## Title

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

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## Abstract

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

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

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

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

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

## Background

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

52 between the two genera of ≈ 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. pyrina* and *P. communis* / *V. inaequalis* [5, 9]. In all cases, conidia germinated
65 and produced appressoria and runner hypheae, 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 [11] and nonhost scab resistance in apple and European pear.
77

## Results and discussion

### Variable symptoms of nonhost resistance

Nonhost interactions were observed in a test performed on 'Gala' and 'Conference', all inoculated by a *V. pyrina* strain (VP102) and a *V. inaequalis strain* (VI EUB05). At the macroscopic level, a total absence of sporulation was observed on all nonhost interactions (Table 1). The apple 'Gala' remained completely symptomless after *V. pyrina* inoculations (Fig. 1C). This is similar to the observation of Chevalier et al [9] after inoculation of 'Gala' with another *V. pyrina* strain. On the contrary, pear plants inoculated with *V. inaequalis* presented occasional pin points symptoms (Fig. 1A) and chlorotic lesions (Fig. 1B). Chlorotic lesions had already been observed by Chevalier et al [9] after inoculation of the pear 'Pierre Corneille' with the *V. inaequalis* strain EUB04, but pin points had never been reported in this nonhost interaction. According to our observations, apple nonhost resistance could be classified as type I and pear as type II according to Mysore and Ryu [12] definition based on the absence/presence of visible HR reaction.

**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 | *V. pyrina* strain VP102 | | *V. inaequalis* 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 |

Class 0: absence of symptoms
Class 1: hypersensitivity (pin points)
Class 2: resistance (chlorotic lesions, slight necrosis, crinkled aspect)
Class 3a: weak resistance (necrotic or chlorotic lesions with occasional very light sporulation)
Class 3b: weak susceptibility (clearly sporulating chlorotic or necrotic lesions
Class 4: susceptibility (sporulation only)

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) (Fig. 1 E and G) in both plant species. No formation of subcuticular stroma and no
106 conidiogenesis were observed in the nonhost interactions, contrary to the host-resistance reactions
107 [11]. These observations are similar to the collapsed cells described by Chevalier et al [9] in apple and
108 pear nonhost reactions, and to the rare HR-like reactions observed by Stehmann et al [5] on apple
109 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. This kinetic
120 experimental design is open to criticism because it does not exclude in results data corresponding on
121 one hand to genes responding not to the infection but to the inoculation method (spray of water),
122 and on the other hand to genes whose expression varies due to leaves ageing. Water spray is
123 effectively perceived as a mechanical stimulus by plants, the Arabidopsis response being largely
124 regulated by the JA pathway induction under the control of MYC2/MYC3/MYC4 transcription factors
125 [13]. But this response is transient and really fast as most of genes differently regulated peak within

126 30 minutes regain untreated transcriptional levels within 3 hours [13]. It seems therefore very
127 unlikely that our analysis at 24 and 72hpi includes genes responding to water spray. Resistance due
128 to leaves ageing i.e. ontogenic resistance has been investigated in *Malus-Venturia* pathosystem at 72
129 and 96hpi, and 5 genes have been identified whose modulation could be linked to this resistance
130 [14]. None of these apple genes, or homologs in pear, were found differentially expressed at 72hpi in
131 our interactions (Table S1), which argue against the presence of ageing responding genes in our
132 results. In total, 60 DEGs in apple and 1857 DEGs in pear were identified, which amounts to 0.19 % of
133 all apple genes on the apple AryANE v2.0 microarray, and 4.23 % of all pear genes on the Pyrus v1.0
134 microarray (Table 2).

135 **Table 2. Number of DEGs identified during apple and pear nonhost response to *V. pyrina* and *V.***
136 ***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 |

137 *: DEGs numbers were calculated using the p-adj values ≤ 0.01 as selection threshold
138 **: 31311 genes on the apple Ariane V2 microarray, 43906 genes on the pear V1 microarray
139

140 The very small number of DEGs detected in the apple nonhost interaction at 24 or 72hpi is in
141 agreement with the total absence of macroscopic symptoms observed during this interaction.
142 However, at the microscopic level, small HR-like reactions were detected in the apple / *V. pyrina*
143 interaction at 14 days post inoculation. Because theses reactions involve only a few cells in the
144 leaves, the changes in gene expression are probably below the threshold of DEG detection applied in
145 this experiment. It is also possible that the mild response of apple to *V. pyrina* occurs later than
146 72hpi, between 72hpi and 6 days after inoculation. Indeed, Chevalier et al [9] observed these rare HR

147 from 6 days post inoculation, with no more evolution until 14 days after inoculation. A longer time (6
148 days post inoculation) would have been necessary to conclude on this last hypothesis.
149 On the contrary, the number of DEGs detected during the pear / *V. inaequalis* interaction is in the
150 same order of magnitude as the number of DEGs detected during pear host resistance to *V. pyrina*
151 (see [11]). This is in agreement with the frequent observation of macroscopic symptoms of resistance
152 (chlorotic lesions or pin points) in this interaction. Among the 1857 pear DEGs, 80.2 % were only
153 detected at 24hpi and 15.4 % only at 72hpi, whereas 4.2 % were upregulated or down regulated
154 similarly at both time points of the kinetics. Among all the pear DEGs observed at 24 and 72hpi, the
155 proportion of up-regulated DEGs was higher (68.8 %) than the proportion of downregulated DEGs
156 (31.2 %). Using MapMan to map the DEGs TAIR names, we observed that the main functional
157 categories represented in this set of DEGs were similar to those observed during pear host resistance
158 to *V. pyrina* (see [11]): protein, RNA, signaling, transport and cell cycle (Fig. 2).
159 To basically validate the transcriptomic data, 12 DEGS with varied ratios (between -1.9 and 2.9) have
160 been tested by quantitative RT-PCR (qPCR; Table S2), on the two biological repeats used for
161 transcriptomic analyses. Considering the weak number of DEGs found for apple in this study, we only
162 tested two of them in qPCR. As seen in Table 2 for pear, at 24hpi, a majority of DEGs are up-regulated
163 and at 72hpi, a majority of DEG are down-regulated. qPCR was then performed essentially on DEGs
164 with positive ratios at 24phi and negative ratios at 72hpi (Table S2). The qPCR results confirmed the
165 induced or repressed status of all tested DEGs.
166

167 Weak involvement of hormone signaling pathways classically associated to resistance

168 Pear DEGs were found that indicate that the jasmonic acid (JA) pathway was repressed. The JA
169 biosynthesis and metabolic conversions were reviewed by Wasternack et al [15]. In our data, at
170 24hpi, the first step of JA biosynthesis, that is the conversion of linoleic acid in 12-oxo-phytodienoic
171 acid (OPDA), is compromised: six out of seven lipoxygenases (LOX) (three LOX1, two LOX2 and two
172 LOX5) are repressed, the last one being induced (Fig. 3). OPDA produced in the chloroplast is then

173 transported to the peroxisome for subsequent conversion to JA via the action of OPR3 (12-oxo-
174 phytodienoic acid reductase) and β-oxidation enzymes (reviewed in [16] and in [15]). In pear, three
175 β-oxidation enzymes were found activated more or less rapidly: ACX4 (24hpi), MFP2 (72hpi) and the
176 thioesterase homolog to *At2g29590* (72hpi), which suggests that constitutive OPDA stocks were
177 turned into JA. But the early and long-lasting induction of *JMT* and *ST2A* genes is in favor of a rapid
178 conversion of JA in inactive compounds, *JMT* induction being reinforced by *BBD1* repression (24hpi).
179 *BBD1* is actually known as a negative regulator of *JMT* [17].
180 The defense response depending on JA was also clearly repressed in pear (Fig. 3). The transcription
181 activator MYC2 of JA-induced genes is known to be repressed by its interaction with JAZ proteins
182 (reviewed in [15]), and two *JAZ1* and one *JAZ3* coding genes were found activated at 24hpi in pear.
183 UBP12 is known as a stabilizer of MYC2 [18]. In our data, *UBP12* was found repressed at 72hpi, which
184 reinforces the inactivation of MYC2. WRKY33 is known as an activator of the JA defense pathway [19]
185 and WRK70 [20] or AS1 (or MYB91; [21]) as inhibitors, and among JA-responsive proteins, the
186 pathogenesis-related PR3, PR4 and PR12 act downstream MYC2 activation [22]. In our data,
187 accordingly with the repression of the activator WRKY33 and the activation of the inhibitors WRK70
188 and AS1, some JA-responsive genes were also found repressed, such as the chitinase coding genes
189 *PR4* (also called *HEL*) and *ATEP3*. Furthermore, no DEGs were found for PR3 and PR12 functions. To
190 conclude, in the nonhost interaction between pear and *V. inaequalis*, some JA seems to be produced,
191 but rapidly converted in inactive compounds and the subsequent defense response is clearly
192 repressed.
193 Pear DEGs were found that seems to indicate that the salicylic acid (SA) pathway was slightly
194 engaged and rapidly repressed (Fig. 3). *WRKY70* was induced at 72hpi in our data. This transcription
195 factor is known as a negative regulator of SA biosynthesis but a positive regulator of SA-mediated
196 defense genes in Arabidopsis ([23]; [24]; [25]), among them *PR2*, *PR5* but not *PR1* [26]. *WRKY33*
197 which is known as a negative regulator of SA-responsive genes [27], was also repressed at 72hpi in
198 our data. PR2 and 5 are well-known anti-fungal proteins ([28]; [29]; [30]). At 24hpi a *PR2*, two *PR2*-

199 *like*, a *PR5* and a *PR5-like* coding genes were found induced in our work, another *PR2-like* and two
200 others *PR5-like* being repressed. The differential expression was maintained at 72hpi for only two of
201 the activated ones. Furthermore no DEG was found for the PR1 function but three *PR1-like* genes
202 were found repressed: *ATPRB1* and genes homolog to *At5g57625* and *At4g33720*. *ATPRB1* was
203 already reported as repressed by SA treatment [31]. In our data, the WRKY70 transcription factor was
204 induced later than the induced PR genes so we could imagine that induced PR genes were activated
205 by another precocious regulation, such as an oxidative burst (see below), rather than by WRKY70.
206 Furthermore, WRKY70 induction seems not sufficient to enable a long lasting induction of these
207 defense genes.
208 SA accumulation was also rather mixed. *CBP60a* [32], *ACA11* [33], *EICBP.B* (or *CATMA1*; [34]), all
209 three coding calcium-sensor proteins, are known as negative regulators of SA accumulation and
210 biosynthesis, as well as the light signaling factor *FAR1* [35] or the SA glucosyltransferase *UGT74F1*
211 which convert SA in inactive SA 2-O-beta-D-glucoside or the glucose ester of SA [36]. On the contrary
212 *EDS1*, *PAD4* (reviewed in [37]) and *MKS1* [38] are known as positive regulators of SA accumulation. In
213 our data, the repression of *CBP60a*, *ACA11* and *EICBP.B* genes sustained a SA biosynthesis and
214 accumulation. In addition, *EDS1* activation allowed to consider a positive feedback loop likely to
215 potentiate SA action via EDS1 cytosolic homodimers, even though *PAD4* was repressed. But, as well
216 as *WRKY70* induction, the repression of the MAPK *MKS1* and the activation of the light signaling
217 factor *FAR1* (2 times) or the SA glucosyltransferase *UGT74F1* were in favor of less free SA. Concerning
218 SAR, *MES1* is known as required in healthy systemic tissues of infected plants to release the active SA
219 from methyl-SA, which serves as a long-distance signal for systemic acquired resistance (SAR) [37]
220 and *ACBP6* may be involved in the generation of SAR inducing signal(s) [39]. In our data, SAR seemed
221 compromised given the repression of *ACBP6* at 24hpi and *MES1* at 72hpi. To conclude, in the nonhost
222 interaction between pear and *V. inaequalis*, the SA pathway could be engaged but transiently and
223 presumably reduced to the few infection sites and not spread by SAR in healthy systemic tissues.
224

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

227 Early responses of plants upon pathogen perception include calcium influx and ROS production,
228 which both act as secondary messengers ([40], reviewed in [10]). Three pear DEGs were found that
229 indicate early increased cytosolic calcium level. The CSC (Calcium permeable Stress-gated cation
230 Channel) ERD4 (found two times) and the two glutamate receptors GLR3.4 and GLR2.7, are known as
231 calcium permeable channels ([41]; [42]). They were induced at 24hpi in our data. An increased
232 cytosolic calcium level can lead to a pre-invasive defense response by stomatal closure and promote
233 the post-invasive defense response ROS accumulation [43].

234 Calcium influx has been reported to promote stomatal closure through the regulation of potassium
235 flux and the activation of anion channels in guard cells (reviewed in [10]). The stomata closure is
236 known to be induced via the inhibition of inward potassium currents which is achieved via activation
237 of calcium dependent protein kinases (CDPK) such as CPK13 and CPK8/CDPK19 ([44]; [45]); but also
238 via activation of CBL1 of the CBL1-CIPK5 complex, which activates the GORK potassium outward
239 channel [46]. CPK13, CPK8/CDPK19 and CBL1 were all activated at 24 hpi in our data.

240 A NADPH oxidase *RBOHB* (respiratory burst oxidase homologs, RBOH) is early (i. e. 24hpi) and
241 sustainably (through to 72hpi) induced in the pear/*V. inaequalis* nonhost interaction suggesting a
242 rapid and maintained apoplastic ROS production. Indeed, the apoplastic ROS are mainly produced by
243 plasma membrane localized NADPH oxidases, cell wall peroxidases and amine oxidases [47]. In
244 addition, posttranslational regulation of RBOH is required for its activation and ROS production.
245 Calcium, phosphatidic acid, and direct interactors such as Rac1 GTPase and RACK1 (Receptor for
246 Activated C-Kinase 1) have been reported to be positive regulators of RBOHs (reviewed in [48]). For
247 example, the Rac-like/ROP GTPase ARAC3 is known to interact with a RBOH to promote ROS
248 production [49]. In our data, RBOHB activity was also supported by the presence of positive
249 regulators such as Rac-like/ROP GTPase. The three Rac-like/ROP GTPase *ARAC1*, *ARAC3* and the
250 homolog of *At4g03100* were induced at 24hpi. CDPKs such as CPK1 are also known to activate RBOHs

251 in response to increased cytosolic calcium level [50]. But repression of *CPK1* in our data seems to
252 indicate that this way of activation did not function.
253 In response to abscisic acid (ABA) or microbe-associated molecular pattern (MAMP) immunity,
254 stomatal closure is known to be regulated by apoplastic ROS production (reviewed in [51]) and
255 cysteine-rich receptor-like kinases (CRK) are also known to be elements between ROS production and
256 downstream signaling leading to stomatal closure, sometimes activated (CRK10), sometimes
257 inhibited (CRK2 and CRK29; [52]). Three DEGs coding for CRK were found in our data and the
258 repression of *CRK2* and *CRK29* (found two times) was consistent with the stomata closure previously
259 found, but the repression of *CRK10* (found two times) was not. Beyond closure, inhibition of stomatal
260 development could be seen as an extreme defense. YODA (found two times) and MPK6 (found two
261 times) MAPKs belong to a pathway involved in the negative regulation of stomata development [53].
262 These two genes were induced early in our data.
263 To conclude, in pear/*V. inaequalis* nonhost interaction, a calcium influx leads to the development of
264 the stomatal closure pre-invasive defense, but also promotes a post-invasive defense: apoplastic ROS
265 accumulation. Apoplastic ROS, acting themselves as messengers, come to strengthen the stomatal
266 closure (Fig. 4).
267

268 Transcription factors and sphingolipids maintain HR under control

269 ROS are known to mediate cellular signaling associated with defense-related gene expression,
270 hypersensitive response (HR) i. e. the programmed cell death (PCD) at the site of infection during a
271 pathogen attack, and phytoalexin production [54]. *Arabidopsis thaliana* RCD1 regulator has been
272 proposed to positively regulate cell death in response to apoplastic ROS by protein-protein
273 interactions with transcription factors (reviewed in [55]) and WRKY70 and SGT1b were identified as
274 cell death positive regulators functioning downstream of RCD1 [55]. *RCD1* and *WRKY70* genes were
275 found induced in our data, at 24hpi and 72hpi respectively.

276 In Arabidopsis, the F-box protein CPR1, in association with the Skp1-Cullin-F-box (SCF) ubiquitin ligase
277 complex, targets for degradation NLR (nucleotide-binding domain and leucine-rich repeats containing
278 proteins) resistance protein such as SNC1, RPM1 or RPS2, to prevent overaccumulation and
279 autoimmunity (reviewed in [56]). A *Skp1-like* (*ASK19*; 72hpi) gene and *CPR1* (24hpi) gene were found
280 induced in our data. A gene coding for RPM1 function was also found repressed at 24hpi. These
281 results are in favor of the hypothesis that NLR receptors do not take part in the HR development
282 observed in the pear/*V. inaequalis* nonhost interaction (Fig. 1A). In addition, the induction of an
283 *AtSerpin1* gene homolog at 24hpi (found two times) in our data is consistent with that hypothesis.
284 Indeed, AtMC1 is a pro-death caspase-like protein required for full HR mediated by intracellular NB-
285 LRR immune receptor proteins such as RPP4 and RPM1 [57] and AtSerpin1 is a protease inhibitor
286 which block AtMC1 self-processing and inhibit AtMC1-mediated cell death [58].
287 The differential expression of two others components of the proteasome pathway is in favor of an HR
288 development: the induction of the *RIN3* ubiquitin E3 ligase (24hpi) and the repression of the *BRG3*
289 ubiquitin E3 ligase (24hpi). Indeed, RIN3 is known as positive regulator of RPM1 dependent HR [59].
290 And BRG3 is known as a negative regulator of HR in plant/necrotrophic pathogen interactions [60].
291 Sphingolipids are involved in the control of PCD, either as structural components of membranes but
292 also as initiators in the cell death regulatory pathway. According to Huby et al [61], free ceramides
293 and long chain/sphingoid base components (LCBs) are able to trigger cell death, via ROS production,
294 whereas their phosphorylated counterparts, ceramide phosphates and long chain base phosphate
295 components (LCB-Ps) promote cell survival. The induction of PCD by LCB is based on the activation of
296 protein kinases, among them MPK6 [62]. As already mentioned, *MPK6* was found early induced in
297 our data and we found numerous DEGs in the nonhost interaction between pear and *V. inaequalis*
298 that indicate the presence of free ceramides and LCB, which possibly participate to the HR
299 development. Free LCB presence is demonstrated by the activations of *SBH1* (24hpi), *SLD1* (24hpi)
300 and another sphingolipid Δ8 long-chain base desaturase homolog to *At2g46210* (24hpi; found two
301 times), and their relative conversion in ceramides is demonstrated by the differential expressions of

302 the ceramide synthases *LOH2* (repressed at 24hpi) and *LOH3/LAG13* (induced at 24 and 72hpi). LCB
303 non-conversion in phosphorylated counterparts is shown by the *AtLCBK1* repression (72hpi) and free
304 ceramides maintenance is attested by their non-conversion in glycosyled ones given the repression
305 of a glucosyl ceramide synthase homolog to *At2g19880* (24hpi).
306 The differential expression of numerous known regulators of HR in our data is again consistent with
307 the HR phenotype observed. The mechanosensor MSL10 and the calmodulin-activated $Ca^{2+}$ pump
308 (autoinhibited $Ca^{2+}$-ATPase [ACA]) ACA11 were found engaged: at 24hpi *MSL10* was induced and
309 *ACA11* was repressed. MSL10 is known as a positive regulator of cell death [63] and ACA11 is known
310 as a negative regulator of SA-dependent programmed cell death [33]. Their modulation is linked with
311 the noticed calcium influx discussed above ([33]; [64]). The participation of the SA pathway in the
312 development of the hypersensitive response could also be supported by the repression of *EDR1* (at
313 72hpi). Indeed, the MAPKKK EDR1 is known as a negative regulator of the SA-dependent HR
314 (reviewed in [65]).
315 Three other regulators of HR were found modulated in our data. The transcription factor *AS1*
316 (*MYB91*) was found induced at 24hpi. It is known as a positive regulator of HR and implicated in JA
317 pathway (reviewed in [20]). The transcription factor *WRKY40* was found repressed at 72hpi. It is
318 known as a negative regulator of HR [66] and implicated in PTI [67]. Another negative regulator of HR
319 is the lipid-binding domains containing protein VAD1 [68]. It was found repressed at 72hpi.
320 The behavior of two others genes in our data seems to indicate that the developed HR was contained
321 due to intracellular ROS production and damages. The function *UGT73B3* and *CAT2* were thus
322 activated (24hpi). UGT73B3 and CAT2 are known as restrictors of HR expansion via their action in
323 ROS scavenging (CAT2; [69]) or in detoxification of ROS-reactive secondary metabolites (UGT73B3;
324 [70]).
325 To conclude, in pear/*V. inaequalis* nonhost interaction, HR was spread out, in link with the calcium
326 influx, but especially following apoplastic ROS production and ROS production via free sphingolipids

327 accumulation and not via NLR receptors. Furthermore, the behavior of not less than eight regulators
328 indicate that the developed HR is under control (Fig. 4).
329
330 Cell wall carbohydrates content and cuticle composition are altered
331
332 The first obstacle encountered by host as well as nonhost pathogens attempting to colonize plant
333 tissues is the plant cell wall, which is often covered with a cuticle. Preinvasive penetration barrier, as
334 a preformed physical barrier, or as the onset place of defensive signaling pathways, is considered an
335 important factor, especially in nonhost resistance in which non adapted pathogens normally fail to
336 penetrate nonhost plant cells when blocked by the cell wall ([10]; [43]). Plant cell wall alterations, of
337 the carbohydrates or the phenolic components, either by impairing or overexpressing cell wall-
338 related genes, have been demonstrated to have a significant impact on disease resistance and/or on
339 abiotic stresses (reviewed in [71] and [72]).
340 We found numerous genes related to the cell wall with a modified expression during nonhost
341 interaction between pear and *V. inaequalis*, among them about thirty related to the biosynthesis or
342 the modification of carbohydrates. These genes are presented in table 3, except those related to the
343 lignin and other phenolic compounds, which will be discussed later. We saw in particular several
344 genes related to cellulose (8) and even more genes related to pectin (14) but no genes related to
345 callose.
346 Concerning these particular carbohydrate components, the model proposed by Bacete et al [71] is as
347 follows. Firstly, alterations in cellulose biosynthesis from primary or secondary cell wall trigger
348 specific defensive responses, such as those mediated by the hormones JA, ET or abscisic acid (ABA),
349 activate biosynthesis of antimicrobial compounds, but also might attenuate pattern triggered
350 immunity (PTI) responses. Secondly, alterations of cell wall pectins, either in their overall content,
351 their degree of acetylation or methylation, activate specific defensive responses, such as those
352 regulated by JA or SA, and trigger PTI responses, probably mediated by damage-associated molecular

353 patterns like oligogalacturonides. Thus, even though our results do not completely support a role of
354 these genes, we think that the modified expression of cell wall related genes during nonhost
355 interaction between pear and *V. inaequalis* is meaningful.
356
357
358 **Table 3: Main DEGs related to cell wall carbohydrates synthesis/modification detected during non-**
359 **host interaction pear/*V. inaequalis*.**
360

| | Gene | Action | Expression* |
|---|---|---|---|
| | Primary cell wall | | |
| Cellulose | *CSLA2* | synthesis | I |
| | *PNT1* | synthesis | I |
| | *COBL2* | deposition (GPI-anchored protein) | R |
| | *AtGH9A4* | catabolism | I |
| | *XTR7* | loosening | I |
| Hemi-cellulose (xyloglucan) | *At5g15490* | synthesis | I |
| Pectin | *At3g42180* | synthesis | I |
| | *At4g01220* | synthesis | I |
| | *GHMP kinase* | synthesis | I |
| | *RHM1* | synthesis | R |
| | *PME At2g45220* | methylesterification | I |
| | *PME At2g46930* | methylesterification | I |
| | *PME At3g05910* | methylesterification | R |
| | *PME At1g02810* | methylesterification | R |
| | *PME44* | methylesterification | R |
| | *PG At3g16850* | depolymerisation | I |
| | *PG At3g59850* | depolymerisation | I |
| | *PG At4g13710* | depolymerisation | I |
| | *PG At3g62110* | depolymerisation | R |
| | *IDA* | degradation | R |
| Arabinogalactan protein | *AGP11* | _ | I |
| | *AGP1* | _ | R |
| | Secondary cell wall | | |
| Cellulose | *CESA09* | synthesis | I |
| | *CESA10* | synthesis | I |
| | *CSLG1* | synthesis | I |
| Hemi-cellulose (xylan) | *FRA8* | synthesis | I |

| | | | |
|---|---|---|---|
| Undetermined | | | |
| Expansin | *EXP15* | loosening | I |
| | *EXPB3* | loosening | I |
| Hemi-cellulose | *ATFUC1* | modification | I |
| | *XTH33* | growth and assembling | R |

361 *I: induced, R: repressed
362

363 Concerning the cuticle layer, most cuticles are composed largely of cutin, an insoluble polyester of
364 primarily long-chain hydroxy fatty acids. This lipophilic cutin framework is associated with
365 hydrophobic compounds collectively referred to as waxes. The cuticle is also thought to contain
366 varying amounts of intermingled cell wall polysaccharides and sometimes also a fraction termed
367 cutan (reviewed in [73]). Cutin monomers are synthesized by the modification of plastid-derived 16C
368 and 18C fatty acids in the endoplasmic reticulum (ER), yielding variously oxygenated fatty acid–
369 glycerol esters referred to as monoacylglycerols, which polymerize upon arrival at the growing cuticle
370 (Fig. 5, reviewed in [73]).
371 C16 and C18 fatty acids are also important precursors of cuticular wax synthesis (Fig. 5). Upon
372 transport to the ER, the C16 and C18 fatty acids are extended to form very-long-chain fatty acids
373 (VCLFAs; C>20), and this extension is carried out by the fatty acid elongase (FAE) complex located on
374 the ER membrane. The very-long-chain FAs are then converted into the varied cuticular waxes
375 (primary alcohols, aldehydes, alkanes, secondary alcohols, ketones) by many ways (reviewed in [74]).
376 Interestingly, we found three genes upregulated 24hpi belonging to the FAS (fatty acid synthase)
377 chloroplastic complex implicated in the production of the C16 precursor (Fig. 5): *ACCD*, *FabG* and
378 *MOD1* (found two times). ACCD encodes the carboxytransferase beta subunit of the Acetyl-CoA
379 carboxylase complex which catalyzes the first committed step in fatty acid synthesis: the
380 carboxylation of acetyl-CoA to produce malonyl-CoA. FabG and MOD1 are respectively a β-ketoacyl
381 ACP-reductase and an enoyl-ACP-reductase which catalyze respectively the conversion of
382 acetoacetyl-ACP into β-hydroxyacyl-ACP and the second reductive step from enoyl-ACP to butyryl-
383 ACP (reviewed in [74]).

384 In the ER, the four functions we found related to waxes biosynthesis in our data were repressed at
385 24hpi: *KCS4* (found two times), *CER1* and *CER3*, or 72hpi: *ECR/CER10*. KCS4 and ECR/CER10 belong to
386 the FAE complex ([75]; [76]). The last two genes are implicated in aldehydes (*CER1*) and alkanes
387 (*CER1 and 3*) generation (reviewed in [74]). On the contrary, the eight genes we found connected to
388 cutin biosynthesis were induced at 24hpi except a gene homolog to *At5g14450*, which was induced
389 at 72hpi. One of them is a glycerol-3-phosphate acyltransferase (GPAT) coding gene: GPAT8, which
390 catalyzes the transfer of a fatty acid from coenzyme A (CoA) to glycerol-3-phosphate (Fig. 4; reviewed
391 in [73]). GPAT8 function in cutin formation has been functionally confirmed in association with
392 GPAT4 [77]. The seven others genes code GDSL-lipases enzyme (*At1g28600*, *At1g28660*, *At1g54790*,
393 *At3g16370*, *At3g48460*, *AtCUS4*: *At4g28780*, *At5g14450*), some of which have been shown to
394 function as cutin synthase (Fig. 4; [78]; reviewed in [73]) and polymerize monoacylglycerols.
395 We also found induced respectively at 24 and 72hpi two genes involved in waxes and cutin
396 biosynthesis positive regulation: *MYB16* and *SHN1*. The SHN genes (*SHN1–SHN3*), a set of three
397 largely redundant APETALA 2 family transcription factors from *A. thaliana*, are regulators of floral
398 cutin and epidermal cell morphology. SHN1 is regulated by the MYB family transcription factor
399 MYB106, which, along with its paralog MYB16, controls many aspects of cuticle and epidermis
400 formation in *A. thaliana* (reviewed in [79] and [73]).
401 Cutin and cuticular waxes play an important role in plant-insect and plant-microbe interactions.
402 Numerous Arabidopsis mutants in cutin and waxes biosynthetic or transport genes, such as Acyl-CoA
403 binding proteins (ACBP), show varying degrees of cuticle impairment, alterations in cutin and/or wax
404 composition, and defects in SAR (reviewed in [74]). We found *ACBP6* repressed at 24hpi. That
405 repression is not inconsistent with the previously described amplification of cutin biosynthesis and
406 polymerization, given that *acbp6* KO mutation is not associated with a defect in that pathway [39].
407 That repression is also consistent with the SAR repression observed above as the *acbp6* KO mutant
408 show compromised SAR [39].

409 To conclude, our analysis of nonhost pear/V. *inaequalis* interaction identified an alteration of the
410 cuticle composition with more cutin and less waxes synthesis. The increase in cutin polymerization
411 could lead to a thickening of the cuticular layer to prevent fungus penetration via its appressoria.
412

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

415 As distinguished from primary metabolism, plant secondary metabolism refers to pathways and small
416 molecule products of metabolism that are non-essential for the survival of the organism. But they are
417 key components for plants to interact with the environment in the adaptation to both biotic and
418 abiotic stress conditions. Plant secondary metabolites are usually classified according to their
419 chemical structure. Several groups of large molecules, including phenolic acids and flavonoids,
420 terpenoids and steroids, and alkaloids have been implicated in the activation and reinforcement of
421 defense mechanisms in plants (reviewed in [80]).
422 Terpenoids and steroids, or isoprenoids, are components of both the primary and secondary
423 metabolisms in cells, and mono-, tri-, sesqui- and polyterpenes are considered as secondary
424 metabolites (reviewed in [81]). Our results on pear identified seven DEGs and five DEGs belonging to
425 the chloroplastic methylerythritol posphate (MEP) and to the cytosolic mevalonic acid (MVA)
426 pathway of isoprenoids production respectively (Table 4), which results, among others compounds,
427 in tri- and sesquiterpenes secondary metabolites. The majority of these genes contribute to produce
428 primary metabolites according to Tetali [81]. Except *SMT2*, that we found induced at 24hpi, there is
429 no report concerning a putative implication of others genes in plant biotic resistance. SMT2 is
430 involved in sterols production and *smt2* mutation was reported to compromise bacterial resistance in
431 *Nicotiana benthamiana* [82]. The hypothesis is that sterols regulate plant innate immunity against
432 bacterial host and nonhost infections by regulating nutrient efflux into the apoplast. *V. inaequalis* is a
433 hemi biotrophic pathogen which colonizes only the apoplast compartment at the beginning of the
434 interaction. strong relative induction of *SMT2* in our data could indicate that a similar mechanism of

435 nutrient efflux regulation via sterols could take place to limit the fungus growth in pear nonhost
436 resistance against *V. inaequalis*.
437
438
439 **Table 4: Main DEGs involved in biosynthetic pathways for terpenes and isoprenoids during pear/*V.***
440 ***inaequalis* non-host interaction.**

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

441
442 In our data, the other DEGs that were linked to secondary metabolism belong to the
443 phenylpropanoid pathway production (Fig. 6). Among them we found four genes belonging to the
444 flavonoid production, all repressed, at 24hpi (*DFR* and *DRM6*) or 72hpi (*TT7* and *UGT71D1*). DFR
445 (dihydroflavonol reductase) is involved in flavan-3,4-ol production and TT7 (flavonoid 3' hydroxylase)
446 in dihydroquercetin production from dihydro-kaempferol, and UGT71D1 (glucosyl transferase) in
447 quercetin-glycoside production from quercetin (TAIR database;
448 https://www.arabidopsis.org/index.jsp). DMR6 (flavone synthase) is involved in flavone production
449 from naringenin [83]. Thus flavonoid production does not seem to be favored, which is not consistent
450 with the induction of *MYB12* at 24hpi, but consistent with *MYB4* induction at 72hpi. MYB12 is
451 actually known as a positive regulator of flavonol biosynthesis in pear and apple fruits ([84]; [85])
452 whereas MYB4 is known as a negative regulator of this biosynthetic pathway [86].

453 Concerning the production of monolignols, precursors of lignin synthesis, some genes were found
454 induced, others repressed. We found *CYP98A3* and *CAD9* (found two times) induced at 24hpi and
455 *HCT*, *CCR1* and a gene homolog to *At2g23910* (found two times, one time repressed at 72hpi)
456 repressed at 24hpi Fig. 6). *CYP98A3* encode a C3H (coumarate 3-hydroxylase), *CAD9* encode a CAD
457 (cinnamyl alcohol dehydrogenase), HCT is an hydroxycinnamoyl-CoA shikimate/quinate
458 hydroxycinnamoyl transferase, *CCR1* encode a CCR (cinnamoyl-CoA reductase) and *At2g23910*
459 encode a CCR-related protein. (TAIR and KEGG databases (https://www.genome.jp/kegg/)).
460 Lignification is obtained by cross-linking reactions of the lignin monomers or by polymer–polymer
461 coupling via radicals produced by oxidases such as peroxidases [87] and laccases [88]. However,
462 while peroxidases are able to oxidize monolignols to produce H, G and S units of lignin, laccases only
463 generate G units [87]. In our data, we found two laccases induced at 24hpi: *LAC11* (found two times,
464 one time induced at 24 and 72hpi) and *LAC17* (found two times), and three peroxidases repressed at
465 24hpi: *PRX17*, *PER47* and *PRX52* (also repressed at 72hpi), which can be linked to lignin biosynthetic
466 process (Fig. 6). According to Zhao et al. [88], LAC11 and LAC17, along with LAC4, play a critical role in
467 lignification, and their results suggests that peroxidase and laccase do not serve redundant functions
468 in lignification, at least in the vascular tissues of the stem and root of Arabidopsis. Participation in
469 lignin formation has also been proved for PRX17 [89], PER47 [90] and PRX52 [91]. But there are
470 currently no reports about a possible involvement of all these genes in lignification linked to biotic or
471 abiotic stresses. Concerning non-host resistance, two reports describe lignin
472 accumulation/deposition involvement: one in apple fruit [92] and the other one in cowpea [93]. In
473 the latest, authors showed that preferentially generated lignin units in this nonhost interaction are G
474 units, just as it seems to be the case in our pear / *V. inaequalis* study. To summarize, it is tempting to
475 think that modifications of expression observed for genes linked to lignin polymerization are relevant
476 for the pear nonhost resistance against *V. inaequalis*, but further functional analysis should be
477 conducted to conclude.

478 The biosynthesis of two others types of phenylpropanoid compounds appears to be favored during
479 pear nonhost resistance against *V. inaequalis*: simple coumarin on one hand and hydroxycinnamic
480 acid amides on the other hand. We found four *BGLU*-like genes induced: *BGLU47* and *BGLC3* (at
481 24hpi), *BGLU16* (at 72hpi); *BGLU42* (at 24 and 72hpi) (Fig. 6). These β-glucosidases could be implied
482 in simple coumarin path production from the cinnamic acid (KEGG database). Some natural simple
483 coumarins are known as antifungal compounds *in vitro* and have been developed as fungicides [94].
484 Previous work on Hevea also reports the correlation between the resistance against pathogenic fungi
485 and the production of some coumarins, with antifungal activity *in vitro* [95]. We also found induced
486 at 24hpi the genes *AACT1/ACT1*, *ATPAO5* and genes homologs to *At4g17830* and *At4g38220* (Fig. 6).
487 AACT1/ACT1 catalyze the first specific step in branch pathway synthesizing hydroxycinnamic acid
488 amides from the p-Coumaroyl CoA or the feruloyl CoA and amines agmatine or putrescine [96].
489 Hydroxycinnamic acid amides are produced in response to pathogenic infections [96] and surface
490 exported. Hydroxycinnamic acid amides are reported to participate in Arabidopsis nonhost resistance
491 against *Phytophthora infestans* via their inhibitory activity on spore germination [97]. The three
492 others genes belong to the arginine biosynthesis path (homologs to *At4g1783* and *At4g38220*) and
493 the arginine and proline metabolisms which produce the amines agmatine and putrescine (*ATPAO5*)
494 (KEGG database). Agmatine is directly produced from arginine thanks to an ADC activity (arginine
495 decarboxylase) and putrescine can be produced from spermidine thanks to a PAO activity (polyamine
496 oxidase). ATPAO5 catalyzes the conversion of spermine in spermidine. The induction of these three
497 last genes is therefore consistent with the hypothesis of amines production in order to enable
498 hydroxycinnamic acid amides synthesis. The induction of *C4H* at 24hpi could also favor
499 hydroxycinnamic acid amides synthesis via p-Coumaroyl CoA biosynthesis promotion. C4H
500 (cinnamate 4-hydroxylase) catalyzes the production of p-Coumaric acid from Cinnamic acid and p-
501 Coumaric acid gives p-Coumaroyl CoA thanks to 4CL (4- coumarate-CoA ligase) (KEGG database).
502 Among the suite of defense components synthetized in nonhost as in host context, a chemical barrier
503 can be established via accumulation of a diverse array of secondary metabolites rapidly produced

504 upon pathogen infection, named phytoalexins, with toxic or inhibitory effects (reviewed in [10]).
505 Phytoalexins can be flavonoids, such as the pisatin of pea (in [98]) but also varied phenylpropanoid
506 compounds. In the nonhost interaction pear / *V. inaequalis*, the production of flavonoid type
507 phytoalexins does not seem to be favored, except simple coumarin and hydroxicinnamic acid amines.
508

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

510 Only 60 DEGs were detected in the apple / *V. pyrina* nonhost interaction at 24 or 72hpi, in agreement
511 with the total absence of macroscopic symptoms and few cells engaged in an HR-like reaction
512 observed at the microscopic level. Among these 60 DEGs, 36 have no known function. Among the 24
513 remaining DEGs, nine DEGS could be relevant in apple / *V. pyrina* nonhost interaction in view of our
514 findings in pear / *V. inaequalis* nonhost interaction. *ORG2* (*BHLH038*), a putative integrator of various
515 stress reactions [99] was induced at 24hpi. Three genes were related to an oxidative stress: *GASA10*
516 was repressed at 24hpi and *NRAMP3* and *AOR* were induced at 24hpi. GASA proteins have been
517 suggested to regulate redox homeostasis via restricting the levels of $OH^{\cdot}$ in the cell wall [100]. The
518 repression of this gene is thus in favor of more $OH^{\cdot}$ in the cell wall. The oxidoreductase coding gene
519 *AOR* is known in the chloroplast to contribute to the detoxification of reactive carbonyls produced
520 under oxidative stress [101]. *NRAMP* genes function as positive regulators of ROS accumulation,
521 especially during Arabidopsis *Erwinia chrisanthemi* resistance [102]. The induction (at 24 and 72hpi)
522 of another gene suggests modifications at the cell wall level: *EXP8*, an expansin coding gene involved
523 in cell wall loosening (Tair database). We also found two genes related to hormone pathways, one
524 induced at 24hpi: *WIN1* and the other one repressed at 72hpi: *UBP12*. WIN1 is known as a negative
525 regulator of SA pathway [103] and UBP12 as a positive regulator of JA pathway via the stabilization of
526 MYC2 [18]. In link with the JA pathway, we also found *TPS21* induced at 24hpi. TPS21 is involved in
527 sesquiterpenes production and is promoted by JA signal via MYC2 [104]. TPS21 is especially involved
528 in the jasmonate-dependent defensive emission of herbivore-induced volatiles in cranberries [105].
529 Finaly the last DEG we found relevant in apple / *V. pyrina* nonhost interaction could promote HR via

530 ceramides accumulation. *ACD11* is repressed at 24hpi in our data. In *acd11* mutants, the relatively
531 abundant cell death inducer phytoceramide rises acutely [106].
532 Because nonhost resistance of apple against *V. pyrina* is of a type I, with a very limited number of
533 cells engaged in an HR-like reaction, it has not been possible for us to exhaustively describe how this
534 interaction is expressed at the transcriptomic level in the first three days of the interaction. Further
535 insight with later points of cinetic and more adapted techniques such as laser-assisted cell picking,
536 prior to micro arrays or RNA sequencing analysis (review in [107]) could provide more information in
537 the future.
538

539 Comparison of pear resistances against the host pathogen *V. pyrina* and the nonhost
540 pathogen *V. inaequalis*

541 Perchepied et al [11] performed a detailed transcriptomic analysis of the host resistance of pear
542 against *V. pyrina* strain VP102, deployed in a transgenic pear bearing the well-known apple *Rvi6*
543 resistance gene against *V. inaequalis*. Comparing this work to ours gives us the rare opportunity to
544 analyze similarities and differences between a host and a nonhost resistance in the same plant. Only
545 four transcriptomic studies involving pear/pathogen interactions have been published so far. Yan et
546 al [108] reported the modulation of expression of 144 pear genes after fruit treatment by
547 *Meyerozyma guilliermondii*, an antagonistic yeast used for biocontrol of natural pear fruit decay.
548 Zhang et al [109] similarly reported the modulation of expression of 1076 pear genes after treatment
549 with *Wickerhamomyces anomalus*, another biocontrol agent. Using RNA-seq, Wang et al. [110]
550 reported a major role of ethylene signalization during the compatible interaction between *P. pyrifolia*
551 and *Alternaria alternata*, a necrotrophic pathogen. Finally, Xu et al. [111] applied RNA-seq to
552 characterize the genes of *Penicillium expansum* activated after infection of pear fruits. None of these
553 studies can be directly compared to our work on host and nonhost scab pear resistance.
554 Concerning the recognition and early signaling steps of the interactions, many receptors and co-
555 receptors have been found induced in the host pear resistance, especially damage-associated

556 molecular patterns receptors such as RLK7, revealing that PTI and ETI must be engaged. We did not
557 find evidence of the mobilization of such receptors in the pear nonhost resistance. PTI and ETI
558 receptors are nonetheless reported as implicated in nonhost resistance (reviewed in [112] and [10]).
559 As we only analyzed post infection transcriptional modulations in the nonhost pear/*V. inaequalis*
560 interaction (at 24 and 72hpi), one hypothesis to explain the lack of PTI and ETI receptors in our data
561 could be that these receptors were already present as preformed defenses and not particularly
562 induced by the infection onset. In pear nonhost interaction, the earliest signaling pathways we were
563 able to highlight are calcium influx and apoplastic ROS production. Calcium signaling seems to be also
564 implicated in pear host resistance, but less obviously than in nonhost resistance.
565 Regarding the hormonal signaling pathways, the JA defense signaling pathway was found repressed
566 in pear nonhost resistance but quite activated in pear host resistance. The JA/ethylene (ET) defense
567 signaling pathway is known as an effective defense against necrotrophic fungi in Arabidopsis [113].
568 Thus, it is not surprising to find the JA pathway repressed in the development of the pear nonhost
569 resistance against the hemi-biotrophic pathogen *V. inaequalis*. But it is very interesting to find this
570 pathway rather induced in the development of the pear host resistance against the other hemi-
571 biotrophic pathogen *V. pyrina*. The SA signaling pathway is commonly seen as the classical one
572 triggered to resist biotrophic fungi in Arabidopsis [113], but only a little engagement in pear nonhost
573 resistance has been observed, SA signaling being repressed in pear host resistance. If this absence of
574 SA implication is quite unexpected in pear host resistance against a hemi-biotrophic fungus, it is
575 consistent with the report that the exact role of these key defense phytohormones is unclear in
576 nonhost resistance and remains to be established [43]. As shown by Tsuda et al [114], an explanation
577 for the hormone pathways behavior in pear host resistance could be that: as both the SA and JA/ET
578 pathways positively contribute to immunity, a loss of signaling flow through the SA pathway can be
579 compensated by a rerouting signal through the JA/ET pathways. In addition, independently of SA
580 signaling, but in positive connection with JA signaling, SAR seems to be engaged in distal tissues
581 during pear host resistance. To conclude, in pear host as well as nonhost resistances, classical

582 resistance hormones SA and JA/ET, and the correlative PR gene defenses, seems differently involved
583 than in Arabidopsis.
584 The carbohydrate content of the cell-wall is modified in response to the attacks by the pathogens.
585 Regarding cell-wall and cuticle, in pear host as well as nonhost resistances, important modifications
586 were highlighted. Similar modifications affected the cellulose and mainly the pectin contents, but no
587 callose production was observed. Regarding cuticle, waxes production was induced in host resistance
588 whereas it was repressed in nonhost resistance, in favor of cutin production / polymerization, which
589 was also induced in host resistance. To conclude, as a first obstacle encountered by host, as well as
590 nonhost, pathogens attempting to colonize plant tissues, the plant cell wall and its cuticle seem to
591 play a leading role in both pear host and nonhost resistances.
592 Finally, the production of secondary metabolites and phenylpropanoids compounds in particular,
593 seems to be a major line of defense, in pear host as well as nonhost resistances, but with
594 divergences. If lignin and flavonoid productions are preponderant in pear host resistance against *V.*
595 *pyrina*, lignin implication in pear nonhost resistance is less clear and flavonoids production is
596 obviously repressed. But the biosynthesis of two other types of phenylpropanoid-derived
597 phytoalexins appears to be favored during pear nonhost resistance: simple coumarin on one hand
598 and hydroxycinnamic acid amides on the other hand.
599 The comparative analysis between a host and a nonhost resistance in pear shows that, even though
600 specificities are observed, the two major defense lines engaged are shared: the cell wall and its
601 cuticle on one hand, the secondary metabolism with the phenylpropanoid pathway on the other
602 hand. Moreover, these defenses seem deployed largely independently of the SA signaling pathway,
603 widely recognized as the main defense hormone against biotrophic pathogens.
604

605 ## Conclusion

606 As far as we know, our work is the first one published regarding a transcriptomic analysis of post-
607 infections events of a nonhost resistance to *Venturia sp.* in apple and pear. Velho and Stadik [115]

608 recently published a detailed description of the apple / *Colletotrichum higginsianum* nonhost
609 resistance, highlighting the accumulation of callose at the sites of penetration of the fungus. But no
610 data on gene expression was included. Here, our molecular work on apple / *V. pyrina* nonhost
611 resistance remains preliminary and in order to allow a deeper deciphering, further analyses must be
612 considered with the aid of tools adapted to this type I nonhost resistance with very few cells engaged
613 in an HR-like reaction, only visible at a microscopic level. In pear, this deciphering allowed us to show
614 that nonhost resistance against *V. inaequalis* is a type II one, which involves enough pathogen
615 penetration in plant tissue to trigger visible HR and develops post-invasive defenses.
616 To summarize our findings on pear with a notion of cascading effect, we can propose the following
617 scenario (Fig. 4): once *V. inaequalis* presence is recognized by pear, a calcium cellular influx is
618 induced and leads to the development of a pre-invasive defense, the stomatal closure, but also
619 promotes an early post-invasive defense, an apoplastic ROS accumulation. Apoplastic ROS, acting
620 themselves as ubiquitous messengers, come to reinforce the stomatal closure but also mediate
621 cellular signaling resulting in two post-invasive defenses: HR development at infection sites, along
622 with phytoalexin (simple coumarin and hydroxicinnamic acid amines) production. The observed
623 alterations of the epidermis composition (cellulose, pectin, lignin for the cell wall, and cutin for the
624 cuticle), are presumed to strengthen this physical barrier and can be seen as the development of
625 another pre-invasive defense. The calcium (action on pectin reviewed in [116]) and the ROS (action
626 on lignin, [117]; [118]; action on cuticle, [119]) have been linked to some type of epidermis
627 modifications and may participate in the proceeding of these defense in pear / *V. inaequalis* nonhost
628 interaction.
629 Nonhost resistance is defined as the resistance of an entire plant species against a specific parasite or
630 pathogen [120] and is seen as the most durable resistance of plant. Thus, understanding the
631 molecular mechanisms underlying nonhost resistance can open up some interesting avenues to
632 create sustainable host resistances in the same plant species. Considering pear, in order to stop the
633 germination and entrance of hemibiotrophic host fungi such as *V. pyrina*, strengthening the cuticle

634 initial barrier via more cutin production and cross-link, or promoting the biosynthesis of phytoalexins
635 like hydroxycinnamic acid amines, appear as promising solutions, relatively easy to engineer
636 regarding recent advances in biotechnology tools on this species ([121]; [122]; [123]).
637

638 ## Material and methods

639 Biological material

640 Apple plants from the cultivar 'Gala' and pear plants from the cultivar 'Conference' were chosen
641 because of their susceptibility to *V. inaequalis* and *V. pyrina*, respectively. The apple and pear
642 genotypes were multiplied in vitro, rooted and acclimatized in greenhouse as described previously
643 ([124]; [125]).
644 For apple scab inoculation, the *V. inaequalis* monoconidial isolate used was EU-B05 from the
645 European collection of *V. inaequalis* of the European project Durable Apple Resistance in Europe
646 [126]. For pear scab inoculation, the monoconidial strain VP102 of *V. pyrina* was chosen for its
647 aggressiveness on 'Conference' [127].
648

649 Scab inoculation procedure

650 Greenhouse growth conditions and mode of inoculum preparation were as described in Parisi and
651 Lespinasse [128] for apple and Chevalier et al [129] for pear. Briefly, the youngest leaf of actively
652 growing shoots was tagged and the plants inoculated with a conidial suspension ($2 \times 10^5$ conidia $ml^{-1}$)
653 of *Venturia pyrina* strain VP102 for apple and *Venturia inaequalis* strain EUB04 for pear. Symptoms
654 were recorded at 14, 21, 28, 35 and 42 days after inoculation on 20 actively growing shoots (one
655 shoot by plant) for each interaction. The type of symptoms was scored using the 6 class-scale of
656 Chevalier et al [130].
657

658 Microscopic observations

659 Histological studies were made on samples stained with the fluorophore solophenylflavine [131]. In
660 brief, leaf discs were rinsed in ethanol 50° before staining in a water solution of solophenylflavine
661 7GFE 500 (SIGMA-Aldrich, St Louis USA) 0.1% (v/v) for 10 min. The samples were first rinsed in
662 deionized water, then in 25% glycerol for 10 min. Finally, the leaf samples were mounted on glass-
663 slides in a few drops of 50% glycerol. They were examined with a wide-field epifluorescence
664 microscope BH2-RFC Olympus (Hamburg, D) equipped with the following filter combination:
665 excitation filter 395 nm and emission filter 504 nm.

666

667 Transcriptomics experiment

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

673 For RNA extraction, frozen leaves were ground to a fine powder in a ball mill (MM301, Retsch, Hann,
674 Germany). RNA was extracted with the kit NucleoSpin RNA Plant (Macherey Nagel, Düren, Germany)
675 according to the manufacturer's instructions but with a modification: 4% of PVP40 (4 g for 100 ml)
676 were mixed with the initial lysis buffer RAP before use. Purity and concentration of the samples were
677 assayed with a Nanodrop spectrophotometer ND-1000 (ThermoFisher Scientific, Waltham, MA, USA)
678 and by visualization on agarose gel (1% (weight/volume) agarose, TAE 0.5x, 3% (volume/volume)
679 Midori green). Intron-spanning primers (forward primer: CTCTTGGTGTCAGGCAAATG, reverse primer:
680 TCAAGGTTGGTGGACCTCTC) designed on the *EF-1α* gene (accession AJ223969 for apple and
681 PCP017051 for pear, available at https://www.rosaceae.org/, with the datasets on "Pyrus communis
682 v1.0 draft genome") were used to check the absence of genomic DNA contamination by PCR. The
683 PCR reaction conditions were as follows: 95°C for 5 min, followed by 35 cycles at 95°C for 30 s, 60°C

684 for 45 s, 72°C for 1 min, with a final extension at 72°C for 5 min. The PCR products were separated on
685 a 2% agarose gel.
686 Amplifications (aRNAs) were produced with MessageAmpII aRNA Kit (Ambion Invitrogen, Waltham,
687 MA, USA), from 300 ng total RNA. Then 5 µg of each aRNA were retrotranscribed and labelled using a
688 SuperScript II reverse transcriptase (Transcriptase inverse SuperScript™ II kit, Invitrogen, Carlsbad,
689 CA, USA) and fluorescent dyes: either cyanine-3 (Cy3) or cyanine-5 (Cy5) (Interchim, Montluçon,
690 France). Labeled samples (30 pmol each, one with Cy3, the other with Cy5) were combined two by
691 two, depending on the experimental design (i. e. for example, the CF/EUB05/24hpi sample and the
692 CF/non inoculated sample are labeled with Cy3 and Cy5 respectively and pooled to co-hybridize the
693 microarray) . For each comparison two biological replicates were analyzed in dye-switch as described
694 in Depuydt et al [132]. Paired labeled samples were then co-hybridized to Agilent microarray AryANE
695 v2.0 (Agilent-070158_IRHS_AryANE-Venise, GPL26767 at GEO: https://www.ncbi.nlm.nih.gov/geo/)
696 for apple, or Pyrus v1.0 (Agilent-078635_IRHS_Pyrus, GPL26768 at GEO) for pear, containing
697 respectively 133584 (66792 sense and 66792 anti-sense probes) and 87812 (43906 sense and 43906
698 anti-sense probes) 60-mer oligonucleotide probes. The hybridizations were performed as described
699 in Celton, Gaillard et al [133] using a MS 200 microarray scanner (NimbleGen Roche, Madison, WI,
700 USA).
701 For microarray analysis we designed two new chips. For apple we used a deduplicated probeset from
702 the AryANE v1.0 ([133]; 118740 probes with 59370 in sense and 59370 in anti-sense) augmented by
703 14844 probes (7422 in sense and 7422 in anti-sense) designed on new gene annotations from *Malus*
704 *domestica* GDDH13 v1.1 (https://iris.angers.inra.fr/gddh13 or
705 https://www.rosaceae.org/species/malus/malus_x_domestica/genome_GDDH13_v1.1). These
706 probes target new coding genes with UTRs when available, manually curated micro-RNA precursors
707 and transposable elements. For transposable elements we used one consensus sequence for each
708 family and a randomly peaked number of elements proportional to their respective abundance in the

709 genome. The microarrays used in this study also have probes for coding genes of *V. inaequalis* but
710 they have not been taken into account.
711 For pear the design was done on the *Pyrus communis* Genome v1.0 Draft Assembly & Annotation
712 available on GDR (https://www.rosaceae.org/species/pyrus/pyrus_communis/genome_v1.0) web
713 site. We have downloaded the reference genome and gene predictions fasta files and structural
714 annotation gff file the 21st of September 2015. Using home-made Biopython scripts we have
715 extracted spliced CDS sequences with 60 nucleotides before start and after stop codons to get UTR-
716 like sequences likely to be found on transcripts resulting in a fasta file containing 44491 sequences.
717 These 60 nucleotides increase the probability of finding specific probes on genes with high similarity.
718 This file was sent to the eArray Agilent probe design tool (https://earray.chem.agilent.com/earray/)
719 to generate one probe per gene prediction. Options used were: Probe Length: 60, Probe per Target:
720 1, Probe Orientation: Sense, Design Options: Best Probe Methodology, Design with 3' Bias. The
721 probeset was then reverse-complemented to generate anti-sense probes and filtered to remove
722 duplicated probes. The final probeset contains 87812 unique probes targeting 1 (73612 probes) or
723 more (14200 probes) potential transcript both in sense and anti-sense.
724 Normalization and statistical analyses performed to get normalized intensity values have been done
725 as in Celton, Gaillard et al [133]. For each comparison and each probe, we retrieved a ratio of the
726 logarithms of the fluorescence intensities (one per compared sample: T0 versus 24hpi or T0 versus
727 72hpi in our case) and an associated p-value. The applied p-value threshold to determine DEGs
728 (differentially expressed genes) was 0.01. Through blast analysis, a TAIR accession number (The
729 Arabidopsis Information Resource; https://www.arabidopsis.org/; [134]) has been linked to a
730 majority of apple or pear "probe/corresponding gene" and the couple "TAIR accession/ratio value"
731 has then been used to make a global analysis of functional categories observed in the Mapman
732 software (https://mapman.gabipd.org/homemapman.gabipd.org; [135]). The detailed analysis of
733 DEGs has been done through TAIR and KEGG (https://www.genome.jp/kegg/) databases, and

734 bibliography. Metadata for the 172 (162 for pear and 10 for apple) DEGs discussed in this work are
735 available in Table S3 and S4 (Online only).
736
737 qPCR validation of transcriptomic data
738 In order to validate transcriptomic data, qPCR was performed on a selection of gene/sample
739 associations. First-strand cDNA was synthesized using total RNA (2.0 µg) in a volume of 30 µl of 5×
740 buffer, 0.5 µg of oligodT15 primer, 5 µl of dNTPs (2.5 mM each), and 150 units of MMLV RTase
741 (Promega, Madison, WI, USA). The mixture was incubated at 42°C for 75 min. qPCR was then
742 performed. Briefly, 2.5 µl of the appropriately diluted samples (1/16 dilution) were mixed with 5 µl of
743 PerfeCTa SYBR Green SuperMix for iQ kit (Quantabio, Beverly, MA, USA) and 0.2 or 0.6 µl of each
744 primer (10 µM) in a final volume of 10 µl. Primers were designed with Primer3Plus, their volumes
745 were according to their optimal concentration (determined for reaction efficiency near to 100%;
746 calculated as the slope of a standard dilution curve; [136]). Accessions, primer sequences and
747 optimal concentrations are indicated in Table S2. The reaction was performed on a CFX Connect Real-
748 Time System (BIO-RAD, Hercules, CA, USA) using the following program: 95°C, 5 min followed by 40
749 cycles comprising 95°C for 3 s, 60°C for 1 min. Melting curves were performed at the end of each run
750 to check the absence of primer-dimers and nonspecific amplification products. Expression levels
751 were calculated using the ΔΔCT method [137] and were corrected as recommended in
752 Vandesompele et al [138], with three internal reference genes (GADPH, TUA and ACTIN 7 for apple,
753 GADPH, TUA and EF1α for pear) used for the calculation of a normalization factor. For each couple
754 DEG/sample (sample defining a plant, time, treatment and biological repeat combination), the ratio
755 was obtained by dividing the mean value of CT calculated from 3 technical repeats by the
756 normalization factor obtained for this sample.
757

758 **Supplementary information**

759 **Additional File 1: Table S1**, **S2, S3** and **S4**.

760

761 ## Abbreviations

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

771

772 ## Declarations

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776 Authors contribution

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

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## Availability of data and materials

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

## Ethics approval and consent to participate

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

## Consent for publication

This section is not applicable.

## Competing interests

The authors declare that they have no competing interests

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811 *americanum; Sphaerella inaequalis (Venturia inaequalis)* against *Spilocaea pomi, Fumago*
812 *mali, Actinone macrataegi, Cladosporium dendriticum, Asteroma mali,* and *Scolicotrichum*
813 *venosum;* and *Venturia pyrina* against *Helminthosporium pyrorum, Fusicladium virescens, F.*
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1159

1160 ## Figure legends

1161

1162 **Fig. 1**: **Macro- and microscopic observations of nonhost interactions.**

1163 Binocular observation 21 days after *V. inaequalis* inoculation on 'Conference' (A) and (B) and *V.*
1164 *pyrina* inoculation on 'Gala' (C). Wide field fluorescence observations of: 'Conference' 3 days (D) and
1165 14 days (E) after *V. inaequalis* inoculation, 'Gala' 3 days (F) and 14 days (G) after *V. pyrina*
1166 inoculation. Ap: appressorium, C: conidia, Gf: germination filament, Pp: pin point
1167
1168 **Fig. 2**: **Functional categories of DEGs at 24 or 72hpi during pear response to *V. inaequalis*.**
1169 The number of up- or down-regulated DEGs is expressed as a percentage of the total number of
1170 genes present in the Pyrus v1.0 (87812 probes) microarray. DEGs are classified in functional
1171 categories according to MapMan 3.5.1R2 bins. Only bins with ≥ 6 DEGs are presented.
1172
1173 **Fig. 3: DEGs involved in hormonal pathways during pear/*V. inaequalis* non-host interaction**.
1174 A: DEGs involved in JA pathway; B: DEGs involved in SA pathway. Genes written in red are induced,
1175 genes written in blue are repressed. ACA11: autoinhibited $Ca2^{+}$-ATPase, calmodulin-activated $Ca2^{+}$
1176 pumps at the plasma membrane, endoplasmic reticulum, and vacuole. ACBP6: acyl-CoA-binding
1177 protein. ACX4: acyl-CoA-oxidase1. AS1/MYB91: Asymmetric leaves 1 transcription factor, CAMTA1:
1178 calmodulin-binding transcription activator, CBP60a: calmodulin-binding protein 60a, EDS1: enhanced
1179 disease suceptibility 1. FAR1: FAR-red impaired response 1. G-box: cis-element in the promoter. JAZ:
1180 jasmonate-zim domain protein, JMT: jasmonic acid carboxyl methyltransferase. LOX: lipoxygenase,
1181 MES1: methylesterase 1. MFP2: multifunctional protein 2. MKS1: MAP kinase substrate 1. MYC2:
1182 transcription factor. NINJA: novel interactor of JAZ. PAD4: phytoalexin deficient 4. UGT74F1:
1183 glucosyltransferase. PR1-like (with ATPRB1), PR2, PR3, PR4 (HEL and ATEP3), PR5, PR12:
1184 pathogenesis-related proteins. ST2A: sulfotransferase 2A. TPL: TOPLESS co-repressor. UBP12:
1185 ubiquitin-specific protease 12. WRKY: transcription factor.
1186
1187 **Fig. 4**: **Scenario of major events observed at three first days of pear/*V. inaequalis* non-host**
1188 **interaction.**

1189 On the left side, events observed in a typical cell, on the right side, events observed in guard cells of a
1190 stomata. A: apoplasm, AP: appressorium, C: cuticle, CBL1: calcineurin B-like protein 1, CDPK: $Ca_2^+$-
1191 dependent protein kinases, CRK: cysteine-rich receptor-like kinase, CY: cytoplasm, CW: cell wall, HAA:
1192 hydroxycinnamic acid amines, HR: hypersensitive response, JA: jasmonic acid, MB: plasma
1193 membrane, LCB: Long Chain/sphingoid Base components, MPK6: Mitogen activated protein kinase 6,
1194 MSL10: mechano-sensitive like 10, N: nucleus, PH: penetration hypha, PR: pathogenesis related
1195 proteins, RBOHB: respiratory burst oxidase homolog B, ROS: reactive oxygen species, S: stomata, SA:
1196 salycilic acid, SC: simple coumarins, SP: spore.
1197
1198 **Fig. 5: Main DEGs involved in cutin and wax biosynthesis during pear/V. inaequalis non-host**
1199 **interaction**.
1200 In green the chloroplast, in brown the endoplastic reticulum (ER) and in yellow the nucleus. Genes
1201 written in red are induced, genes written in blue are repressed. FAS: Fatty Acid Synthase complex to
1202 which belong ACCD (carboxytransferase beta subunit of the Acetyl-CoA carboxylase complex), FabG
1203 (β-ketoacyl ACP-reductase) and MOD1 (enoyl-ACP-reductase) functions. FAE: fatty acid elongase
1204 complex. KCS4 (3-ketoacyl-CoA synthase 4) and ECR/CER10 (trans-2-enoyl-CoA reductase) belong to
1205 the FAE complex. CER1 (octadecanal decarbonylase) and CER3 are implicated in aldehydes (CER1)
1206 and alkanes (CER1 and 3) generation in waxes biosynthesis. In cutin monomers synthesis, the ω-
1207 hydroxylation of C16:0 and C18:1 is catalyzed by cytochrome P450 monooxygenase (CYP86A) and
1208 LACS-encoded acyl-CoA synthetase may be required either to synthesize 16-hydroxy 16:0-CoA, a
1209 substrate for ω-hydroxylase, or for membrane transfer of monomers. Finally, the mature
1210 monoacylglycerol cutin monomers are generated by transfer of the acyl group from acyl-CoA to
1211 glycerol-3-phosphate by glycerol-3-phosphate acyltransferase (GPAT) enzymes such as GPAT8. Some
1212 GDSL-lipases enzyme (such as At1g28600, At1g28660, At1g54790, At3g16370, At3g48460, AtCUS4:
1213 At4g28780, At5g14450) are then functioning as cutin synthase and polymerize cutin

1214 monoacylglycerols. Transcription factors such as MYB16 and SHN1 are positive regulators of wax and
1215 cutin biosynthesis. Adapted from Xia et al, 2009, [73] and [74].
1216
1217 **Fig. 6: Main DEGs involved in the phenylpropanoid pathway during Pear / *V. inaequalis* non-host**
1218 **interaction.**
1219 Genes framed in red are induced, genes frames in blue are repressed. Framed in black, the detail of
1220 genes involved in flavonoids production and found in this interaction. Abbreviations: 4CL, 4-
1221 coumarate-CoA ligase; AACT, anthocyanin 5-aromatic acyltransferase; ANR, anthocyanidin reductase;
1222 ANS, anthocyanin synthase; BGLC or BGLU, β-glucosidases; C3H, coumarate 3-hydroxylase; C4H,
1223 cinnamate 4-hydroxylase; CAD, cinnamyl alcohol dehydrogenase; CCoAOMT, caffeoyl-CoA O-
1224 methyltransferase; CCR, cinnamoyl-CoA reductase; CHI, chalcone isomerase; CHS, chalcone synthase;
1225 COMT, caffeic acid 3-O-methyltransferase; CPK, calcium-dependent protein kinase ; DFR,
1226 dihydroflavonol reductase; DMR6, downy mildiou resistant 6; F3H, flavanone 3-hydroxylase; F3'H
1227 flavonoid 3'-hydroxylase; FLS, flavonol synthase; FNS, flavone synthase; GGT1, gamma-glutamyl
1228 transpeptidase 1; GT, glucosyl transferase; HCT, hydroxycinnamoyl-CoA shikimate/quinate
1229 hydroxycinnamoyl transferase; LAC, laccase; LAR, leucoanthocyanidin reductase; OMT1, O-
1230 methyltransferase 1; PAL, phenylalanine ammonia-lyase; PER or PRX, peroxidase; TT7, transparent
1231 testa 7; UGFT, UDP-glucose flavonoid-3-O-glucosyltransferase; UGT71D1, UDP-glycosyltransferase
1232 71D1.

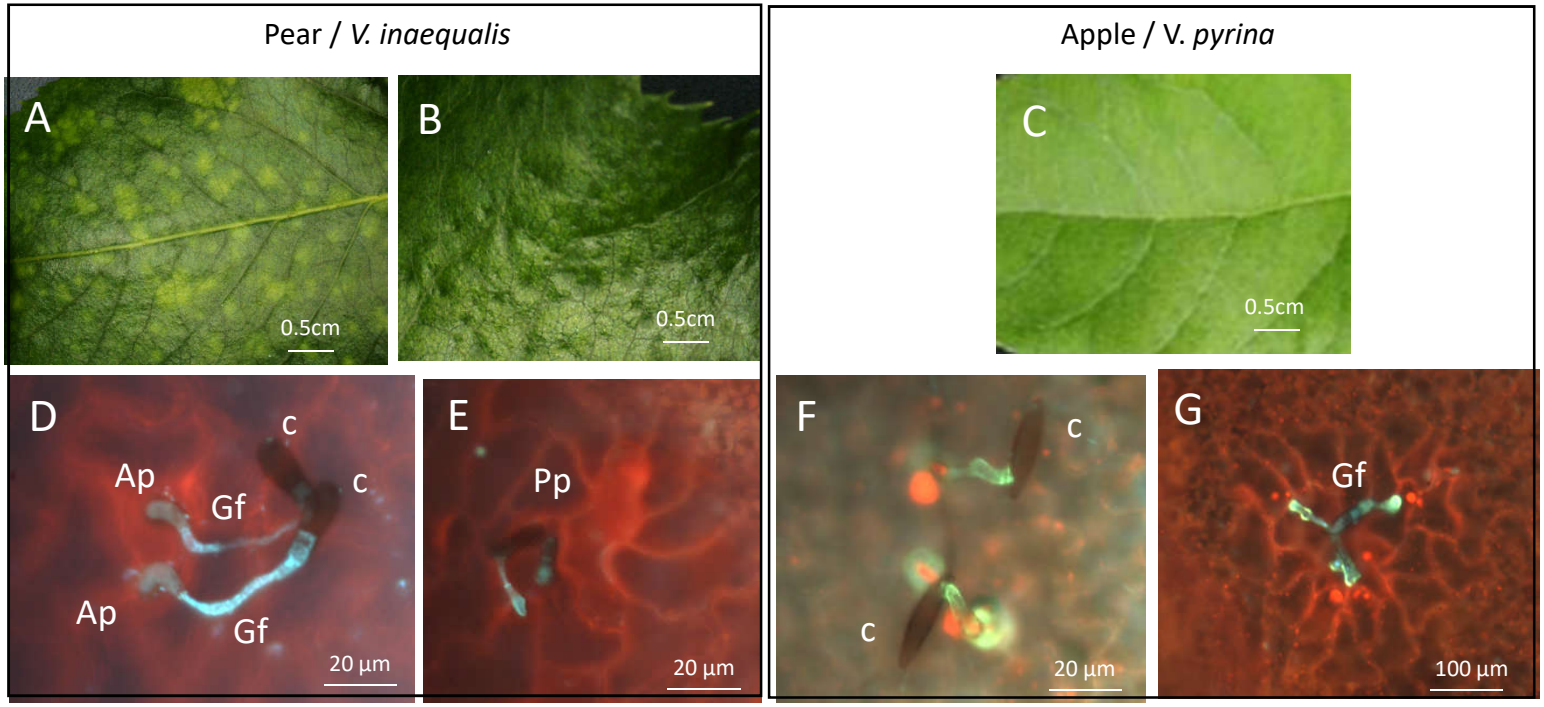
Pear / V. inaequalis
Apple / V. pyrina
A
0.5cm
B
0.5cm
C
0.5cm
D
c
Ap
Gf
c
Ap
Gf
20 µm
E
Pp
20 µm
F
c
c
20 µm
G
Gf
100 µm

Fig. 1

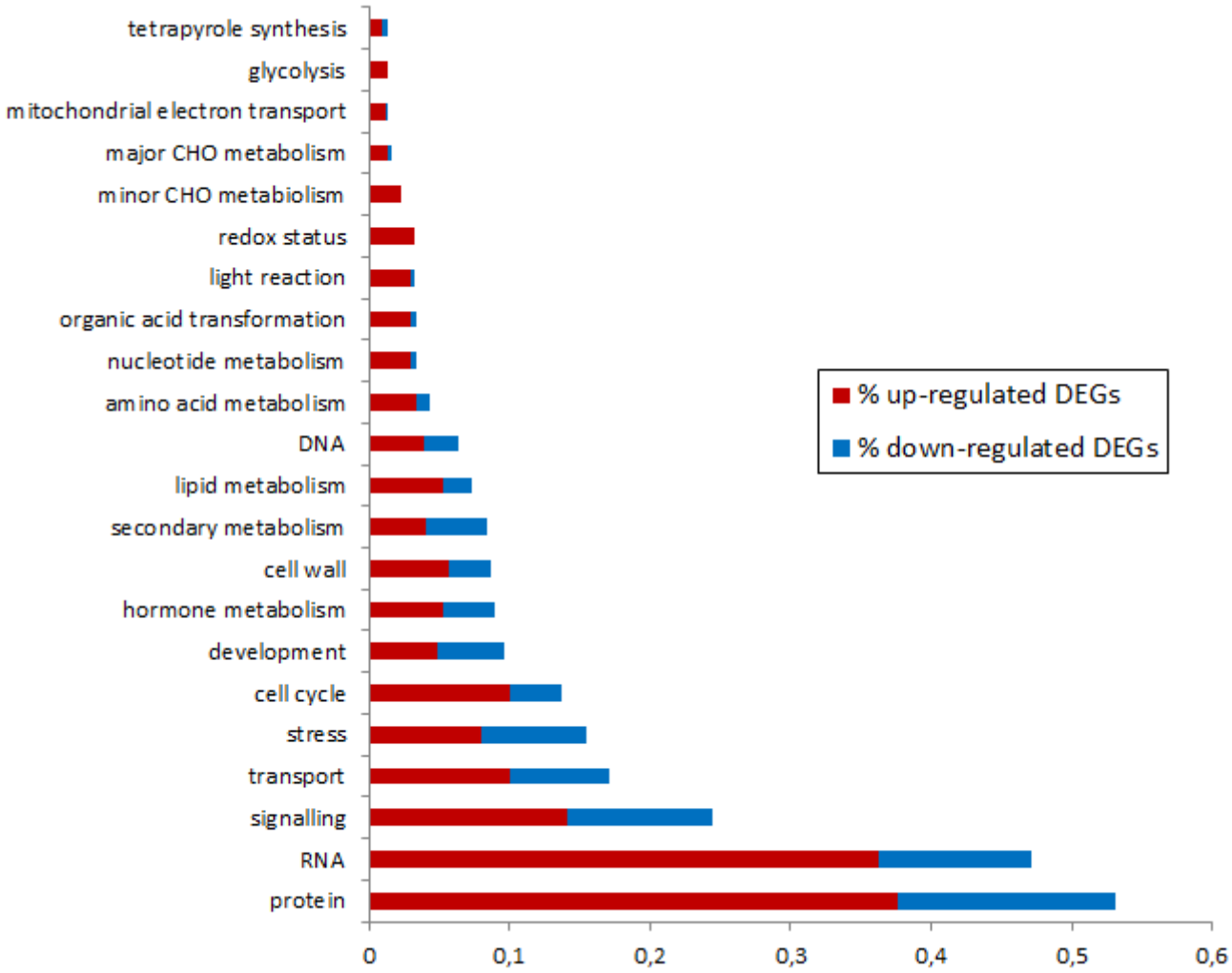
tetrapyrole synthesis
glycolysis
mitochondrial electron transport
major CHO metabolism
minor CHO metabiolism
redox status
light reaction
organic acid transformation
nucleotide metabolism
amino acid metabolism
DNA
lipid metabolism
secondary metabolism
cell wall
hormone metabolism
development
cell cycle
stress
transport
signalling
RNA
protein
0
0,1
0,2
0,3
0,4
0,5
0,6
% up-regulated DEGs
% down-regulated DEGs

Fig. 2

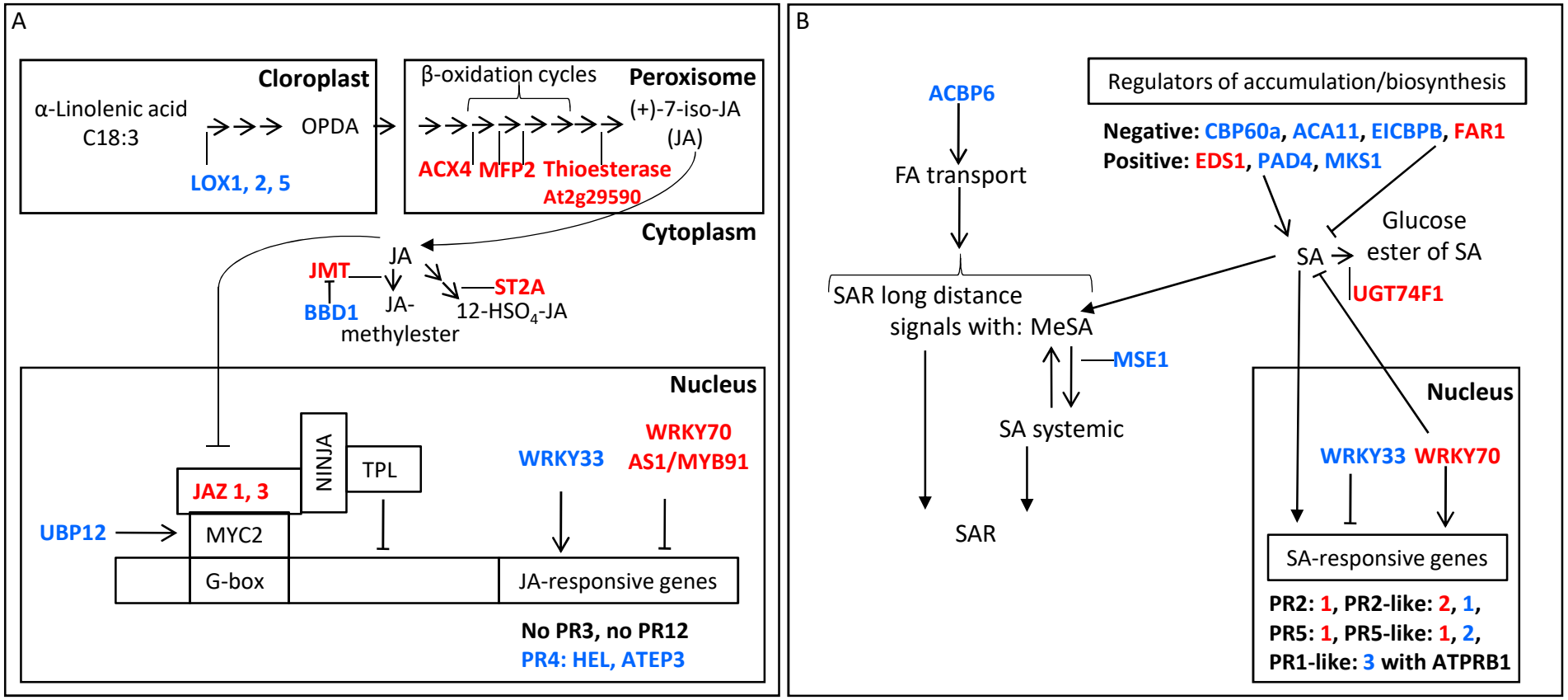
A
Cloroplast
α-Linolenic acid
C18:3
OPDA
LOX1, 2, 5
β-oxidation cycles
Peroxisome
(+)-7-iso-JA
(JA)
ACX4
MFP2
Thioesterase
At2g29590
Cytoplasm
JA
JMT
BBD1
JA-
methylester
ST2A
12-HSO4-JA
Nucleus
NINJA
TPL
JAZ 1, 3
UBP12
MYC2
G-box
WRKY33
WRKY70
AS1/MYB91
JA-responsive genes
No PR3, no PR12
PR4: HEL, ATEP3
B
ACBP6
FA transport
SAR long distance
signals with: MeSA
MSE1
SA systemic
SAR
Regulators of accumulation/biosynthesis
Negative: CBP60a, ACA11, EICBPB, FAR1
Positive: EDS1, PAD4, MKS1
SA
Glucose
ester of SA
UGT74F1
Nucleus
WRKY33
WRKY70
SA-responsive genes
PR2: 1, PR2-like: 2, 1,
PR5: 1, PR5-like: 1, 2,
PR1-like: 3 with ATPRB1

Fig. 3

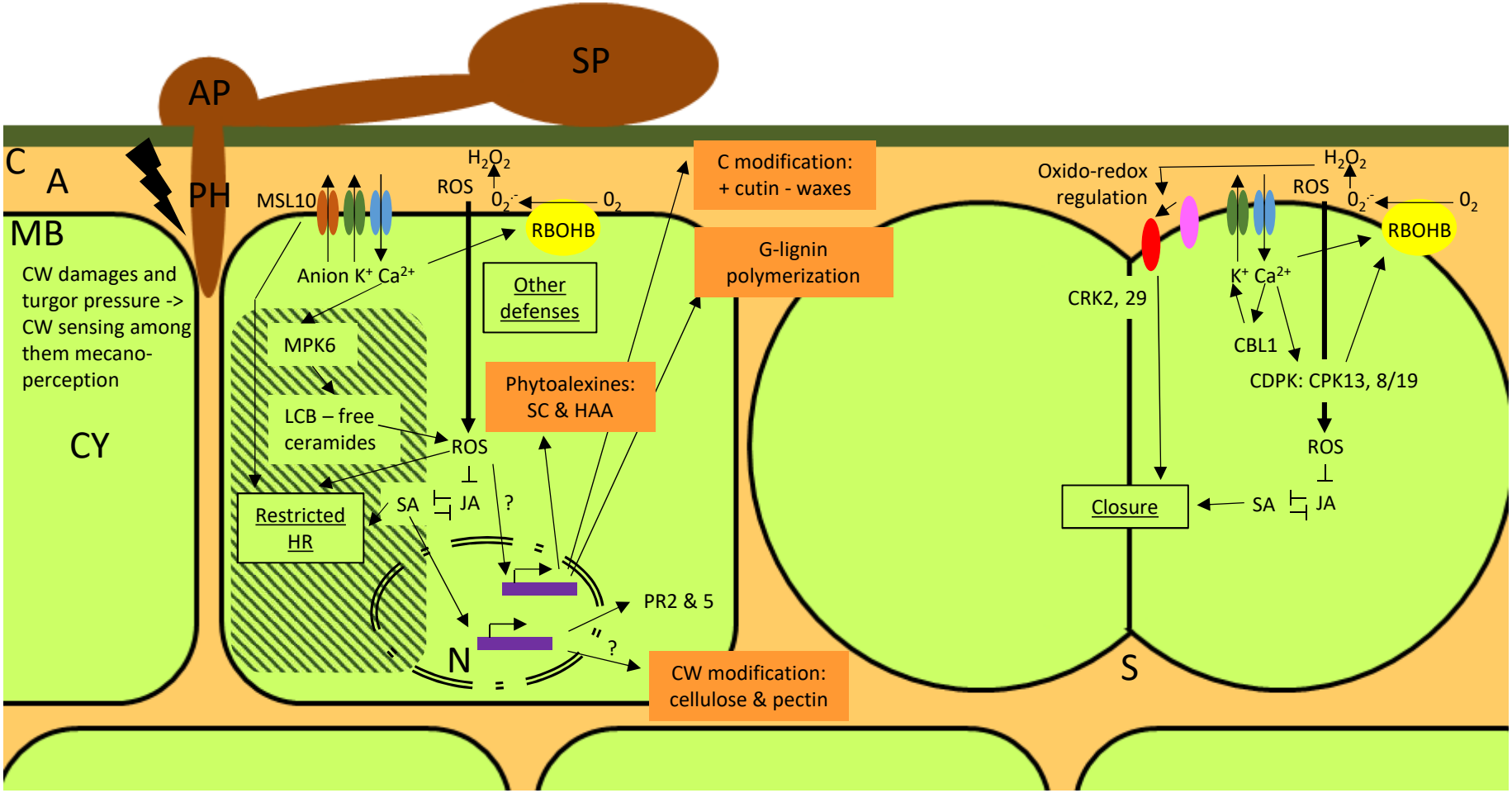
SP
AP
C
A
PH
MB
CW damages and turgor pressure -> CW sensing among them mecano-perception
CY
MSL10
Anion K+ Ca2+
MPK6
LCB – free ceramides
Restricted HR
ROS
H2O2
O2.-
O2
RBOHB
Other defenses
Phytoalexines: SC & HAA
SA
JA
?
N
PR2 & 5
C modification: + cutin - waxes
G-lignin polymerization
CW modification: cellulose & pectin
Oxido-redox regulation
CRK2, 29
K+ Ca2+
CBL1
CDPK: CPK13, 8/19
Closure
S

Fig. 4

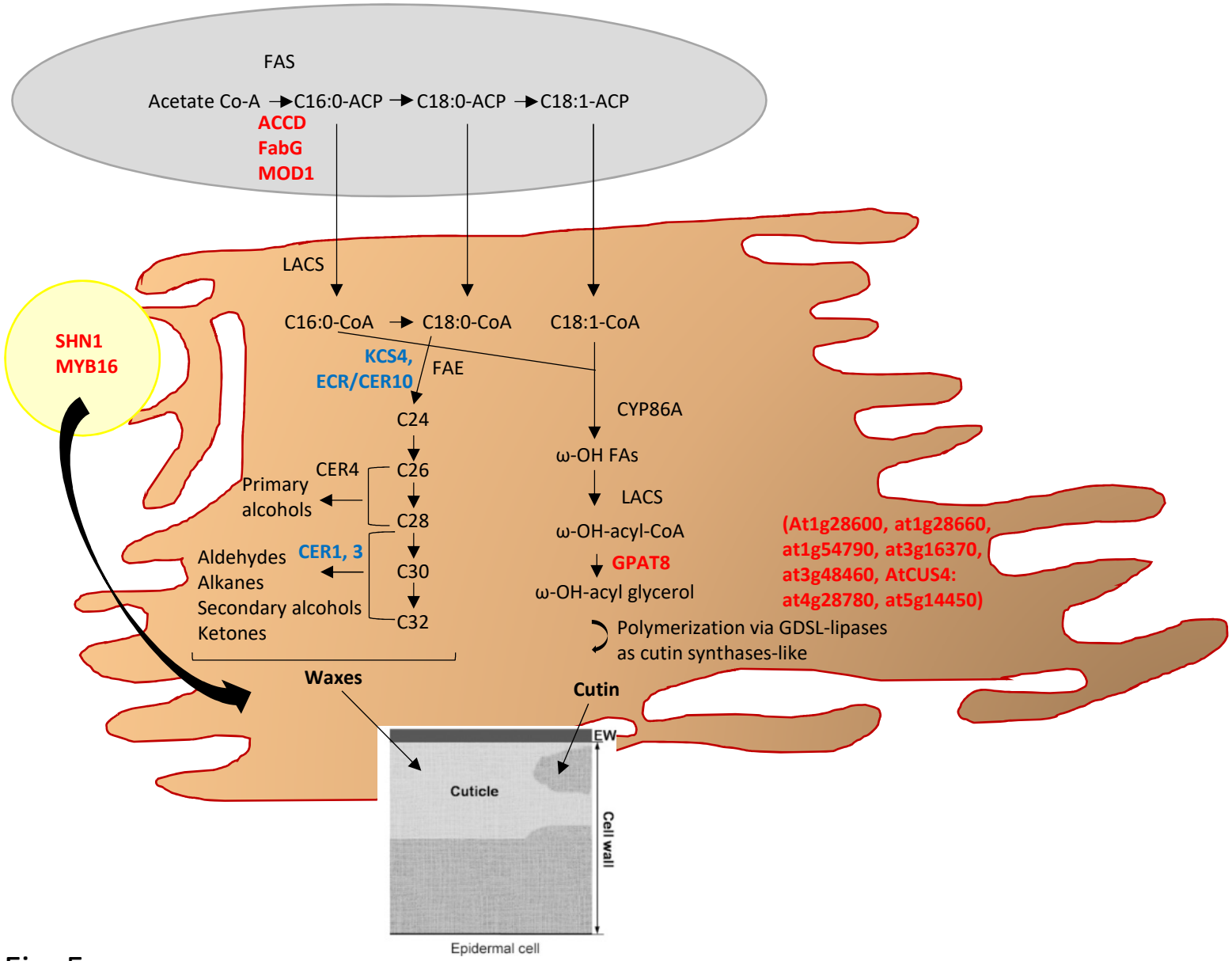
FAS
Acetate Co-A
C16:0-ACP
C18:0-ACP
C18:1-ACP
ACCD
FabG
MOD1
LACS
C16:0-CoA
C18:0-CoA
C18:1-CoA
KCS4,
ECR/CER10
FAE
C24
C26
C28
C30
C32
CER4
Primary alcohols
CER1, 3
Aldehydes
Alkanes
Secondary alcohols
Ketones
Waxes
CYP86A
ω-OH FAs
LACS
ω-OH-acyl-CoA
GPAT8
ω-OH-acyl glycerol
(At1g28600, at1g28660, at1g54790, at3g16370, at3g48460, AtCUS4: at4g28780, at5g14450)
Polymerization via GDSL-lipases as cutin synthases-like
Cutin
SHN1
MYB16
EW
Cuticle
Cell wall
Epidermal cell

Fig. 5

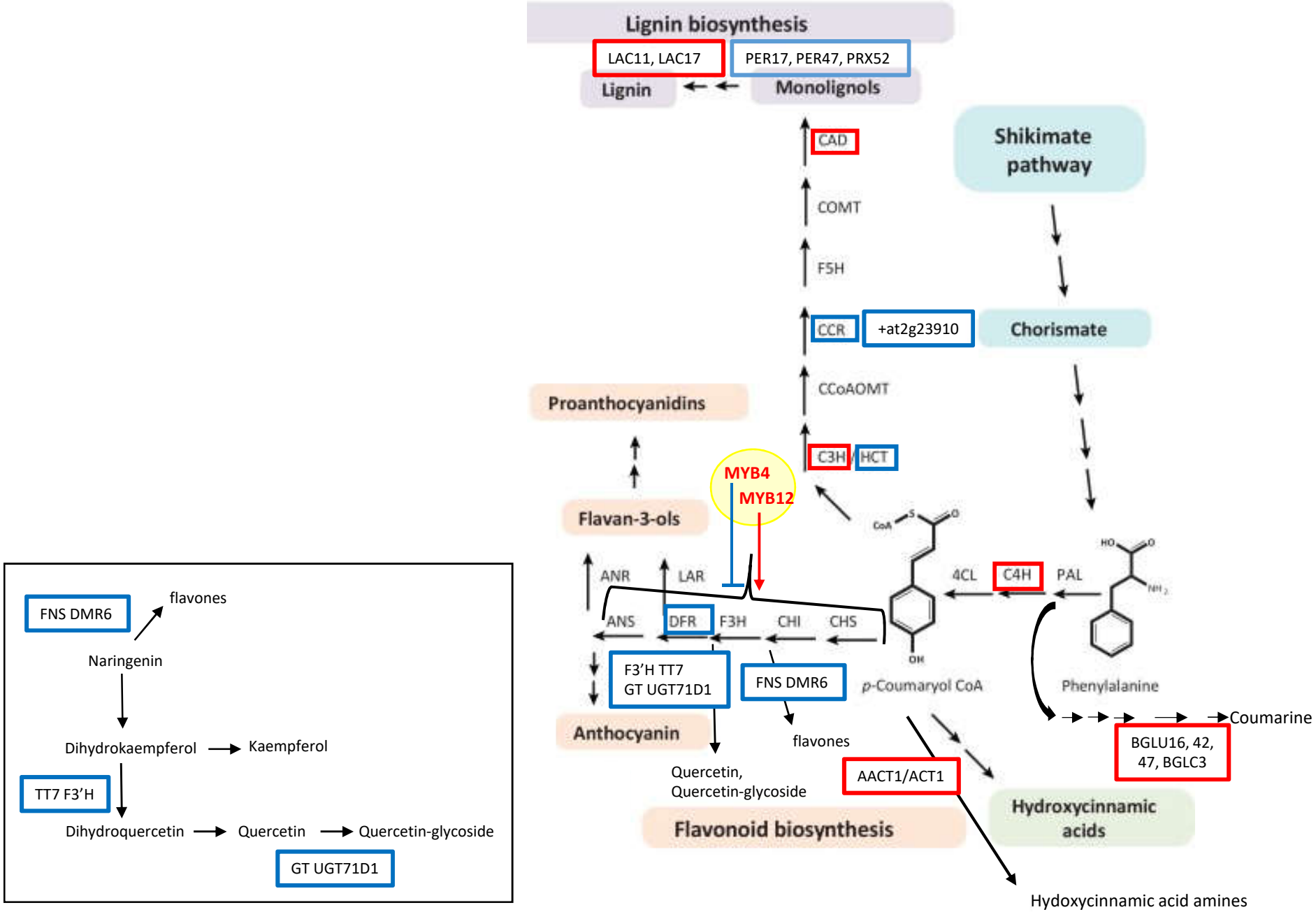
Lignin biosynthesis
LAC11, LAC17
PER17, PER47, PRX52
Lignin
Monolignols
CAD
COMT
F5H
CCR
+at2g23910
CCoAOMT
C3H
HCT
Shikimate pathway
Chorismate
Proanthocyanidins
MYB4
MYB12
Flavan-3-ols
ANR
LAR
ANS
DFR
F3H
CHI
CHS
4CL
C4H
PAL
F3'H TT7
GT UGT71D1
FNS DMR6
p-Coumaryol CoA
Phenylalanine
Coumarine
BGLU16, 42, 47, BGLC3
Anthocyanin
flavones
Quercetin, Quercetin-glycoside
AACT1/ACT1
Hydroxycinnamic acids
Flavonoid biosynthesis
Hydoxycinnamic acid amines
FNS DMR6
flavones
Naringenin
Dihydrokaempferol
Kaempferol
TT7 F3'H
Dihydroquercetin
Quercetin
Quercetin-glycoside
GT UGT71D1

Fig. 6