, all repressed, at 24hpi (*DFR* and *DRM6*) or 72hpi (*TT7* and *UGT71D1*). *DFR*
 428 (dihydroflavonol reductase) is involved in flavan-3,4-ol production and *TT7* (flavonoid 3' hydroxylase)
 429 in dihydroquercetin production from dihydro-kaempferol, and *UGT71D1* (glucosyl transferase) in
 430 quercetin-glycoside production from quercetin (TAIR database;

431 <https://www.arabidopsis.org/index.jsp>). DMR6 (flavone synthase) is involved in flavone production
432 from naringenin [81]. Thus flavonoid production does not seem to be favored, which is not consistent
433 with the induction of *MYB12* at 24hpi, but consistent with *MYB4* induction at 72hpi. MYB12 is
434 actually known as a positive regulator of flavonol biosynthesis in pear and apple fruits ([82]; [83])
435 whereas MYB4 is known as a negative regulator of this biosynthetic pathway [84].

436 Concerning the production of monolignols, precursors of lignin synthesis, some genes were found
437 induced, others repressed. We found *CYP98A3* and *CAD9* (found two times) induced at 24hpi and
438 *HCT*, *CCR1* and a gene homolog to *At2g23910* (found two times, one time repressed at 72hpi)
439 repressed at 24hpi Fig. 6). *CYP98A3* encode a C3H (coumarate 3-hydroxylase), *CAD9* encode a CAD
440 (cinnamyl alcohol dehydrogenase), *HCT* is an hydroxycinnamoyl-CoA shikimate/quinate
441 hydroxycinnamoyl transferase, *CCR1* encode a CCR (cinnamoyl-CoA reductase) and *At2g23910*
442 encode a CCR-related protein. (TAIR and KEGG databases (<https://www.genome.jp/kegg/>)).

443 Lignification is obtained by cross-linking reactions of the lignin monomers or by polymer–polymer
444 coupling via radicals produced by oxidases such as peroxidases [85] and laccases [86]. However,
445 while peroxidases are able to oxidize monolignols to produce H, G and S units of lignin, laccases only
446 generate G units [85]. In our data, we found two laccases induced at 24hpi: *LAC11* (found two times,
447 one time induced at 24 and 72hpi) and *17* (found two times), and three peroxidases repressed at
448 24hpi: *PRX17*, *PER47* and *PRX52* (also repressed at 72hpi), which can be linked to lignin biosynthetic
449 process (Fig. 6). According to Zhao et al. [86], *LAC11* and *17*, along with *LAC4*, play a critical role in
450 lignification, and their results suggests that peroxidase and laccase do not serve redundant functions
451 in lignification, at least in the vascular tissues of the stem and root of Arabidopsis. Participation in
452 lignin formation has also been proved for *PRX17* [87], *PER47* [88] and *PRX52* [89]. But there are
453 currently no reports about a possible involvement of all these genes in lignification linked to biotic or
454 abiotic stresses. Concerning non-host resistance, two reports describe lignin
455 accumulation/deposition involvement: one in apple fruit [90] and the other one in cowpea [91]. In
456 the latest, authors showed that preferentially generated lignin units in this nonhost interaction are G

457 units, just as it seems to be the case in our pear / *V. inaequalis* study. To summarize, it is tempting to
458 think that modifications of expression observed for genes linked to lignin polymerization are relevant
459 for the pear nonhost resistance against *V. inaequalis*, but further functional analysis should be
460 conducted to conclude.

461 The biosynthesis of two others types of phenylpropanoid compounds appears to be favored during
462 pear nonhost resistance against *V. inaequalis*: simple coumarin on one hand and hydroxycinnamic
463 acid amides on the other hand. We found four *BGLU*-like genes induced at 24hpi: *BGLU42* (also
464 induced at 72hpi), *47* and *BGLC3*, or 72hpi: *BGLU16* (Fig. 6). These β -glucosidases could be implied in
465 simple coumarin path production from the cinnamic acid (KEGG database). Some natural simple
466 coumarins are known as antifungal compounds *in vitro* and have been developed as fungicides [92].
467 Ancient work on Hevea also reports the correlation between the resistance against pathogenic fungi
468 and the production of some coumarins, with antifungal activity *in vitro* [93]. We also found induced
469 at 24hpi the genes *AACT1/ACT1*, *ATPAO5* and genes homologs to *At4g17830* and *At4g38220* (Fig. 6).
470 *AACT1/ACT1* catalyze the first specific step in branch pathway synthesizing hydroxycinnamic acid
471 amides from the p-Coumaroyl CoA or the feruloyl CoA and amines agmatine or putrescine [94].
472 Hydroxycinnamic acid amides are produced in response to pathogenic infections [94] and surface
473 exported. Hydroxycinnamic acid amides are reported to participate in Arabidopsis nonhost resistance
474 against *Phytophthora infestans* via their inhibitory activity on spore germination [95]. The three
475 others genes belong to the arginine biosynthesis path (homologs to *At4g1783* and *At4g38220*) and
476 the arginine and proline metabolisms which produce the amines agmatine and putrescine (*ATPAO5*)
477 (KEGG database). Agmatine is directly produced from arginine thanks to an ADC activity (arginine
478 decarboxylase) and putrescine can be produced from spermidine thanks to a PAO activity (polyamine
479 oxidase). *ATPAO5* catalyzes the conversion of spermine in spermidine. The induction of these three
480 last genes is therefore consistent with the hypothesis of amines production in order to enable
481 hydroxycinnamic acid amides synthesis. The induction of *C4H* at 24hpi could also favor
482 hydroxycinnamic acid amides synthesis via p-Coumaroyl CoA biosynthesis promotion. *C4H*

483 (cinnamate 4-hydroxylase) catalyzes the production of p-Coumaric acid from Cinnamic acid and p-
484 Coumaric acid gives p-Coumaroyl CoA thanks to 4CL (4- coumarate-CoA ligase) (KEGG database).
485 Among the suite of defense components synthesized in nonhost as in host context, a chemical barrier
486 can be established via accumulation of a diverse array of secondary metabolites rapidly produced
487 upon pathogen infection, named phytoalexins, with toxic or inhibitory effects (reviewed in [10]).
488 Phytoalexins can be flavonoids, such as the pisatin of pea (in [96]) but also varied phenylpropanoid
489 compounds. In the nonhost interaction pear / *V. inaequalis*, the production of flavonoid type
490 phytoalexins does not seem to be favored, except simple coumarin and hydroxycinnamic acid amines.

491

492 Very limited transcriptomic modulation during apple / *V. pyrina* nonhost interaction

493 Only 60 DEGs were detected in the apple / *V. pyrina* nonhost interaction at 24 or 72hpi, in agreement
494 with the total absence of macroscopic symptoms and few cells engaged in an HR-like reaction
495 observed at the microscopic level. Among these 60 DEGs, 36 have no known function. Among the 24
496 remaining DEGs, nine DEGS could be relevant in apple / *V. pyrina* nonhost interaction in view of our
497 findings in pear / *V. inaequalis* nonhost interaction. *ORG2 (BHLH038)*, a putative integrator of various
498 stress reactions [97] was induced at 24hpi. Three genes were related to an oxidative stress: *GASA10*
499 was repressed at 24hpi and *NRAMP3* and *AOR* were induced at 24hpi. GASA proteins have been
500 suggested to regulate redox homeostasis via restricting the levels of OH[·] in the cell wall [98]. The
501 repression of this gene is thus in favor of more OH[·] in the cell wall. The oxidoreductase coding gene
502 *AOR* is known in the chloroplast to contribute to the detoxification of reactive carbonyls produced
503 under oxidative stress [99]. *NRAMP* genes function as positive regulators of ROS accumulation,
504 especially during Arabidopsis *Erwinia chrysanthemi* resistance [100]. The induction (at 24 and 72hpi)
505 of another gene suggests modifications at the cell wall level: *EXP8*, an expansin coding gene involved
506 in cell wall loosening (Tair database). We also found two genes related to hormone pathways, one
507 induced at 24hpi: *WIN1* and the other one repressed at 72hpi: *UBP12*. WIN1 is known as a negative
508 regulator of SA pathway [101] and UBP12 as a positive regulator of JA pathway via the stabilization of

509 MYC2 [16]. In link with the JA pathway, we also found *TPS21* induced at 24hpi. *TPS21* is involved in
510 sesquiterpenes production and is promoted by JA signal via MYC2 [102]. *TPS21* is especially involved
511 in the jasmonate-dependent defensive emission of herbivore-induced volatiles in cranberries [103].
512 Finally the last DEG we found relevant in apple / *V. pyrina* nonhost interaction could promote HR via
513 ceramides accumulation. *ACD11* is repressed at 24hpi in our data. In *acd11* mutants, the relatively
514 abundant cell death inducer phytoceramide rises acutely [104].
515 Because nonhost resistance of apple against *V. pyrina* is of a type I, with a very limited number of
516 cells engaged in an HR-like reaction, it has not been possible for us to exhaustively describe how this
517 interaction is expressed at the transcriptomic level. Further insight with more adapted technics such
518 as laser-assisted cell picking, prior to micro arrays or RNA sequencing analysis (review in [105]) could
519 provide more information in the future.

520

521 Comparison of pear resistances against the host pathogen *V. pyrina* and the nonhost
522 pathogen *V. inaequalis*

523 Perchepped et al [12] performed a detailed transcriptomic analysis of the host resistance of pear
524 against *V. pyrina* strain VP102, deployed in a transgenic pear bearing the well-known apple *Rvi6*
525 resistance gene against *V. inaequalis*. Comparing this work to our gives us the rare opportunity to
526 analyze similarities and differences between a host and a nonhost resistance in the same plant. Only
527 four transcriptomic studies involving pear/pathogen interactions have been published so far. Yan et
528 al [106] reported the modulation of expression of 144 pear genes after fruit treatment by
529 *Meyerozyma guilliermondii*, an antagonistic yeast used for biocontrol of natural pear fruit decay.
530 Zhang et al [107] similarly reported the modulation of expression of 1076 pear genes after treatment
531 with *Wickerhamomyces anomalus*, another biocontrol agent. Using RNA-seq, Wang et al. [108]
532 reported a major role of ethylene signalization during the compatible interaction between *P. pyrifolia*
533 and *Alternaria alternata*, a necrotrophic pathogen. Finally, Xu et al. [109] applied RNA-seq to

534 characterize the genes of *Penicillium expansum* activated after infection of pear fruits. None of these
535 studies can be directly compared to our work on host and nonhost scab pear resistance.

536 Concerning the recognition and early signaling steps of the interactions, many receptors and co-
537 receptors have been found induced in the host pear resistance, especially damage-associated
538 molecular patterns receptors such as RLK7, revealing that PTI and ETI must be engaged. We did not
539 found evidence of the mobilization of such receptors in the pear nonhost resistance. PTI and ETI
540 receptors are nonetheless reported as implicated in nonhost resistance (reviewed in [110] and [10]).

541 As we only analyzed post infection transcriptional modulations in the nonhost pear/*V. inaequalis*
542 interaction (at 24 and 72hpi), one hypothesis to explain the lack of PTI and ETI receptors in our data
543 could be that these receptors were already present as preformed defenses and not particularly
544 induced by the infection onset. In pear nonhost interaction, the earliest signaling pathways we were
545 able to highlight are calcium influx and apoplastic ROS production. Calcium signaling seems to be also
546 implicated in pear host resistance, but less obviously than in nonhost resistance.

547 About the hormonal signaling pathways, the JA defense signaling pathway was found repressed in
548 pear nonhost resistance but quite activated in pear host resistance. The JA/ethylene (ET) defense
549 signaling pathway is known as an effective defense against necrotrophic fungi in Arabidopsis [111].

550 Thus, it is not surprising to find the JA pathway repressed in the development of the pear nonhost
551 resistance against the hemi-biotrophic pathogen *V. inaequalis*. But it is very interesting to find this
552 pathway rather induced in the development of the pear host resistance against the other hemi-
553 biotrophic pathogen *V. pyrina*. The SA signaling pathway is commonly seen as the classical one
554 triggered to resist biotrophic fungi in Arabidopsis [111], but only a little engagement in pear nonhost
555 resistance has been observed, SA signaling being repressed in pear host resistance. If this absence of
556 SA implication is quite unexpected in pear host resistance against a hemi-biotrophic fungus, it is
557 consistent with the report that the exact role of these key defense phytohormone is unclear in
558 nonhost resistance and remains to be established [41]. As shown by Tsuda et al [112], an explanation
559 for the hormone pathways behavior in pear host resistance could be that: as both the SA and JA/ET

560 pathways positively contribute to immunity, a loss of signaling flow through the SA pathway can be
561 compensated by a rerouting signal through the JA/ET pathways. In addition, independently of SA
562 signaling, but in positive connection with JA signaling, SAR seems to be engaged in distal tissues
563 during pear host resistance. To conclude, in pear host as well as nonhost resistances, classical
564 resistance hormones SA and JA/ET, and the correlative PR gene defenses, seems differently involved
565 than in Arabidopsis.

566 The carbohydrate content of the cell-wall is modified in response to the attacks by the pathogens.
567 Regarding cell-wall and cuticle, in pear host as well as nonhost resistances, important modifications
568 were highlighted. Similar modifications affected the cellulose and mainly the pectin contents, but no
569 callose production was observed. Regarding cuticle, waxes production was induced in host resistance
570 whereas it was repressed in nonhost resistance, in favor of cutin production / polymerization, which
571 was also induced in host resistance. To conclude, as a first obstacle encountered by host as well as
572 nonhost pathogens attempting to colonize plant tissues, the plant cell wall and its cuticle seem to
573 play a foreground role in pear host as well as nonhost resistances.

574 Finally, the production of secondary metabolites and phenylpropanoids compounds in particular,
575 seems to be a major line of defense, in pear host as well as nonhost resistances, but with
576 divergences. If lignin and flavonoid productions are preponderant in pear host resistance against *V.*
577 *pyrina*, lignin implication in pear nonhost resistance is less clear and flavonoids production is
578 obviously repressed. But the biosynthesis of two other types of phenylpropanoid-derived
579 phytoalexins appears to be favored during pear nonhost resistance: simple coumarin on one hand
580 and hydroxycinnamic acid amides on the other hand.

581 The comparative analysis between a host and a nonhost resistance in pear shows that, even though
582 specificities are observed, the two major defense lines engaged are shared: the cell wall and its
583 cuticle on one hand, the secondary metabolism with the phenylpropanoid pathway on the other
584 hand. Moreover, these defenses seem deployed largely independently of the SA signaling pathway,
585 widely recognized as the main defense hormone against biotrophic pathogens.

586

587 **Conclusion**

588 As far as we know, our work is the first one published regarding a transcriptomic analysis of post-
589 infections events of a nonhost resistance to *Venturia sp.* in apple and pear. Velho and Stadik [113]
590 recently published a detailed description of the apple / *Colletotrichum higginsianum* nonhost
591 resistance, highlighting the accumulation of callose at the sites of penetration of the fungus. But no
592 data on gene expression was included. Here, our molecular work on apple / *V. pyrina* nonhost
593 resistance remains preliminary and in order to allow a deeper deciphering, further analyses must be
594 considered with the aid of tools adapted to this type I nonhost resistance with very few cells engaged
595 in an HR-like reaction, only visible at a microscopic level. In pear, this deciphering allowed us to show
596 that nonhost resistance against *V. inaequalis* is a type II one, which involves enough pathogen
597 penetration in plant tissue to trigger visible HR and develops post-invasive defenses.

598 To summarize our findings on pear with a notion of cascading effect, we can propose the following
599 scenario (Fig. 4): once *V. inaequalis* presence is recognized by pear, a calcium cellular influx is
600 induced and leads to the development of a pre-invasive defense, the stomatal closure, but also
601 promotes an early post-invasive defense, an apoplastic ROS accumulation. Apoplastic ROS, acting
602 themselves as ubiquitous messengers, come to reinforce the stomatal closure but also mediate
603 cellular signaling resulting in two post-invasive defenses: HR development at infection sites, along
604 with phytoalexin (simple coumarin and hydroxycinnamic acid amines) production. The observed
605 alterations of the epidermis composition (cellulose, pectin, lignin for the cell wall, and cutin for the
606 cuticle), are presumed to strengthen this physical barrier and can be seen as the development of
607 another pre-invasive defense. The calcium (action on pectin reviewed in [114]) and the ROS (action
608 on lignin, [115]; [116]; action on cuticle, [117]) have been linked to some type of epidermis
609 modifications and may participate in the proceeding of these defense in pear / *V. inaequalis* nonhost
610 interaction.

611 Nonhost resistance is defined as the resistance of an entire plant species against a specific parasite or
612 pathogen [118] and is seen as the most durable resistance of plant. Thus, understanding the
613 molecular mechanisms underlying nonhost resistance can open up some interesting avenues to
614 create sustainable host resistances in the same plant species. Considering pear, in order to stop the
615 germination and entrance of hemibiotrophic host fungi such as *V. pyrina*, strengthening the cuticle
616 initial barrier via more cutin production and cross-link, or promoting the biosynthesis of phytoalexins
617 like hydroxycinnamic acid amines, appear as promising solutions, relatively easy to engineer
618 regarding recent advances in biotechnology tools on this species ([119]; [120]; [121]).

619

620 **Material and methods**

621 Biological material

622 Apple plants from the cultivar 'Gala' and pear plants from the cultivar 'Conference' were chosen
623 because of their susceptibility to *V. inaequalis* and *V. pyrina*, respectively. The apple and pear
624 genotypes were multiplied in vitro, rooted and acclimatized in greenhouse as described previously
625 ([122]; [123]).

626 For apple scab inoculation, the *V. inaequalis* monoconidial isolate used was EU-B05 from the
627 European collection of *V. inaequalis* of the European project Durable Apple Resistance in Europe
628 [124]. For pear scab inoculation, the monoconidial strain VP102 of *V. pyrina* was chosen for its
629 aggressiveness on 'Conference' [125].

630

631 Scab inoculation procedure

632 Greenhouse growth conditions and mode of inoculum preparation were as described in Parisi and
633 Lespinasse [126] for apple and Chevalier et al [127] for pear. Briefly, the youngest leaf of actively
634 growing shoots was tagged and the plants inoculated with a conidial suspension (2×10^5 conidia ml⁻¹)
635 of *Venturia pyrina* strain VP102 for apple and *Venturia inaequalis* strain EUB04 for pear. Symptoms

636 were recorded at 14, 21, 28, 35 and 42 days after inoculation. The type of symptoms was scored
637 using the 6 class-scale of Chevalier et al [128].

638

639 Microscopic observations

640 Histological studies were made on samples stained with the fluorophore solophenylflavine [129]. In
641 brief, leaf discs were rinsed in ethanol 50° before staining in a water solution of solophenylflavine
642 7GFE 500 (SIGMA-Aldrich, St Louis USA) 0.1% (v/v) for 10 min. The samples were first rinsed in
643 deionized water, then in glycerol 25% for 10 min. Finally, the leaf samples were mounted on glass-
644 slides in a few drops of glycerol 50%. They were examined with a wide-field epifluorescence
645 microscope BH2-RFC Olympus (Hamburg, D) equipped with the following filter combination:
646 excitation filter 395 nm and emission filter 504 nm.

647

648 Transcriptomics experiment

649 Leaf samples were immediately frozen in liquid nitrogen and kept at -80°C until analysis. Sampling
650 concerned the youngest expanded leaf of each plant labeled the day of the inoculation. Each sample
651 is a pool of leaves from three different plants and two biological repeats (n=2) have been made by
652 condition (genotype x treatment x time). Leaf samples taken just before inoculation (T0) and at 24
653 and 72hpi, were then used to perform transcriptomics analyses.

654 For RNA extraction, frozen leaves were ground to a fine powder in a ball mill (MM301, Retsch, Hann,
655 Germany). RNA was extracted with the kit NucleoSpin RNA Plant (Macherey Nagel, Düren, Germany)
656 according to the manufacturer's instructions but with a modification: 4% of PVP40 (4 g for 100 ml)
657 were mixed with the initial lysis buffer RAP before use. Purity and concentration of the samples were
658 assayed with a Nanodrop spectrophotometer ND-1000 (ThermoFisher Scientific, Waltham, MA, USA)
659 and by visualization on agarose gel (1% (weight/volume) agarose, TAE 0.5x, 3% (volume/volume)
660 Midori green). Intron-spanning primers (forward primer: CTCTGGTGTCAGGCAAATG, reverse primer:
661 TCAAGGTTGGTGGACCTCTC) designed on the *EF-1α* gene (accession AJ223969 for apple and

662 PCP017051 for pear, available at <https://www.rosaceae.org/>, with the datasets on "Pyrus communis
663 v1.0 draft genome") were used to check the absence of genomic DNA contamination by PCR. The
664 PCR reaction conditions were as follows: 95°C for 5 min, followed by 35 cycles at 95°C for 30 s, 60°C
665 for 45 s, 72°C for 1 min, with a final extension at 72°C for 5 min. The PCR products were separated on
666 a 2% agarose gel.

667 Amplifications (aRNAs) were produced with MessageAmpII aRNA Kit (Ambion Invitrogen, Waltham,
668 MA, USA), from 300 ng total RNA. Then 5 µg of each aRNA were retrotranscribed and labelled using a
669 SuperScript II reverse transcriptase (Transcriptase inverse SuperScript™ II kit, Invitrogen, Carlsbad,
670 CA, USA) and fluorescent dyes: either cyanine-3 (Cy3) or cyanine-5 (Cy5) (Interchim, Montluçon,
671 France). Labeled samples (30 pmol each, one with Cy3, the other with Cy5) were combined two by
672 two, depending on the experimental design. For each comparison two biological replicates were
673 analyzed in dye-switch as described in Depuydt et al [130]. Paired labeled samples were then co-
674 hybridized to Agilent microarray AryANE v2.0 (Agilent-070158_IRHS_AryANE-Venise, GPL26767 at
675 GEO: <https://www.ncbi.nlm.nih.gov/geo/>) for apple, or Pyrus v1.0 (Agilent-078635_IRHS_Pyrus,
676 GPL26768 at GEO) for pear, containing respectively 133584 (66792 sense and 66792 anti-sense
677 probes) and 87812 (43906 sense and 43906 anti-sense probes) 60-mer oligonucleotide probes. The
678 hybridizations were performed as described in Celton, Gaillard et al [131] using a MS 200 microarray
679 scanner (NimbleGen Roche, Madison, WI, USA).

680 For microarray analysis we designed two new chips. For apple we used a deduplicated probeset from
681 the AryANE v1.0 ([131]; 118740 probes with 59370 in sense and 59370 in anti-sense) augmented by
682 14844 probes (7422 in sense and 7422 in anti-sense) designed on new gene annotations from *Malus*
683 *domestica* GDDH13 v1.1 (<https://iris.angers.inra.fr/gddh13> or
684 https://www.rosaceae.org/species/malus/malus_x_domestica/genome_GDDH13_v1.1). These
685 probes target new coding genes with UTRs when available, manually curated micro-RNA precursors
686 and transposable elements. For transposable elements we used one consensus sequence for each
687 family and a randomly peaked number of elements proportionally to their respective abundance in

688 the genome. The microarray used in this study also have probes for coding genes of *V. inaequalis* but
689 they have not been taken into account.

690 For pear the design was done on the *Pyrus communis* Genome v1.0 Draft Assembly & Annotation
691 available on GDR (https://www.rosaceae.org/species/pyrus/pyrus_communis/genome_v1.0) web
692 site. We have downloaded the reference genome and gene predictions fasta files and structural
693 annotation gff file the 21st of September 2015. Using home-made Biopython scripts we have
694 extracted spliced CDS sequences with 60 nucleotides before start and after stop codons to get UTR-
695 like sequences likely to be found on transcripts resulting in a fasta file containing 44491 sequences.
696 These 60 nucleotides size increase the probability of finding specific probes on genes with high
697 similarity. This file was sent to the eArray Agilent probe design tool
698 (<https://earray.chem.agilent.com/earray/>) to generate one probe per gene prediction. Options used
699 were: Probe Length: 60, Probe per Target: 1, Probe Orientation: Sense, Design Options: Best Probe
700 Methodology, Design with 3' Bias. The probeset was then reverse-complemented to generate anti-
701 sense probes and filtered to remove duplicated probes. The final probeset contains 87812 unique
702 probes targeting 1 (73612 probes) or more (14200 probes) potential transcript both in sense and
703 anti-sense.

704 Normalization and statistical analyses performed to get normalized intensity values have been done
705 as in Celton, Gaillard et al [131]. For each comparison and each probe, we retrieved a ratio of the
706 logarithms of the fluorescence intensities (one per compared sample: T0 versus 24hpi or T0 versus
707 72hpi in our case) and an associated p-value. The applied p-value threshold to determine DEGs
708 (differentially expressed genes) was 0.05. Through blast analyze, a TAIR accession number (The
709 Arabidopsis Information Resource; <https://www.arabidopsis.org/>; [132]) has been linked to a
710 majority of apple or pear “probe/corresponding gene” and the couple “TAIR accession/ratio value”
711 has then been used to make a global analyze of functional categories observed in the Mapman
712 software (<https://mapman.gabipd.org/homemapman.gabipd.org>; [133]). The detailed analyze of
713 DEGs has been done through TAIR and KEGG (<https://www.genome.jp/kegg/>) databases, and

714 bibliography. Metadata for the 172 (162 for pear and 10 for apple) DEGs discussed in this work are
715 available in Table S2 and S3 (Online only).

716

717 QPCR validation of transcriptomic data

718 In order to validate transcriptomic data, QPCR was performed on a selection of gene/sample
719 associations. First-strand cDNA was synthesized using total RNA (2.0 µg) in a volume of 30 µl of 5×
720 buffer, 0.5 µg of oligodT15 primer, 5 µl of dNTPs (2.5 mM each), and 150 units of MMLV RTase
721 (Promega, Madison, WI, USA). The mixture was incubated at 42°C for 75 min. Quantitative RT-PCR
722 (QPCR) was then performed. Briefly, 2.5 µl of the appropriately diluted samples were mixed with 5 µl
723 of PerfeCTa SYBR Green SuperMix for iQ kit (Quantabio, Beverly, MA, USA) and 0.2 or 0.6 µl of each
724 primer (10 µM) in a final volume of 10 µl. Primers were designed with Primer3Plus, their volumes
725 were according to their optimal concentration (determined for reaction efficiency near to 100%;
726 calculated as the slope of a standard dilution curve; [134]). Accessions, primer sequences and
727 optimal concentrations are indicated in Table S1. The reaction was performed on a CFX Connect Real-
728 Time System (BIO-RAD, Hercules, CA, USA) using the following program: 95°C, 5 min followed by 40
729 cycles comprising 95°C for 3 s, 60°C for 1 min. Melting curves were performed at the end of each run
730 to check the absence of primer-dimers and nonspecific amplification products. Expression levels
731 were calculated using the $\Delta\Delta CT$ method [135] and were corrected as recommended in
732 Vandesompele et al [136], with three internal reference genes (GADPH, TUA and ACTIN 7 for apple,
733 GADPH, TUA and EF1 α for pear) used for the calculation of a normalization factor. For each couple
734 DEG/sample (sample defining a plant, time, treatment and biological repeat combination), the ratio
735 was obtained by dividing the mean value of CT calculated from 3 technical repeats by the
736 normalization factor obtained for this sample.

737

738 **Supplementary information**

739 **Additional File 1: Table S1, S2 and S3.**

740

741 **Abbreviations**

742 ABA: abscisic acid; CDPK: calcium dependent protein kinase; CRK: cysteine-rich receptor-like kinase;
743 DEG: differentially expressed gene; DFR: dihydroflavonol 4-reductase; DGDG:
744 digalactosyldiacylglycerol; ET: ethylene; ER: endoplasmic reticulum; ETI: effector triggered immunity;
745 FAE: fatty acid elongase; GPAT: glycerol-3-phosphate acyltransferase; hpi: hours post inoculation; HR:
746 hypersensitive reaction; JA; jasmonic acid; LCB: long chain/sphingoid base component; LCB-Ps: long
747 chain base phosphate component; LOX: lipoxygenase; MAMP: microbe-associated molecular pattern;
748 OPDA: 12-oxo-phytodienoic acid; PCD: programmed cell death; PTI: pattern triggered immunity; RBOH:
749 respiratory burst oxidase homolog; ROS: reactive oxygen species; SA: salicylic acid; SAR: systemic
750 acquired resistance

751

752 **Declarations**

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756 Authors contribution

757 EC, LP, and EV conceived the study. EC and EV supervised the study. ER and MB performed the
758 biological experiments. SG and SP performed the database work and assisted with the bioinformatics
759 analysis. EV wrote the original manuscript. EV and EC edited the manuscript. All authors have read
760 and agreed to the published version of the manuscript.

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764 Availability of data and materials

765 The datasets supporting the conclusion of this article are available in the Gene Expression Omnibus
766 (GEO) repository [<https://www.ncbi.nlm.nih.gov/geo/>] with GSE159179 and GSE159180 accession
767 numbers for apple and pear respectively.

768 Ethics approval and consent to participate

769 Experimental research on plants in this work comply with relevant institutional, national, and
770 international guidelines and legislation.

771 Consent for publication

772 This section is not applicable.

773 Competing interests

774 The authors declare that they have no competing interests

775

776 **References**

- 777 1. Potter D, Eriksson T, Evans RC, Oh S, Smedmark JEE, Morgan DR, Kerr M, Robertson KR, et al.
778 Phylogeny and classification of *Rosaceae*. *Plant Syst Evol.* 2007;266:5-43.
- 779 2. Campbell CS, Evans RC, Morgan DR, Dickinson TA, Arsenault MP. Phylogeny of subtribe *Pyrinae*
780 (formerly the *Maloideae*, *Rosaceae*): Limited resolution of a complex evolutionary history.
781 *Plant Syst Evol.* 2007;266: 119-145.
- 782 3. Wu J, Wang Z, Shi Z, Zhang S, Ming R, Zhu SL et al. The genome of pear (*Pyrus bretschneideri*
783 Redh.). *Genome Res.* 2013;23:396-408.
- 784 4. Velasco R, Zharkikh A, Affourtit J, Dhingra A, Cestaro A, Kalyanaraman A et al. The genome of the
785 domesticated apple (*Malus × domestica* Borkh.). *Nature Genet.* 2010;42:833–839.
- 786 5. Stehmann C, Pennycook S, Plummer K. Molecular identification of a sexual interloper: the pear
787 pathogen *Venturia pirina*, has sex on apple. *Phytopathol.* 2001;91:663-541.

- 788 6. Rossman A, Castlebury L, Aguirre-Hudson B, Berndt R, Edwards. (2647–2651) Proposals to
789 conserve the name *Venturia acerina* against *Cladosporium humile*; *Venturia borealis* against
790 *Torulama culicola*; *Venturia carpophila* against *Fusicladium amygdali* and *Cladosporium*
791 *americanum*; *Sphaerella inaequalis* (*Venturia inaequalis*) against *Spilocaea pomi*, *Fumago*
792 *mali*, *Actinone macrataegi*, *Cladosporium dendriticum*, *Asteroma mali*, and *Scolicotrichum*
793 *venosum*; and *Venturia pyrina* against *Helminthosporium pyrorum*, *Fusicladium virescens*, *F.*
794 *fuscescens*, *Cladosporium polymorphum* and *Passalora pomi* (Ascomycota: Dothideomycetes).
795 *Taxon*. 2018;67:1209-1211.
- 796 7. Gonzalez-Dominguez E, Armengol J, Rossi V. Biology and epidemiology of *Venturia* species
797 affecting fruit crops: a review. *Front Plant Sci*. 2017;8: 1496.
- 798 8. Jiang S, Park P, Ishii H. Penetration behaviour of *Venturia nashicola* , associated with hydrogen
799 peroxide generation, in Asian and European pear leaves. *J Phytopathol*. 2014;162: 770-778.
- 800 9. Chevalier M, Bernard C, Tellier M, Audrain C, Durel CE. Host and non-host interactions of *Venturia*
801 *inaequalis* and *Venturia pirina* on *Pyrus communis* and *Malus x domestica*. *Acta Hortic*.
802 2004;663: 205-208.
- 803 10. Lee HA, Lee HY, Seo E, Lee J, Kim SB, Oh S, Choi E, Choi E, Lee SE, Choi D. Current understanding
804 of plant nonhost resistance. *Mol Plant-Microbe Interact*. 2017a;30:5-5.
- 805 11. Mysore JS, Ryu CM. Nonhost resistance: how much do we know? *Trends Plant Sci*. 2004;9: 97-
806 104.
- 807 12. Perchepied L, Chevreau E, Ravon E, Gaillard S, Pelletier S, Bahut M, Berthelot P, Cournol R,
808 Schouten HJ, Vergne E. Successful intergeneric transfer of a major apple scab resistance gene
809 (Rvi6) from apple to pear and precise comparison of the downstream molecular mechanisms
810 of this resistance in both species. *bioRxiv* 2021.05.31.446424; doi:
811 <https://doi.org/10.1101/2021.05.31.446424>.
- 812 13. Wasternack C, Feussner I. The oxylipin pathways: biochemistry and function. *Annu Rev Plant Biol*.
813 2018;69:363-386.

- 814 14. Li C, Schillmiller AL, Liu G, Lee GI, Jayanty S, Sageman C, et al. Role of beta-oxidation in jasmonate
815 biosynthesis and systemic wound signaling in tomato. *Plant Cell*. 2005;17:971-986.
- 816 15. Seo JS, Koo YJ, Jung C, Yeu SY, Song JT, Kim JK, Choi Y, Lee JS, Do Choi Y. Identification of a novel
817 jasmonate-responsive element in the AtJMT promoter and its binding protein for AtJMT
818 repression. *PLoS One*. 2013;8:e55482.
- 819 16. Jeong JS, Jung C, Seo JS, Kim JK, Chua NH. The deubiquitinating enzymes UBP12 and UBP13
820 positively regulate MYC2 levels in jasmonate responses. *Plant Cell*. 2017;29:1406-1424.
- 821 17. Birkenbihl RP, Diezel C, Somssich IE. Arabidopsis WRKY33 is a key transcriptional regulator of
822 hormonal and metabolic responses toward *Botrytis cinerea* infection. *Plant Physiol*.
823 2012;159:266-85.
- 824 18. Kaurilind E, Xu E, Brosché M. A genetic framework for H₂O₂ induced cell death in *Arabidopsis*
825 *thaliana*. *BMC Genomics*. 2015;16:837.
- 826 19. Nurnberg PL, Knox KA, Yun BW, Morris PC, Shafiei R, Hudson A, Loake GJ. The developmental
827 selector AS1 is an evolutionarily conserved regulator of the plant immune response. *Proc*
828 *Natl Acad Sci U.S.A.* 2007;104:18795-187800.
- 829 20. Ali S, Ganai BA, Kamili AN, Bhat AA, Mir ZA, Bhat JA et al. Pathogenesis-related proteins and
830 peptides as promising tools for engineering plants with multiple stress tolerance. *Microb Res*.
831 2018; 212-213:29-37.
- 832 21. Li J, Brader G, Palva ET. The WRKY70 transcription factor: a node of convergence for jasmonate-
833 mediated and salicylate-mediated signals in plant defense. *Plant Cell*. 2004;16:319–331.
- 834 22. Li J, Brader G, Kariola T, Palva ET. WRKY70 modulates the selection of signaling pathways in plant
835 defense. *Plant J*. 2006;46:477–491.
- 836 23. Wang D, Amornsiripanitch N, Dong X. A genomic approach to identify regulatory nodes in the
837 transcriptional network of systemic acquired resistance in plants. *PLoS Pathog*. 2006;2:e123.
- 838 24. Li J, Zhong R, Palva ET. WRKY70 and its homolog WRKY54 negatively modulate the cell wall-
839 associated defenses to necrotrophic pathogens in Arabidopsis. *PLoS One*. 2017;12:e0183731.

- 840 25. Genot B, Lang J, Berriri S, Garmier M, Gilard F, Pateyron S, et al. Constitutively active Arabidopsis
841 MAP kinase 3 triggers defense responses involving salicylic acid and SUMM2 resistance
842 protein. *Plant Physiol.* 2017;174:1238-1249.
- 843 26. Hu X, Reddy AS. Cloning and expression of a PR5-like protein from Arabidopsis: inhibition of
844 fungal growth by bacterially expressed protein. *Plant Mol Biol.* 1997;34:949-59.
- 845 27. Mestre P, Arista G, Piron MC, Rustenholz C, Ritzenthaler C, Merdinoglu D, Chich JF. Identification
846 of a *Vitis vinifera* endo- β -1,3-glucanase with antimicrobial activity against *Plasmopara*
847 *viticola*. *Mol Plant Pathol.* 2017;18:708-719.
- 848 28. Zhang SB, Zhang WJ, Zhai HC, Lv YY, Cai JP, Jia F, Wang JS, Hu YS. Expression of a wheat β -1,3-
849 glucanase in *Pichia pastoris* and its inhibitory effect on fungi commonly associated with
850 wheat kernel. *Protein Expres Purif.* 2019;154:134-139.
- 851 29. Santamaria M, Thomson CJ, Read ND, Loake GJ; The promoter of a basic PR1-like gene, AtPRB1,
852 from Arabidopsis establishes an organ-specific expression pattern and responsiveness to
853 ethylene and methyl jasmonate. *Plant Mol Biol.* 2001;47:641-52.
- 854 30. Truman W, Sreekanta S, Lu Y, Bethke G, Tsuda K, Katagiri F, Glazebrook J. The CALMODULIN-
855 BINDING PROTEIN60 family includes both negative and positive regulators of plant immunity.
856 *Plant Physiol.* 2013;163:1741-1751.
- 857 31. Boursiac Y, Lee SM, Romanowsky S, Blank R, Sladek C, Chung WS, Harper JF. Disruption of the
858 vacuolar calcium-ATPases in Arabidopsis results in the activation of a salicylic acid-dependent
859 programmed cell death pathway. *Plant Physiol.* 2010;154:1158-71.
- 860 32. Huang J, Sun Y, Orduna AR, Jetter R, Li X. The Mediator kinase module serves as a positive
861 regulator of salicylic acid accumulation and systemic acquired resistance. *Plant J.* 2019;98:842-
862 852.
- 863 33. Wang W, Tang W, Ma T, Niu D, Jin JB, Wang H, Lin R. A pair of light signaling factors FHY3 and
864 FAR1 regulates plant immunity by modulating chlorophyll biosynthesis. *Journal of Integrative*
865 *Plant Biol.* 2015;58:91-103.

- 866 34. Song JT, Koo YJ, Seo HS, Kim MC, Choi YD, Kim JH. Overexpression of AtSGT1, an Arabidopsis
867 salicylic acid glucosyltransferase, leads to increased susceptibility to *Pseudomonas syringae*.
868 Phytochemistry. 2008;69:1128-34.
- 869 35. Vlot AC, Dempsey DA, Klessig DF. Salicylic acid, a multifaceted hormone to combat disease. Annu
870 Rev Phytopathol. 2009;47:177-206.
- 871 36. Andreasson E, Jenkins T, Brodersen P, Thorgrimsen S, Petersen NH, Zhu S et al. The MAP kinase
872 substrate MKS1 is a regulator of plant defense responses. EMBO J. 2005;24:2579-89.
- 873 37. Xia Y, Yu K, Gao QM, Wilson EV, Navarre D, Kachroo P, Kachroo A. Acyl CoA binding proteins are
874 required for cuticle formation and plant responses to microbes. Front Plant Sci. 2012;3:224.
- 875 38. Boller T, Felix G. A renaissance of elicitors: perception of microbe-associated molecular patterns
876 and danger signals by pattern recognition receptors. Annu Rev Plant Biol. 2009;60:379-406.
- 877 39. Vincill ED, Bieck AM, Spalding EP. Ca(2+) conduction by an amino acid-gated ion channel related
878 to glutamate receptors. Plant Physiol. 2012;159:40-46.
- 879 40. Hou C, Tian W, Kleist T, He K, Garcia V, Bai F, Hao Y, Luan S, Li L. DUF221 proteins are a family of
880 osmosensitive calcium-permeable cation channels conserved across eukaryotes. Cell Res.
881 2014;24:632-635.
- 882 41. Fonseca JP, Mysore KS. Genes involved in nonhost disease resistance as a key to engineer durable
883 resistance in crops. Plant Sci. 2019;279:108-116.
- 884 42. Ronzier E, Corratgé-Faillie C, Sanchez F, Prado K, Brière C, Leonhardt N, Thibaud JB, Xiong TC.
885 CPK13, a noncanonical Ca²⁺-dependent protein kinase, specifically inhibits KAT2 and KAT1
886 shaker K⁺ channels and reduces stomatal opening. Plant Physiol. 2014;166:314-26.
- 887 43. Zou JJ, Li XD, Ratnasekera D, Wang C, Liu WX, Song LF, Zhang WZ, Wu WH. Arabidopsis CALCIUM-
888 DEPENDENT PROTEIN KINASE8 and CATALASE3 function in abscisic acid-mediated signaling
889 and H₂O₂ homeostasis in stomatal guard cells under drought stress. Plant Cell.
890 2015;27:1445-1460.

- 891 44. Förster S, Schmidt LK, Kopic E, Anschutz U, Huang S, Schlücking K, et al. Wounding-induced
892 stomatal closure requires jasmonate-mediated activation of GORK K(+) channels by a Ca(2+)
893 sensor-kinase CBL1-CIPK5 complex. *Dev Cell*. 2019;48:87-99.e6.
- 894 45. Kadota Y, Shirasu K, Zipfel C. Regulation of the NADPH oxidase RBOHD during plant immunity.
895 *Plant Cell Physiol*. 2015;56: 1472–1480.
- 896 46. Adachi H, Yoshioka H. Kinase-mediated orchestration of NADPH oxidase in plant immunity. *Brief*
897 *Funct Genomics*. 2015;14:253-259.
- 898 47. Zhai L, Sun C, Feng Y, Li D, Chai X, Wang L, Sun Q, et al. AtROP6 is involved in reactive oxygen
899 species signaling in response to iron-deficiency stress in *Arabidopsis thaliana*. *FEBS Lett*.
900 2018;592:3446-3459.
- 901 48. Gao X, Chen X, Lin W, Chen S, Lu D, Niu Y, Li L, Cheng C, McCormack M, Sheen J, Shan L, He P.
902 Bifurcation of *Arabidopsis* NLR immune signaling via Ca²⁺-dependent protein kinases. *PLoS*
903 *Pathog*. 2013;9:e1003127.
- 904 49. Qi J, Wang J, Gong Z, Zhou JM. Apoplastic ROS signaling in plant immunity. *Curr Opin Plant Biol*.
905 2017;38:92-100.
- 906 50. Bourdais G, Burdiak P, Gauthier A, Nitsch L, Salojärvi J, Rayapuram C et al. CRK consortium large-
907 scale phenomics identifies primary and fine-tuning roles for CRKs in responses related to
908 oxidative stress. *PLoS Genet*. 2015;11:e1005373.
- 909 51. Sun T, Nitta Y, Zhang Q, Wu D, Tian H, Lee JS, Zhang Y. Antagonistic interactions between two
910 MAP kinase cascades in plant development and immune signaling. *EMBO Rep*.
911 2018;19:e45324.
- 912 52. O'Brien JA, Daudi A, Butt VS, Bolwell GP. Reactive oxygen species and their role in plant defence
913 and cell wall metabolism. *Planta*. 2012;236:765-779.
- 914 53. Brosché M, Blomster T, Salojärvi J, Cui F, Sipari N, Leppälä J et al. Transcriptomics and functional
915 genomics of ROS-induced cell death regulation by RADICAL-INDUCED CELL DEATH1. *PLoS*
916 *Genet*. 2014;10:e1004112.

- 917 54. Cheng YT, Li Y, Huang S, Huang Y, Dong X, Zhang Y, Li X. Stability of plant immune-receptor
918 resistance proteins is controlled by SKP1-CULLIN1-F-box (SCF)-mediated protein degradation.
919 Proc Natl Acad Sci U.S.A. 2011;108:14694-14699.
- 920 55. Coll NS, Vercammen D, Smidler A, Clover C, Van Breusegem F, Dangl JL, Epple P. Arabidopsis type
921 I metacaspases control cell death. Science. 2010;330:1393-1397.
- 922 56. Lema-Asqui S, Vercammen D, Serrano I, Valls M, Rivas S, Van Breusegem F, et al. AtSERPIN1 is an
923 inhibitor of the metacaspase AtMC1-mediated cell death and autocatalytic processing in
924 planta. New Phytol. 2018;218:1156-1166.
- 925 57. Kawasaki T, Nam J, Boyes DC, Holt BF, Hubert DA, Wiig A, Dangl JL. A duplicated pair of
926 Arabidopsis RING-finger E3 ligases contribute to the RPM1-and RPS2-mediated
927 hypersensitive response. Plant J. 2005;44: 258-270.
- 928 58. Luo H, Laluk K, Lai Z, Veronese P, Song F, Mengiste T. The Arabidopsis Botrytis Susceptible1
929 interactor defines a subclass of RING E3 ligases that regulate pathogen and stress responses.
930 Plant Physiol. 2010;154:1766-1782.
- 931 59. Huby E, Napier JA, Baillieul F, Michaelson LV, Dhondt-Cordelier S. Sphingolipids: towards an
932 integrated view of metabolism during the plant stress response. New Phytol. 2020;225:659-
933 670.
- 934 60. Saucedo-Garcia M, Guevara-Garcia A, Gonzalez-Solis A, Cruz-Garcia F, VazquezSantana S,
935 Markham JE, et al. MPK6, sphinganine and the LCB2a gene from serine palmitoyltransferase
936 are required in the signaling pathway that mediates cell death induced by long chain bases in
937 Arabidopsis. New Phytol. 2011;191:943–957.
- 938 61. Veley KM, Maksaev G, Frick EM, January E, Kloepper SC, Haswell ES. Arabidopsis MSL10 has a
939 regulated cell death signaling activity that is separable from its mechanosensitive ion channel
940 activity. Plant Cell. 2014;26:3115-3131.
- 941 62. Guerringue Y, Thomine S, Frachisse JM. Sensing and transducing forces in plants with MSL10 and
942 DEK1 mechanosensors. FEBS Lett. 2018;592:1968-1979.

- 943 63. Zhao C, Nie H, Shen Q, Zhang S, Lukowitz W, Tang D. EDR1 physically interacts with MKK4/MKK5
944 and negatively regulates a MAP kinase cascade to modulate plant innate immunity. *PLoS*
945 *Genet.* 2014;10:e1004389.
- 946 64. Lee MH, Jeon HS, Kim HG, Park OK. An Arabidopsis NAC transcription factor NAC4 promotes
947 pathogen-induced cell death under negative regulation by microRNA164. *New Phytol.*
948 2017b;214:343-360.
- 949 65. Najafi J, Brembu T, Vie AK, Viste R, Winge P, Somssich IE, Bones AM. PAMP-INDUCED SECRETED
950 PEPTIDE 3 modulates immunity in Arabidopsis. *J Exp Bot.* 2020;71:850-864.
- 951 66. Khafif M, Balagué C, Huard-Chauveau C, Roby D. An essential role for the VAS1 domain of the
952 Arabidopsis VAD1 protein in the regulation of defense and cell death in response to
953 pathogens. *PLoS One.* 2017;12:e0179782.
- 954 67. Mittler R, Herr EH, Orvar BL, van Camp W, Willekens H, Inzé D, Ellis BE. Transgenic tobacco plants
955 with reduced capability to detoxify reactive oxygen intermediates are hyperresponsive to
956 pathogen infection. *Proc Natl Acad Sci U.S.A.* 1999;96:14165-70.
- 957 68. Simon C, Langlois-Meurinne M, Didierlaurent L, Chaouch S, Bellvert F, Massoud K, et al. The
958 secondary metabolism glycosyltransferases UGT73B3 and UGT73B5 are components of redox
959 status in resistance of Arabidopsis to *Pseudomonas syringae* pv. *tomato*. *Plant Cell Environ.*
960 2014;37:1114-1129.
- 961 69. Bacete L, Mélida H, Miedes E, Molina A. Plant cell wall-mediated immunity: cell wall changes
962 trigger disease resistance responses. *Plant J.* 2018;93:614-636.
- 963 70. Miedes E, Vanholme R, Boerjan W, Molina A. The role of the secondary cell wall in plant
964 resistance to pathogens. *Front Plant Sci.* 2014;5:358.
- 965 71. Fich EA, Segerson NA, Rose JK. The plant polyester cutin: biosynthesis, structure, and biological
966 roles. *Ann Rev Plant Biol.* 2016;67:207-233.
- 967 72. Lim GH, Singhal R, Kachroo A, Kachroo P. Fatty acid- and lipid-mediated signaling in plant defense.
968 *Annu Rev Phytopathol.* 2017;55:505-536.

- 969 73. Joubès J, Raffaele S, Bourdenx B, Garcia C, Laroche-Traineau J, Moreau P, Domergue F, Lessire R.
970 The VLCFA elongase gene family in *Arabidopsis thaliana*: phylogenetic analysis, 3D modelling
971 and expression profiling. *Plant Mol Biol.* 2008;67:547-66.
- 972 74. Lee SB and Suh MC. Advances in the understanding of cuticular waxes in *Arabidopsis thaliana* and
973 crop species. *Plant Cell Rep.* 2015;34:557-572.
- 974 75. Li Y, Beisson F, Koo AJ, Molina I, Pollard M, Ohlrogge J. Identification of acyltransferases required
975 for cutin biosynthesis and production of cutin with suberin-like monomers. *Proc Natl Acad Sci*
976 *U.S.A.* 2007;104:18339-18344.
- 977 76. Yeats TH, Huang W, Chatterjee S, Viart HM, Clausen MH, Stark RE, Rose JK. Tomato Cutin
978 Deficient 1 (CD1) and putative orthologs comprise an ancient family of cutin synthase-like
979 (CUS) proteins that are conserved among land plants. *Plant J.* 2014;77:667-675.
- 980 77. Cui F, Brosche M, Lehtonen MT, Amiryousefi A, Xu E, Punkkinen M, Valkonen JPT, Fujii H,
981 Overmyer K. Dissecting abscisic acid signaling pathways involved in cuticle formation. *Mol*
982 *Plant.* 2016;9: 926-938.
- 983 78. Yang L, Wen KS, Ruan X, Zhao YX, Wei F, Wang Q. Response of plant secondary metabolites to
984 environmental factors. *Molecules.* 2018;23i:e762.
- 985 79. Tetali S. Terpenes and isoprenoids: a wealth of compounds for global use. *Planta.* 2019;249:1-8.
- 986 80. Wang K, Senthil-Kumar M, Ryu CM, Kang L, Mysore KS. Phytosterols play a key role in plant innate
987 immunity against bacterial pathogens by regulating nutrient efflux into the apoplast. *Plant*
988 *Physiol.* 2012;158:1789-1802.
- 989 81. Falcone Ferreyra ML, Emiliani J, Rodriguez EJ, Campos-Bermudez VA, Grotewold E, Casati P. The
990 identification of maize and *Arabidopsis* type I FLAVONE SYNTHASEs links flavones with
991 hormones and biotic interactions. *Plant Physiol.* 2015;169:1090-107.
- 992 82. Wang N, Xu H, Jiang S, Zhang Z, Lu N, Qiu H, Qu C, Wang Y, Wu S, Chen X. MYB12 and MYB22 play
993 essential roles in proanthocyanidin and flavonol synthesis in red-fleshed apple (*Malus*
994 *sieversii* f. *niedzwetzkyana*). *Plant J.* 2017a;90:276-292.

- 995 83. Zhai R, Zhao Y, Wu M, Yang J, Li X, Liu H, Wu T, Liang F, Yang C, Wang Z, Ma F, Xu L. The MYB
996 transcription factor PbMYB12b positively regulates flavonol biosynthesis in pear fruit. BMC
997 Plant Biol. 2019;19:85.
- 998 84. Wang XC, Wu J, Guan ML, Zhao CH, Geng P, Zhao Q. Arabidopsis MYB4 plays dual roles in
999 flavonoid biosynthesis. Plant J. 2020;101:637-652.
- 1000 85. Fernández-Pérez F, Vivar T, Pomar F, Pedreño MA, Novo-Uzal E. Peroxidase 4 is involved in
1001 syringyl lignin formation in *Arabidopsis thaliana*. J Plant Physiol. 2015a;175:86-94.
- 1002 86. Zhao Q, Nakashima J, Chen F, Yin Y, Fu C, Yun J, Shao H, Wang X, Wang ZY, Dixon RA. Laccase is
1003 necessary and nonredundant with peroxidase for lignin polymerization during vascular
1004 development in Arabidopsis. Plant Cell. 2013;25:3976-3987.
- 1005 87. Cosio C, Ranocha P, Francoz E, Burlat V, Zheng Y, Perry SE, Ripoll JJ, Yanofsky M, Dunand C. The
1006 class III peroxidase PRX17 is a direct target of the MADS-box transcription factor AGAMOUS-
1007 LIKE15 (AGL15) and participates in lignified tissue formation. New Phytol. 2017;213:250-263.
- 1008 88. Tokunaga N, Kaneta T, Sato S, Sato Y. Analysis of expression profiles of three peroxidase genes
1009 associated with lignification in Arabidopsis thaliana. Physiol Plantarum. 2009;136:237-249.
- 1010 89. Fernández-Pérez F, Pomar F, Pedreño MA, Novo-Uzal E. The suppression of AtPrx52 affects fibers
1011 but not xylem lignification in Arabidopsis by altering the proportion of syringyl units. Physiol
1012 Plantarum. 2015b;154:395-406.
- 1013 90. Vilanova L, Teixidó N, Torres R, Usall J, Viñas I. The infection capacity of *P. expansum* and *P.*
1014 *digitatum* on apples and histochemical analysis of host response. International Journal of
1015 Food Microbiol. 2012;157:360-367.
- 1016 91. Fink W, Haug M, Deising H, Mendgen K. Early defence responses of cowpea (*Vigna sinensis* L.)
1017 induced by non-pathogenic rust fungi. Planta. 1991;185:246-254.
- 1018 92. Song PP, Zhao J, Liu ZL, Duan YB, Hou YP, Zhao CQ, Wu M, Wei M, Wang NH, Lv Y, Han ZJ.
1019 Evaluation of antifungal activities and structure-activity relationships of coumarin derivatives.
1020 Pest Manag. Sci. 2017;73:94-101.

- 1021 93. Giesemann A, Biehl B, Lieberei R. Identification of scopoletin as a phytoalexin of the rubber tree
1022 *Hevea brasiliensis*. J Phytopathol. 1986;117:373–376.
- 1023 94. Muroi A, Ishihara A, Tanaka C, Ishizuka A, Takabayashi J, Miyoshi H, Nishioka T. Accumulation of
1024 hydroxycinnamic acid amides induced by pathogen infection and identification of agmatine
1025 coumaroyltransferase in *Arabidopsis thaliana*. Planta. 2009;230:517-527.
- 1026 95. Dobritzsch M, Lübken T, Eschen-Lippold L, Gorzolka K, Blum E, Matern A et al. MATE transporter-
1027 dependent export of hydroxycinnamic acid amides. Plant Cell. 2016;28:583-96.
- 1028 96. Celoy RM, VanEtten HD. (+)-Pisatin biosynthesis: from (-) enantiomeric intermediates via an
1029 achiral 7,2'-dihydroxy-4',5'-methylenedioxyisoflav-3-ene. Phytochem. 2014;98:120-127.
- 1030 97. Vorwieger A, Gryczka C, Czihal A, Douchkov D, Tiedemann J, Mock HP, et al. Iron assimilation and
1031 transcription factor controlled synthesis of riboflavin in plants. Planta. 2007;226:147-158.
- 1032 98. Trapalis M, Li SF, Parish RW. The Arabidopsis GASA10 gene encodes a cell wall protein strongly
1033 expressed in developing anthers and seeds. Plant Sci. 2017;260:71-79.
- 1034 99. Yamauchi Y, Hasegawa A, Mizutani M, Sugimoto Y. Chloroplastic NADPH-dependent alkenal/one
1035 oxidoreductase contributes to the detoxification of reactive carbonyls produced under
1036 oxidative stress. FEBS Lett. 2012;586:1208-1213.
- 1037 100. Segond D, Dellagi A, Lanquar V, Rigault M, Patrit O, Thomine S, Expert D. NRAMP genes function
1038 in *Arabidopsis thaliana* resistance to *Erwinia chrysanthemi* infection. Plant J. 2009;58:195-
1039 207.
- 1040 101. Lee MW, Jelenska J, Greenberg JT. Arabidopsis proteins important for modulating defense
1041 responses to *Pseudomonas syringae* that secrete HopW1-1. Plant J. 2008;54:452-445.
- 1042 102. Hong GJ, Xue XY, Mao YB, Wang LJ, Chen XY. Arabidopsis MYC2 interacts with DELLA proteins in
1043 regulating sesquiterpene synthase gene expression. Plant Cell. 2012;24:2635-2648.
- 1044 103. Rodriguez-Saona CR, Polashock J, Malo EA. Jasmonate-mediated induced volatiles in the
1045 American cranberry, *Vaccinium macrocarpon*: from gene expression to organismal
1046 interactions. Front Plant Sci. 2013;4:115.

- 1047 104. Simanshu DK, Zhai X, Munch D, Hofius D, Markham JE, Bielawski J, et al. Arabidopsis accelerated
1048 cell death 11, ACD11, is a ceramide-1-phosphate transfer protein and intermediary regulator
1049 of phytoceramide levels. *Cell Rep.* 2014;6:388-399.
- 1050 105. Fink L, Kwapiszewska G, Wilhelm J, Bohle RM. Laser-microdissection for cell type- and
1051 compartment-specific analyses on genomic and proteomic level. *Exp Toxicol Pathol.* 2006;57
1052 Suppl 2:25-259.
- 1053 106. Yan Y, Zheng XF, Apaliya MT, Yang HJ, Zhang HY. Transcriptome characterization and expression
1054 profile of defense-related genes in pear induced by *Meyerozyma guilliermondii*. *Postharvest*
1055 *Biol Technol.* 2018;141:63-70.
- 1056 107. Zhang Q, Zhao L, Li B, Gu X, Zhang X, Boateng NS, Zhang H. Molecular dissection of defense
1057 response of pears induced by the biocontrol yeast, *Wickerhamomyces anomalus* using
1058 transcriptomics and proteomics approaches. *Biol Control.* 2020;148:104305.
- 1059 108. Wang H, Lin J, Chang YH, Jiang CZ. Comparative transcriptomic analysis reveals that
1060 ethylene/H₂O₂-mediated hypersensitive response and programmed cell death determine
1061 the compatible interaction of pear and *Alternaria alternata*. *Front Plant Sci.*
1062 2017b;8:196.
- 1063 109. Xu M, Yang Q, Boateng NAS, Ahima J, Dou Y, Zhang H. Ultrastructure observation and
1064 transcriptome analysis of *Penicillium expansum* invasion in postharvest pears. *Postharvest*
1065 *Biol Technol.* 2020;165:111198.
- 1066 110. Gill US, Lee S, Mysore KS. Host versus nonhost resistance: distinct wars with similar arsenals.
1067 *Phytopathol.* 2015;105:580-587.
- 1068 111. Pieterse CM, Van der Does D, Zamioudis C, Leon-Reyes A, Van Wees SC. Hormonal modulation
1069 of plant immunity. *Annu Rev Cell Dev Biol.* 2012;28:489-521.
- 1070 112. Tsuda K, Sato M, Stoddard T, Glazebrook J, Katagiri F. Network properties of robust immunity in
1071 plants. *PLoS Genet.* 2009;5(12):e1000772.

- 1072 113. Velho AC, Stadnik MJ. Non-host resistance of arabidopsis and apple is associated with callose
1073 accumulation and changes in preinfective structures of *Colletotrichum* species. *Physiol Mol*
1074 *Plant Pathol.* 2020;110:101463.
- 1075 114. Thor K. Calcium-nutrient and messenger. *Front Plant Sci.* 2019 ;10:440.
- 1076 115. Das K and Roychoudhury A. Reactive oxygen species (ROS) and response of antioxidants as ROS-
1077 scavengers during environmental stress in plants. *Front Envir Sci.* 2014;2:53.
- 1078 116. Lehmann S, Serrano M, L'Haridon F, Tjamos SE, Metraux JP. Reactive oxygen species and plant
1079 resistance to fungal pathogens. *Phytochemistry.* 2015;112:54-62.
- 1080 117. Survila M, Davidsson PR, Pennanen V, Kariola T, Broberg M, Sipari N, Heino P, Palva ET.
1081 Peroxidase-generated apoplastic ROS impair cuticle integrity and contribute to DAMP-elicited
1082 defenses. *Front Plant Sci.* 2016; 7:1945.
- 1083 118. Heath MC. Nonhost resistance and nonspecific plant defenses. *Curr Opin Plant Biol.* 2000;3:315-
1084 319.
- 1085 119. Charrier A, Vergne E, Dousset N, Richer A, Petiteau A, Chevreau E. Efficient targeted
1086 mutagenesis in apple and first time edition of pear using the CRISPR/Cas9 system. *Front Plant*
1087 *Sci.* 2019;10:40.
- 1088 120. Chevreau E, Evans K, Montanari S Chagné D. Ch.19.9 *Pyrus* spp. Pear and *Cydonia* spp. Quince.
1089 In: Litz RE, Pliego-Alfaro F, Hormaza JL, editors. *Biotechnology of Fruit and Nut Crops*, 2nd
1090 edition. C.A.B. International, Wallingford, UK; 2020;p. 581-605.
- 1091 121. Malabarba J, Chevreau E, Dousset N, Veillet F, Moizan J, Vergne E. New strategies to overcome
1092 present CRISPR/Cas9 limitations in apple and pear: efficient dechimerization and base
1093 editing. *Int J Mol Sci.* 2020;22:319.
- 1094 122. Faize M, Malnoy M, Dupuis F, Chevalier M, Parisi L, Chevreau E. Chitinases of *Trichoderma*
1095 *atroviridae* induce scab resistance and some metabolic changes in two cultivars of apple.
1096 *Phytopathol.* 2003;93:1496-1504.

- 1097 123. Leblay C, Chevreau E, Raboin LM. Adventitious shoot regeneration from in vitro leaves of several
1098 pear cultivars (*Pyrus communis* L.). *Plant Cell Tissue Organ Cult.* 1991;25:99-105.
- 1099 124. Lespinasse Y, Durel CE, Parisi L, Laurens F, Chevalier M, Pinet C. A European project: D.A.R.E.
1100 Durable apple resistance in Europe. *Acta Hortic.* 2000;538:197–200.
- 1101 125. Chevalier M, Tellier M, Lespinasse Y, Bruynincks M, Georgeault S. Behaviour studies of new
1102 races of *Venturia pirina* isolated from 'Conference' cultivar on a range of pear cultivars. *Acta*
1103 *Hortic.* 2008a;800:817-824.
- 1104 126. Parisi L, Lespinasse Y. Pathogenicity of *Venturia inaequalis* strains of race 6 on apple clone
1105 (*Malus sp.*). *Plant Dis.* 1996;80: 1179-1183.
- 1106 127. Chevalier M, Tellier M, Lespinasse Y, Caffier V. How to optimize the *Venturia pirina* inoculation
1107 on pear leaves in greenhouse conditions? *Acta Hortic.* 2008b;800: 913-920.
- 1108 128. Chevalier M, Lespinasse Y, Renaudin S. A microscopic study of different classes of symptoms
1109 coded by the *Vf* gene in apple resistance to scab (*Venturia inaequalis*). *Plant Pathol.* 1991;40:
1110 249–256.
- 1111 129. Hoch HC, Galvani CD, Szarowski DH, Turner JN. Two new fluorescent dyes applicable for
1112 visualization of fungal cell walls. *Mycologia.* 2005;97: 580-588.
- 1113 130. Depuydt S, Trenkamp S, Fernie AR, Elftieh S, Renou J-P, Vuylsteke M, Holster M, Vereecke D. An
1114 integrated genomic approach to define niche establishment by *Rhodococcus fascians* . *Plant*
1115 *Physiol.* 2009;149: 1366–1386.
- 1116 131. Celton JM, Gaillard S, Bruneau M, Pelletier S, Aubourg S, Martin-Magniette ML, Navarro L,
1117 Laurens F, Renou JP. Widespread anti-sense transcription in apple is correlated with siRNA
1118 production and indicates a large potential for transcriptional and/or post-transcriptional
1119 control. *New Phytol.* 2014;203:287-99.
- 1120 132. Berardini TZ, Reiser L, Li D, Mezheritsky Y, Muller R, Strait E, Huala E. The Arabidopsis
1121 information resource: Making and mining the "gold standard" annotated reference plant
1122 genome. *Genesis.* 2015;53:474-85.

- 1123 133. Thimm O, Bläsing O, Gibon Y, Nagel A, Meyer S, Krüger P, Selbig J, Müller LA, Rhee SY, Stitt M.
1124 MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic
1125 pathways and other biological processes. *Plant J.* 2004;37:914-939.
- 1126 134. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic
1127 Acids Res.* 2001;29, e45.
- 1128 135. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative
1129 PCR and the $2^{-\Delta\Delta CT}$ method. *Methods.* 2001;25:402-408.
- 1130 136. Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A. et al. Accurate
1131 normalization of real-time quantitative RT-PCR data by geometric averaging of multiple
1132 internal control genes. *Genome Biol.* 2002;3(7):00341-003411.

1133

1134 **Figure legends**

1135

1136 **Fig. 1: Macro- and microscopic observations of nonhost interactions.**

1137 Binocular observation 21 days after *V. inaequalis* inoculation on 'Conference' (A) and (B) and *V.*
1138 *pyrina* inoculation on 'Gala' (C). Wide field fluorescence observations of: 'Conference' 3 days (D) and
1139 14 days (E) after *V. inaequalis* inoculation, 'Gala' 3 days (F) and 14 days (G) after *V. pyrina*
1140 inoculation. Ap: appressorium, C: conidia, Gf: germination filament, Pp: pin point

1141

1142 **Fig. 2: Functional categories of DEGs at 24 or 72hpi during pear response to *V. inaequalis*.**

1143 The number of up- or down-regulated DEGs is expressed as a percentage of the total number of
1144 genes present in the *Pyrus* v1.0 (87812 probes) microarray. DEGs are classified in functional
1145 categories according to MapMan 3.5.1R2 bins. Only bins with ≥ 6 DEGs are presented.

1146

1147 **Fig. 3: DEGs involved in hormonal pathways during pear/*V. inaequalis* non-host interaction.**

1148 A: DEGs involved in JA pathway; B: DEGs involved in SA pathway. Genes written in red are induced,
1149 genes written in blue are repressed. ACA11: autoinhibited Ca²⁺-ATPase, calmodulin-activated Ca²⁺
1150 pumps at the plasma membrane, endoplasmic reticulum, and vacuole. ACBP6: acyl-CoA-binding
1151 protein. ACX4: acyl-CoA-oxidase1. AS1/MYB91: Asymmetric leaves 1 transcription factor, CAMTA1:
1152 calmodulin-binding transcription activator, CBP60a: calmodulin-binding protein 60a, EDS1: enhanced
1153 disease susceptibility 1. FAR1: FAR-red impaired response 1. G-box: cis-element in the promoter. JAZ:
1154 jasmonate-zim domain protein, JMT: jasmonic acid carboxyl methyltransferase. LOX: lipoxygenase,
1155 MES1: methylesterase 1. MFP2: multifunctional protein 2. MKS1: MAP kinase substrate 1. MYC2:
1156 transcription factor. NINJA: novel interactor of JAZ. PAD4: phytoalexin deficient 4. UGT74F1:
1157 glucosyltransferase. PR1-like (with ATPRB1), PR2, PR3, PR4 (HEL and ATEP3), PR5, PR12:
1158 pathogenesis-related proteins. ST2A: sulfotransferase 2A. TPL: TOPLESS co-repressor. UBP12:
1159 ubiquitin-specific protease 12. WRKY: transcription factor.

1160

1161 **Fig. 4: Scenario of major events observed while three first days of pear/*V. inaequalis* non-host**
1162 **interaction.**

1163 On the left side, events observed in a typical cell, on the right side, events observed in guard cells of a
1164 stomata. A: apoplast, AP: appressorium, C: cuticle, CBL1: calcineurin B-like protein 1, CDPK: Ca²⁺-
1165 dependent protein kinases, CRK: cysteine-rich receptor-like kinase, CY: cytoplasm, CW: cell wall, HAA:
1166 hydroxycinnamic acid amines, HR: hypersensitive response, JA: jasmonic acid, MB: plasma
1167 membrane, LCB: Long Chain/sphingoid Base components, MPK6: Mitogen activated protein kinase 6,
1168 MSL10: mechano-sensitive like 10, N: nucleus, PH: penetration hypha, PR: pathogenesis related
1169 proteins, RBOHB: respiratory burst oxidase homolog B, ROS: reactive oxygen species, S: stomata, SA:
1170 salicylic acid, SC: simple coumarins, SP: spore.

1171

1172 **Fig. 5: Main DEGs involved in cutin and wax biosynthesis during pear/*V. inaequalis* non-host**
1173 **interaction.**

1174 In green the chloroplast, in brown the endoplasmic reticulum (ER) and in yellow the nucleus. Genes
1175 written in red are induced, genes written in blue are repressed. FAS: Fatty Acid Synthase complex to
1176 which belong ACCD (carboxytransferase beta subunit of the Acetyl-CoA carboxylase complex), FabG
1177 (β -ketoacyl ACP-reductase) and MOD1 (enoyl-ACP-reductase) functions. FAE: fatty acid elongase
1178 complex. KCS4 (3-ketoacyl-CoA synthase 4) and ECR/CER10 (trans-2-enoyl-CoA reductase) belong to
1179 the FAE complex. CER1 (octadecanal decarbonylase) and CER3 are implicated in aldehydes (CER1)
1180 and alkanes (CER1 and 3) generation in waxes biosynthesis. In cutin monomers synthesis, the ω -
1181 hydroxylation of C16:0 and C18:1 is catalyzed by cytochrome P450 monooxygenase (CYP86A) and
1182 LACS-encoded acyl-CoA synthetase may be required either to synthesize 16-hydroxy 16:0-CoA, a
1183 substrate for ω -hydroxylase, or for membrane transfer of monomers. Finally, the mature
1184 monoacylglycerol cutin monomers are generated by transfer of the acyl group from acyl-CoA to
1185 glycerol-3-phosphate by glycerol-3-phosphate acyltransferase (GPAT) enzymes such as GPAT8. Some
1186 GDSL-lipases enzyme (such as At1g28600, At1g28660, At1g54790, At3g16370, At3g48460, AtCUS4:
1187 At4g28780, At5g14450) are then functioning as cutin synthase and polymerize cutin
1188 monoacylglycerols. Transcription factors such as MYB16 and SHN1 are positive regulators of wax and
1189 cutin biosynthesis. Adapted from Xia et al, 2009, [71] and [72].

1190

1191 **Fig. 6: Main DEGs involved in the phenylpropanoid pathway during Pear / *V. inaequalis* non-host**
1192 **interaction.**

1193 Genes framed in red are induced, genes frames in blue are repressed. Framed in black, the detail of
1194 genes involved in flavonoids production and found in this interaction. Abbreviations: 4CL, 4-
1195 coumarate-CoA ligase; AACT, anthocyanin 5-aromatic acyltransferase; ANR, anthocyanidin reductase;
1196 ANS, anthocyanin synthase; BGLC or BGLU, β -glucosidases; C3H, coumarate 3-hydroxylase; C4H,
1197 cinnamate 4-hydroxylase; CAD, cinnamyl alcohol dehydrogenase; CCoAOMT, caffeoyl-CoA O-
1198 methyltransferase; CCR, cinnamoyl-CoA reductase; CHI, chalcone isomerase; CHS, chalcone synthase;
1199 COMT, caffeic acid 3-O-methyltransferase; CPK, calcium-dependent protein kinase ; DFR,

1200 dihydroflavonol reductase; DMR6, downy mildiou resistant 6; F3H, flavanone 3-hydroxylase; F3'H
1201 flavonoid 3'-hydroxylase; FLS, flavonol synthase; FNS, flavone synthase; GGT1, gamma-glutamyl
1202 transpeptidase 1; GT, glucosyl transferase; HCT, hydroxycinnamoyl-CoA shikimate/quinate
1203 hydroxycinnamoyl transferase; LAC, laccase; LAR, leucoanthocyanidin reductase; OMT1, O-
1204 methyltransferase 1; PAL, phenylalanine ammonia-lyase; PER or PRX, peroxidase; TT7, transparent
1205 testa 7; UGFT, UDP-glucose flavonoid-3-O-glucosyltransferase; UGT71D1, UDP-glycosyltransferase
1206 71D1.

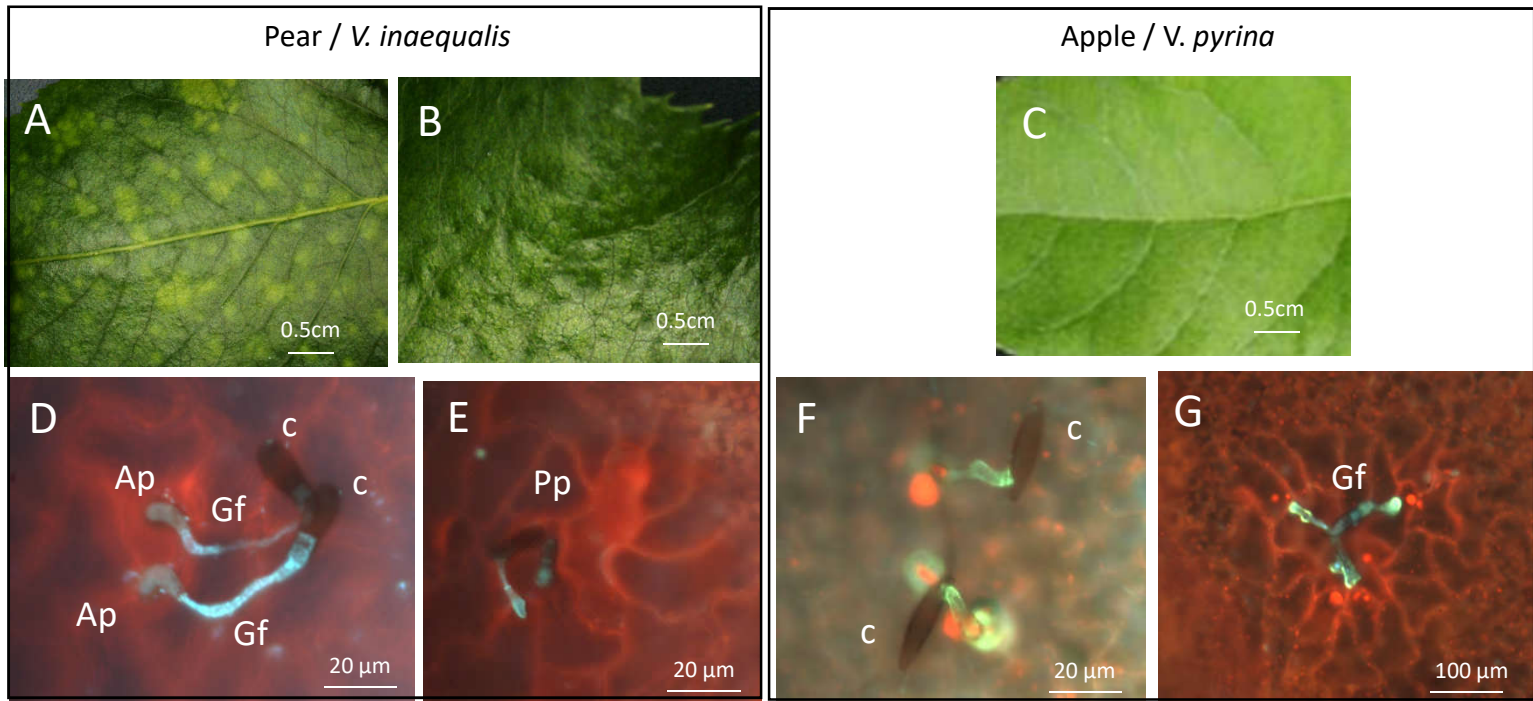


Fig. 1

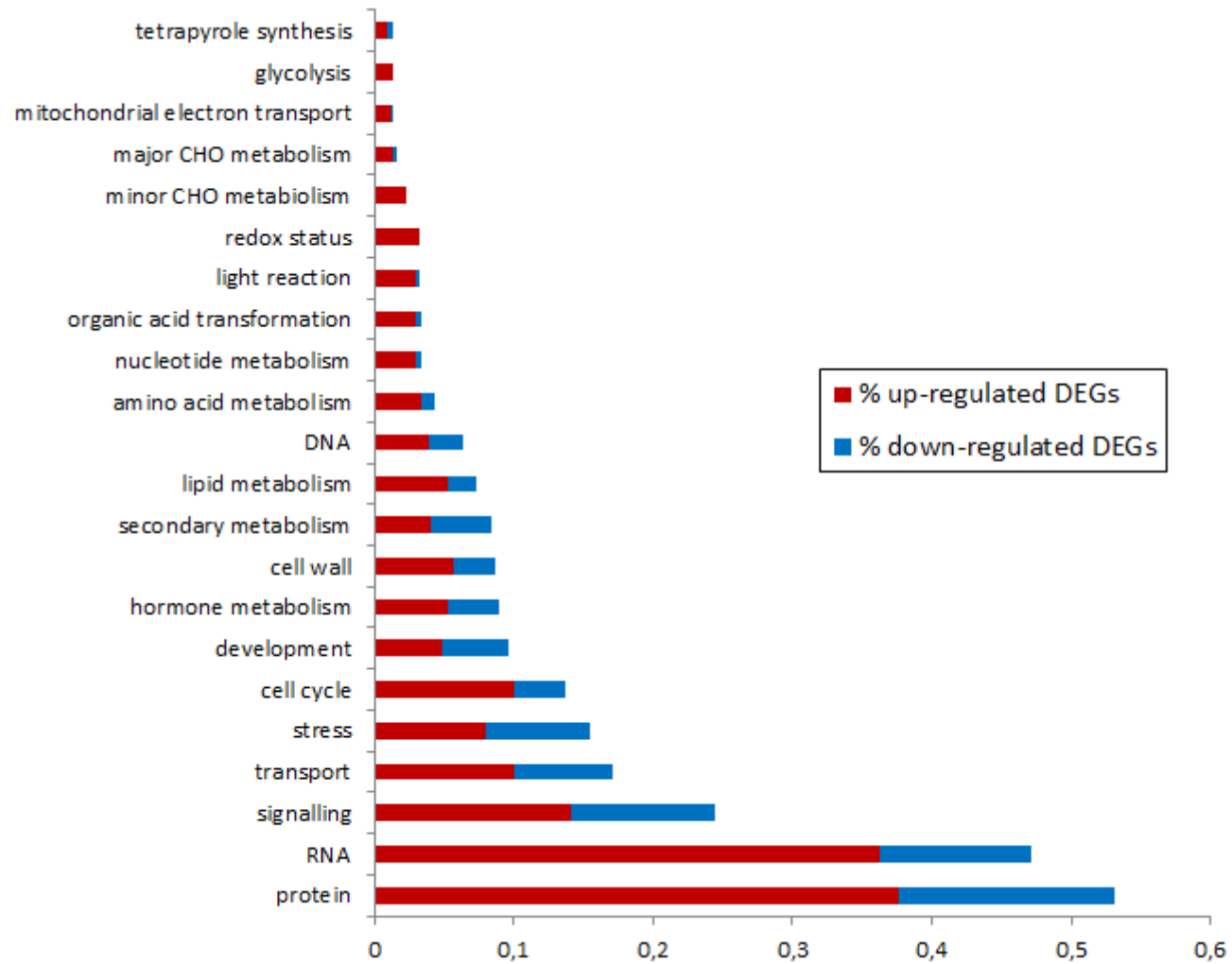


Fig. 2

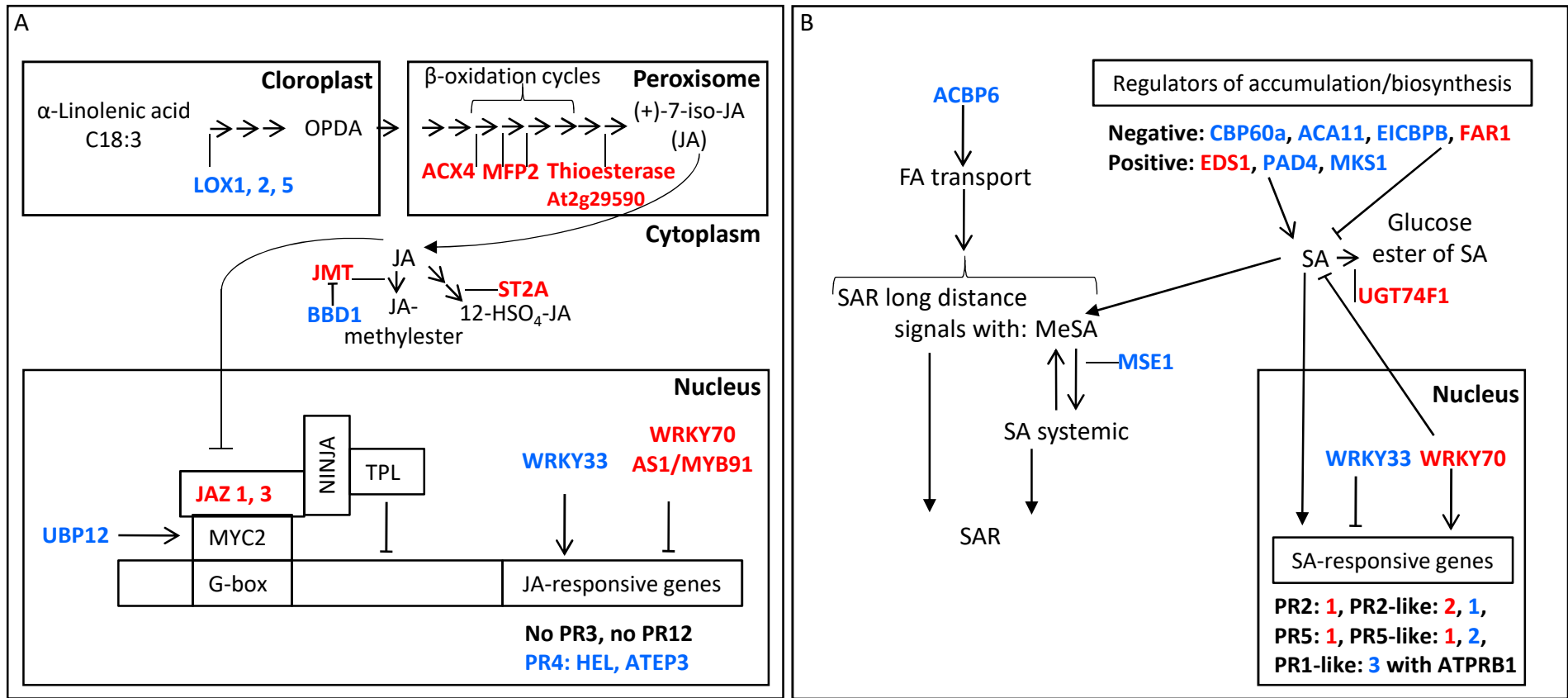


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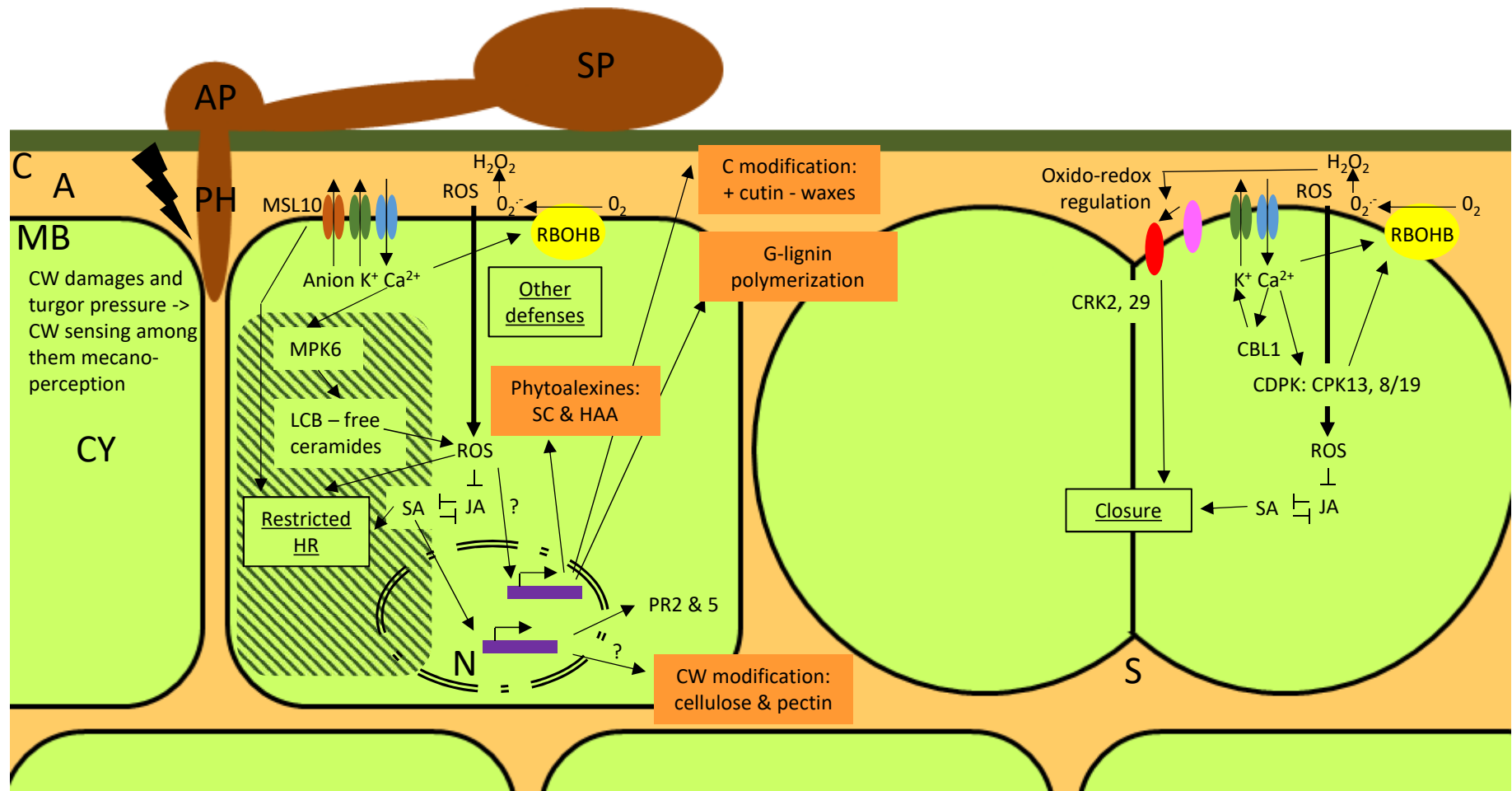


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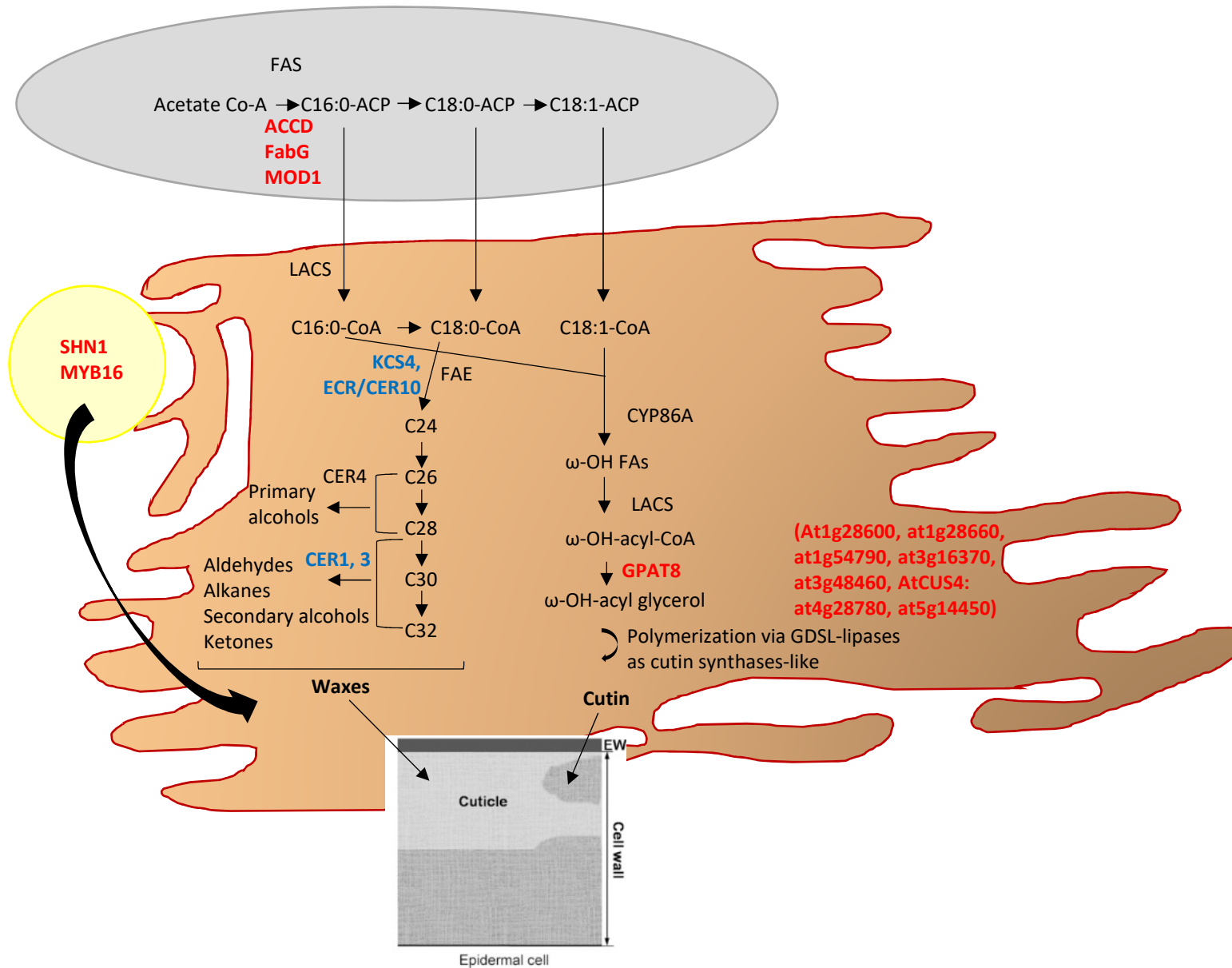


Fig. 5

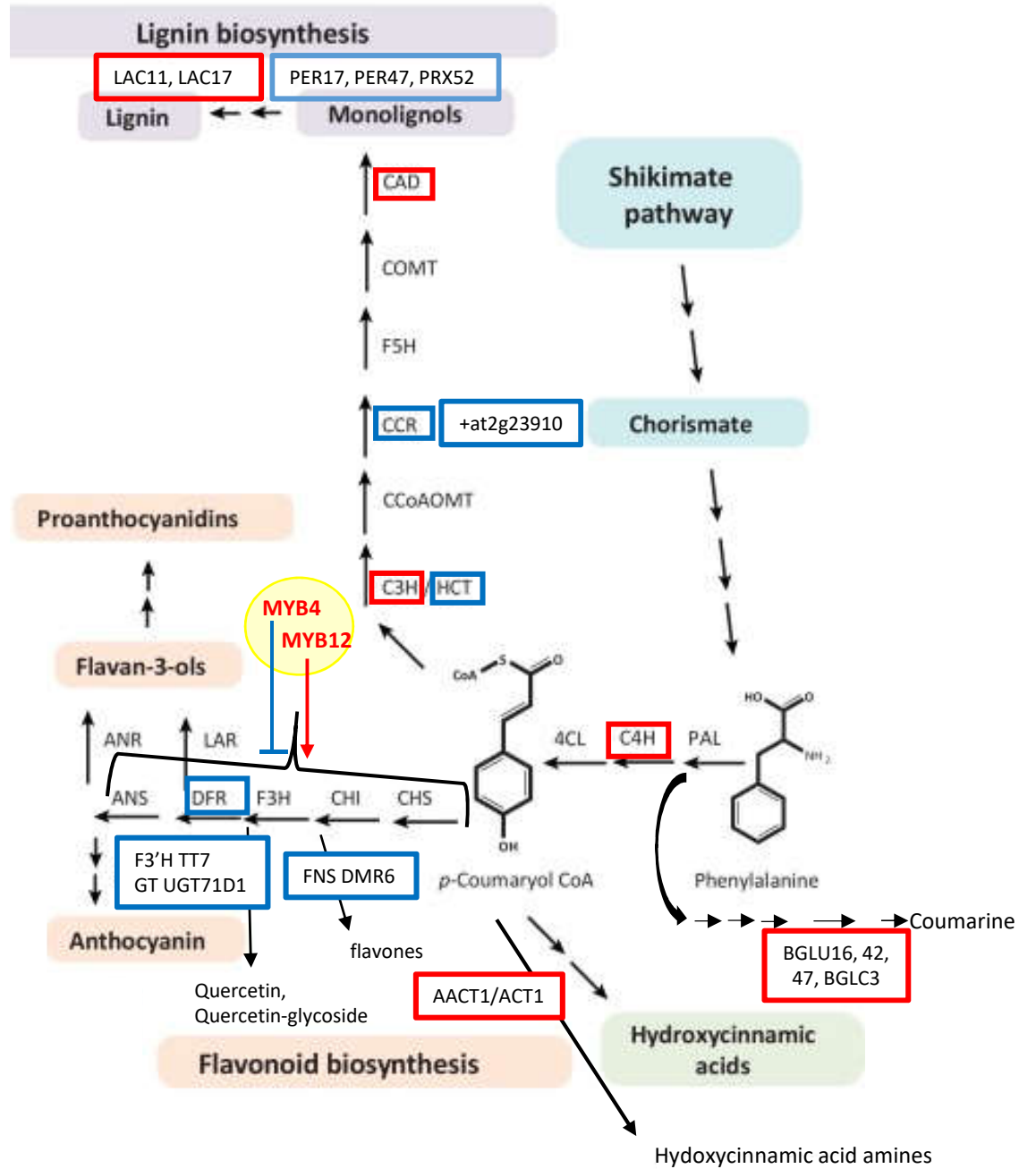
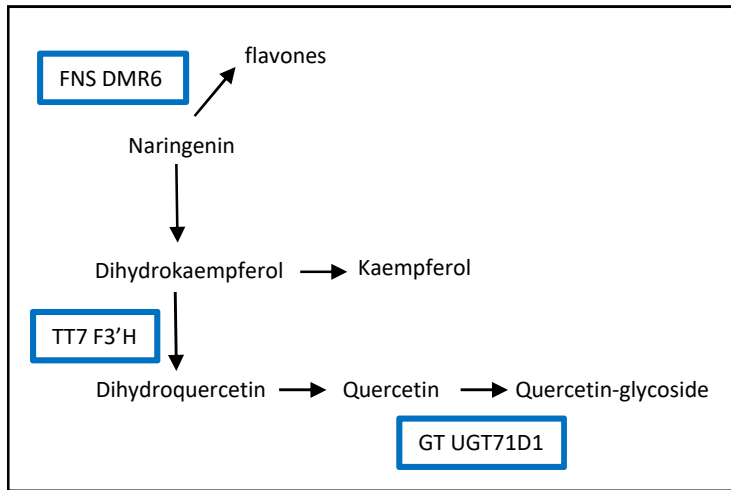


Fig. 6