



**HAL**  
open science

# FINE-TUNING AND REMODELLING OF PECTINS PLAY A KEY ROLE IN THE MAINTENANCE OF CELL ADHESION

Cyril Grandjean, Aline Voxeur, Salem Chabout, François Jobert, Laurent Gutierrez, Jérôme Pelloux, Gregory Mouille, Sophie Bouton

► **To cite this version:**

Cyril Grandjean, Aline Voxeur, Salem Chabout, François Jobert, Laurent Gutierrez, et al.. FINE-TUNING AND REMODELLING OF PECTINS PLAY A KEY ROLE IN THE MAINTENANCE OF CELL ADHESION. 2024. hal-04444310

**HAL Id: hal-04444310**

**<https://hal.science/hal-04444310>**

Preprint submitted on 7 Feb 2024

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

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

1 **FINE-TUNING AND REMODELLING OF PECTINS PLAY A KEY ROLE IN THE**  
2 **MAINTENANCE OF CELL ADHESION**

3 **Cyril Grandjean<sup>1,2</sup>, Aline Voxeur<sup>2</sup>, Salem Chabout<sup>2</sup>, François Jobert<sup>1,3</sup>, Laurent**  
4 **Gutierrez<sup>3</sup>, Jérôme Pelloux<sup>1</sup>, Gregory Mouille<sup>2</sup> et Sophie Bouton<sup>1</sup>.**

5  
6 <sup>1</sup> **UMR INRAE 1158 BioEcoAgro – BIOPI Biologie des Plantes et Innovation, Université de**  
7 **Picardie Jules Verne, 33 Rue St Leu, 80039 Amiens, France.**

8 <sup>2</sup> **Université Paris-Saclay, INRAE, AgroParisTech, Institut Jean-Pierre Bourgin (IJPB), 78000**  
9 **Versailles, France.**

10 <sup>3</sup> **Centre de Ressources Régionales en Biologie Moléculaire (CRRBM), Université de Picardie Jules**  
11 **Verne, 33 Rue St Leu, 80039 Amiens, France.**

12

13 **ABSTRACT**

14 Plant cell adhesion is essential for development and stress response, mediated by pectin-rich  
15 middle lamella deposition between cell walls. However, the precise control mechanism of cell  
16 adhesion remains unclear. The *qua2-1* and *esmd1-1* mutants provide a better understanding of  
17 this process and suggest a signaling pathway triggering the loss and restoration of adhesion via  
18 cell wall modifications. This study attempts to characterize the potential regulatory role of  
19 endogenous oligogalacturonides (OGs) and pectin modifications in the control of cell adhesion  
20 in Arabidopsis.

21 From dark-grown hypocotyls, our extraction revealed seven distinct endogenous OGs with  
22 varying polymerization and modifications. Abundance variations of OGs were observed among  
23 wild type, *qua2-1*, *esmd1-1*, and *qua2-1/esmd1-1* mutants. The structure of homogalacturonans  
24 was analyzed by enzymatic fingerprint, in order to identify changes in esterification patterns.  
25 Expression analysis of pectin-modifying enzymes showed significant variations in *PME*, *PMEI*,  
26 and *PAE* genes. Gene expressions correlate with homogalacturonans modifications and cell  
27 adhesion phenotypes.

28 This study enhances our understanding of a feedback loop between the endogenous OGs,  
29 homogalacturonans esterification fine tuning, and pectin remodeling enzymes expression in  
30 controlling cell adhesion.

31

32

## 33 INTRODUCTION

34 Cell adhesion plays a crucial role in plant development and in response to stress (Somerville et  
35 al., 2004). The adhesion between adjacent plant cells is facilitated by the deposition of a pectin-  
36 rich middle lamella (Daher and Braybrook, 2015). The middle lamella primarily consists of  
37 homogalacturonans (HGs), and the degree of methyl and O acetyl esterification of HGs is  
38 regulated by cell wall localized proteins such as pectin methylesterases (PMEs), pectin  
39 methylesterase inhibitors (PMEIs), and pectin acetylerases (PAEs) encoded by large  
40 multigenic families (Pelloux et al., 2007; Philippe et al., 2017). While some molecular  
41 regulators involved in cell adhesion have been identified (Atakhani et al., 2022), the mechanism  
42 by which plants control and maintain cell adhesion in response to changes in cell wall chemistry  
43 remains poorly understood. Studies on the *Arabidopsis quasimodo2 (qua2)* mutant, which is  
44 affected in a pectin methyltransferase gene and exhibits cellular adhesion defects and reduced  
45 amount of HG (Du et al., 2020; Mouille et al., 2007), provided insights into this process.  
46 Interestingly, the *esmeralda1 (esmd1-1)* mutant, carrying a point mutation in a putative O-  
47 fucosyltransferase, rescues *quasimodo2-1 (qua2-1)* cell adhesion phenotypes without restoring  
48 HG levels (Stéphane Verger et al., 2016). Based on these findings, it has been proposed that  
49 *qua2-1* triggers a signal leading to the loss of cell adhesion, and *esmd1* could affect this pathway  
50 and restore cell adhesion through cell wall modifications (Stéphane Verger et al., 2016).  
51 Identifying the potential signal modified by the *ESMERALDA1* mutation in *qua2-1* and  
52 uncovering new actors in this signal transduction pathway will enhance our understanding of  
53 the control of cell adhesion in plants.

54

55

## 56 RESULTS AND DISCUSSION

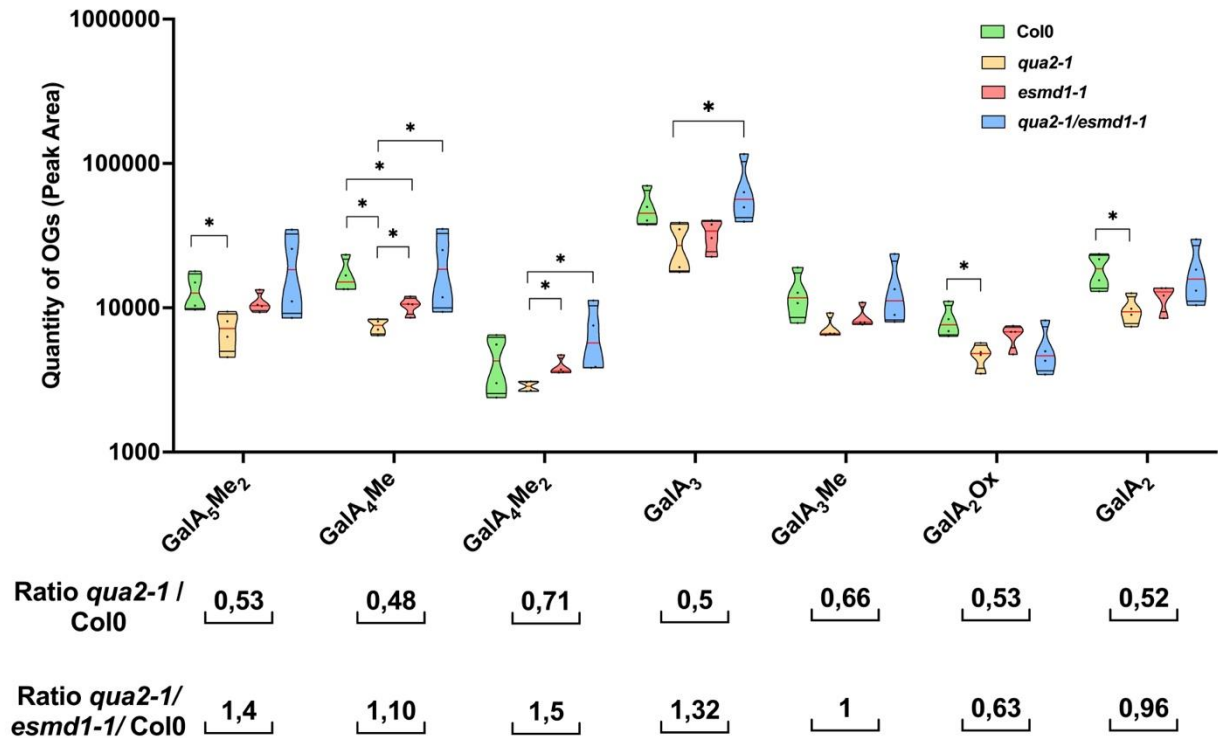
### 57 Are endogenous oligogalacturonides (OGs) involved in cell adhesion?

58 Oligogalacturonides (OGs) are oligomers released from plant cell walls upon partial  
59 degradation of homogalacturonans and can activate various signaling pathways when perceived  
60 by cell wall receptors (Wolf, 2022). Consequently, OGs are considered as endogenous elicitors  
61 that activate plant immunity and control developmental processes (Ferrari et al., 2013; Lin et  
62 al., 2022). OGs are the most likely candidates for mediating different signals that could control  
63 cell adhesion in *Arabidopsis* (Stéphane Verger et al., 2016). Therefore, understanding the  
64 diversity and roles of endogenous OGs in cell adhesion is essential. In this study, we extracted

65 endogenous OGs from dark-grown hypocotyls and analyzed them by high-performance size  
66 exclusion chromatography (HP-SEC) coupled with mass spectrometry (Voxeur et al., 2019).  
67 We identified seven different OGs with degrees of polymerization (DP) ranging from 2 to 5,  
68 decorated with various methylation and oxidation statuses in the wild type Col-0 and in *qua2-*  
69 *1*, *esmd1-1*, and *qua2-1/esmd1-1* mutants (Figure 1). Although the OG quantities differed  
70 among the genotypes, their identities were consistent. Some of these OGs have previously been  
71 characterized as potential elicitors, such as the trimer of galacturonic acid (GalA), which  
72 triggers a dark-grown signal (Sinclair et al., 2017), and GalA<sub>2</sub>Ox, an OG that accumulates in  
73 the later stages of infection by *Botrytis cinerea* (Voxeur et al., 2019). Notably, all the extracted  
74 OGs were less abundant in *qua2-1*, with a 50% to 30% decrease compared to the wild type,  
75 depending on the OG considered. However, the average quantity of most OGs was partially or  
76 fully restored by the *esmd1-1* mutation, and in some cases, the double mutant exhibited even  
77 higher levels than the wild type. Statistically, only GalA<sub>4</sub>Me and GalA<sub>4</sub>Me<sub>2</sub> were significantly  
78 more abundant in *esmd1-1* and the double mutant compared to *qua2-1* (Figure 1). The  
79 dispersion of values was very low for both mutants. Conversely, there was a wide dispersion of  
80 values in the double mutant, as in the wild type, suggesting a recovery of the ability to respond  
81 to a stress. As there is no difference in the identity of endogenous OGs, our results suggest there  
82 is maybe an involvement of overall amount of these OGs in the recovery of *qua2-1/esmd1-1*  
83 cell adhesion.

84 As pectin digestibility is directly influenced by the esterified pattern and/or the action of various  
85 polygalacturonases, pectin and pectate lyases, we further investigated the fine structure of  
86 homogalacturonans in the different genetic backgrounds.

87



88

89 **Figure 1 : Endogenous OGs.**

90 Truncated violin plot of peak area of endogenous OGs of 150 4-day-old dark grown dissected  
 91 hypocotyls of Col-0, *qua2-1*, *qua2-1/esmd1-1* and *esmd1-1*. Oligogalacturonides (OGs) are  
 92 named as follow: GalA<sub>x</sub>Me<sub>y</sub> where the numbers x and y in subscripts indicates the degree of  
 93 polymerization and the number of methylations respectively. GalA: galacturonic acid; Me:  
 94 methyl ester group Ox: oxidation. Red line represents the median, black line the quartiles, black  
 95 dot represents biological replicate (n = 4 biological replicates per genotype). \*, P < 0.05,  
 96 Mann & Whitney test.

97

98 **Does the *esmeralda1* mutation alters Homogalacturonans pattern?**

99 To explore the esterification pattern of HGs in different genotypes, we enzymatically digested  
 100 the cell walls of dark-grown seedlings with a commercial endo-polygalacturonase from  
 101 *Aspergillus aculeatus*. The released fragments were then analyzed by high-performance size  
 102 exclusion chromatography (HP-SEC) coupled with mass spectrometry. We identified 92 HG  
 103 fragments, including monomers with distinct degrees of methyl and O-acetyl esterification,  
 104 some of which were oxidized. Our results revealed that, independently of the total amount of  
 105 GalA hydrolyzed by TFA, less HG fragments were released from the total dried cell wall  
 106 fractions in *qua2-1* compared to the wild type and *esmd1-1* (Supp. Data 1B). Conversely, this  
 107 digestible fraction of HG, relative to the amount of GalA hydrolyzed by TFA (Figure 2A),

108 remains unchanged in the mutants compared to the wild type, despite a slight decrease being  
109 observed. Since the *qua2-1/esmd1-1* double mutant shows a similar pectin defect to *qua2-1*  
110 (Verger et al., 2016), this suggests that the fraction of HGs sensitive to PGs digestion, relative  
111 to the total amount of HGs, is not the main factor responsible of the restoration of adhesion, as  
112 it is more or less maintained across the different genotypes.

113 The identified fragments were grouped into five different categories based on their substitution  
114 and oxidation state, reflecting the homogalacturonans pattern. In *qua2-1*, there was a relative  
115 decrease (50%) in the amount of methylesterified HGs released compared to the wild type, as  
116 previously reported (Du et al., 2020) and to *esmd1-1* (Figure 2B). In contrast, all other fragment  
117 categories were 1.5- to 4-fold more abundant in *qua2-1*. This fingerprint analysis revealed a  
118 previously unidentified pectin modification in *qua2-1*, indicating a decrease in methyl-  
119 esterification of HGs sensitive to polygalacturonase digestion. The proportions of HGs pattern  
120 in the *qua2-1/esmd1-1* double mutant suggest that *esmd1-1* restores the relative content of  
121 fragment categories, resulting in a proportion of pattern almost indistinguishable from the wild  
122 type (Figure 2B), without recovering the HGs quantity (Verger et al., 2016).

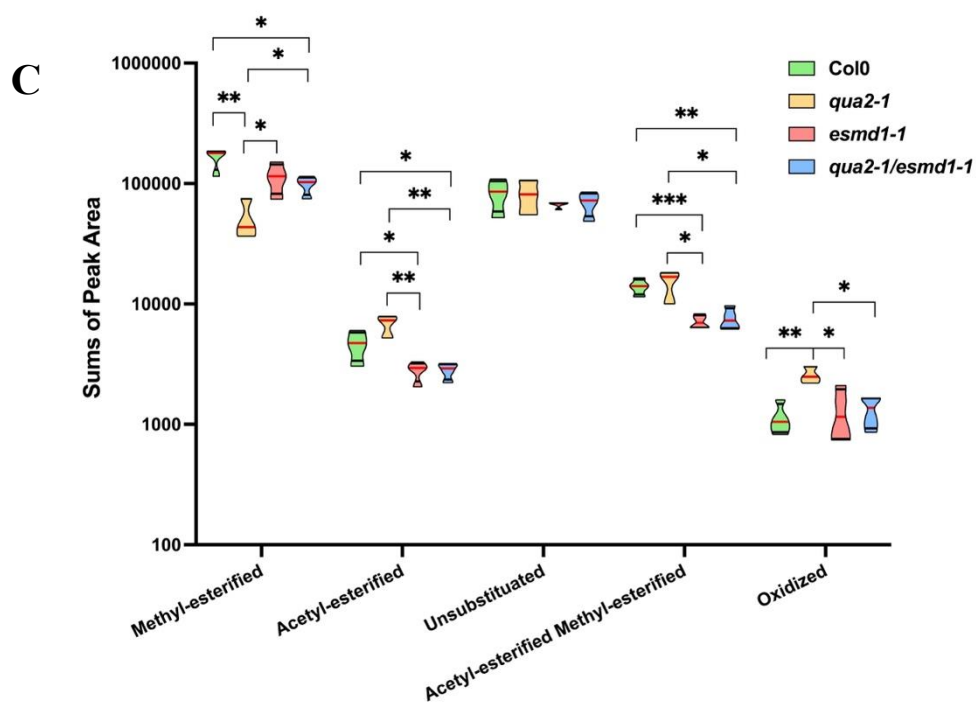
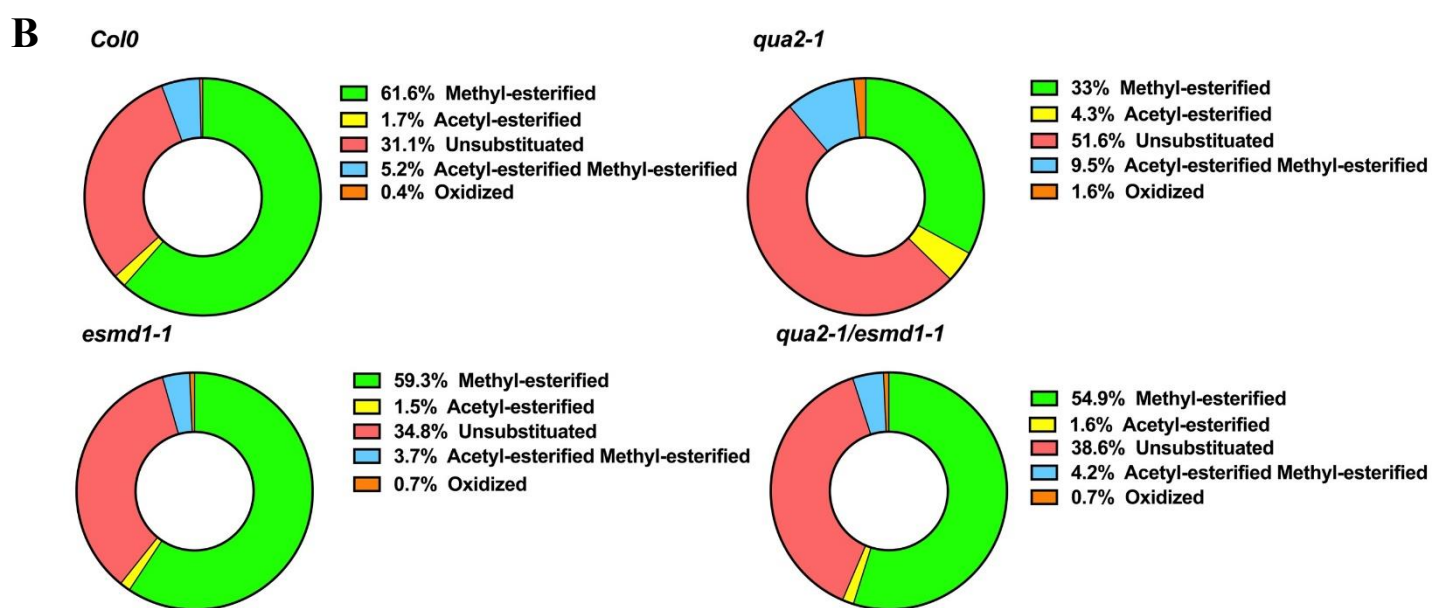
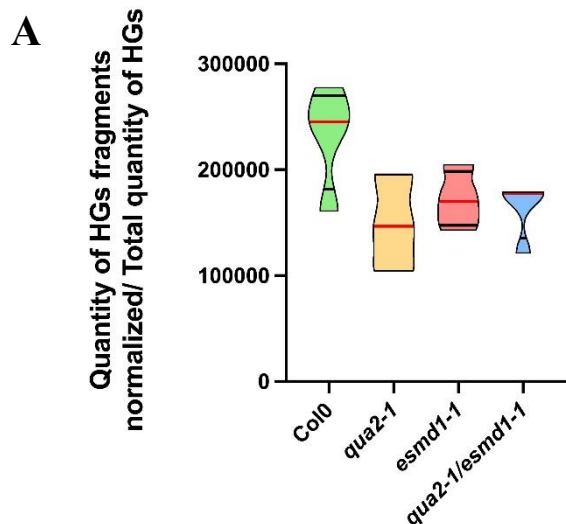
123 Further examination of the quantity of each of the five groups of released HG fragments  
124 revealed that the oxidized fragments were more abundant in *qua2-1* compared to the wild type  
125 (Figure 2C). In contrast, the group of methyl-esterified fragments were less abundant in *qua2-1*.  
126 Except for the oxidized and unsubstituted fragments, the quantities of the other three groups  
127 were altered in the *qua2-1/esmd1-1* double mutant. It is worth mentioning that the pattern of  
128 HG substitution was also altered in the single *esmd1-1* mutant, where the digestion of its cell  
129 wall released fewer acetyl and/or methyl-esterified fragments compared to the wild type (Figure  
130 2C).

131 Taking a closer look at the 49 significantly different HG fragments between the wild type and  
132 *qua2-1*, 30 were more abundant in the wild type, while 19 were more abundant in *qua2-1*  
133 (Supplementary data 2-A). In the wild type, the accumulated fragments exhibited moderate  
134 degree of methyl-esterification (2 to 5) with or without mono-acetyl-esterification, whereas in  
135 *qua2-1*, they were highly methyl-esterified (5 to 10), with or without acetyl-esterification (1-  
136 3). Thus, the observed modifications in the methyl-esterified pattern in *qua2-1* suggest a defect  
137 in PME expression or activity.

138 Analyzing the HG fragments released that were significantly different between *qua2-1* and the  
139 double mutant revealed 52 HGs fragments differentially accumulated between the two mutants

140 (Supp.data 2-C). Among them, 13 methyl-esterified (\*) and 2 acetyl-esterified (°) and 2 methyl-  
141 acetyl-esterified (●) fragments were specific to *esmd1-1*, while 23 methyl-esterified fragments  
142 exhibited a similar increase in the wild type compared to *qua2-1* (Supp. data 2-A). Furthermore,  
143 10 methyl- acetyl-esterified, 1 acetyl-esterified and 1 oxidized HG fragments that were  
144 significantly more abundant in *qua2-1* compared to the wild type (Supp. data 2-A) remained  
145 significantly more abundant in *qua2-1* compared to the double mutant (Supplementary data 2-  
146 C). Both *esmd1-1* HGs pattern features, contribute to the restoration of the proportion of methyl-  
147 esterified group in *qua2-1* background (Figure 2B). These results also indicate that the *esmd1-1*  
148 *I* mutation reduces the acetyl-esterified pattern in the *qua2-1* background, suggesting a higher  
149 activity of PAEs that restore the proportion of acetyl-esterified and methyl- acetyl-esterified  
150 patterns respectively (Figure 2B). The indirect regulation of HG methyl and O-acetyl  
151 esterification patterns by the *O*-fucosyltransferase ESMEALDA is further supported by the  
152 comparison between *esmd1-1* and the wild type (Supp.data 2-B).

153 Overall, since the fraction of HGs sensitive to PGs digestion, relative to the total amount of  
154 HGs, remains unchanged, *esmd1-1* in the *qua2-1* background restored the proportion of methyl  
155 and/or acetylerified pattern as well as the oxidized categories of HGs, through a specific  
156 modulation of HG pattern including some similarities and divergencies with wild-type. This  
157 may lead to a more favorable pattern for proper crosslinking in *qua2-1* and suggests that pectin  
158 pattern modulation allows for more cross-linkable microdomains interspaced within the  
159 homogalacturonan domain, enhancing adhesion restoration. It also contributes to the recovery  
160 of the overall amount of endogenous OGs (Figure 1).





162 **Figure 2 Fine structure of homogalacturonans.**

163 (A) Truncated violin plot of the quantity of HGs fragments & monomers released by PG  
164 *Aspergillus aculeatus* digestion of dried cell wall seedlings 4 days dark grown of Col-0, *qua2-*  
165 *1*, *qua2-1/esmd1-1* and *esmd1-1*. The quantity was calculated for 1mg of dried cell wall and  
166 normalized by the GalA released by TFA hydrolysis. The quantity of GalA hydrolyzed for 1mg  
167 of dried cell wall of same samples was analyzed by GC/MS (Supp. data 1A) and subtracted to  
168 the equivalent quantity of rhamnose to get the quantity of HGs. HGs fragments were quantified  
169 by LC/MS-MS according to Voxeur et al., 2019. Red line represents the median, black line the  
170 quartiles ( $n \leq 4$  biological replicates per genotype). \*,  $P < 0.05$ , \*\*,  $P < 0.001$ , t-Test.

171 (B) Relative content of HGs fragments family released by *Aspergillus aculeatus* PG digestion  
172 of dried cell wall of 4-days-old dark grown of seedlings Col-0, *qua2-1*, *qua2-1/esmd1-1* and  
173 *esmd1-1* ( $n \leq 4$  biological replicates per genotype).

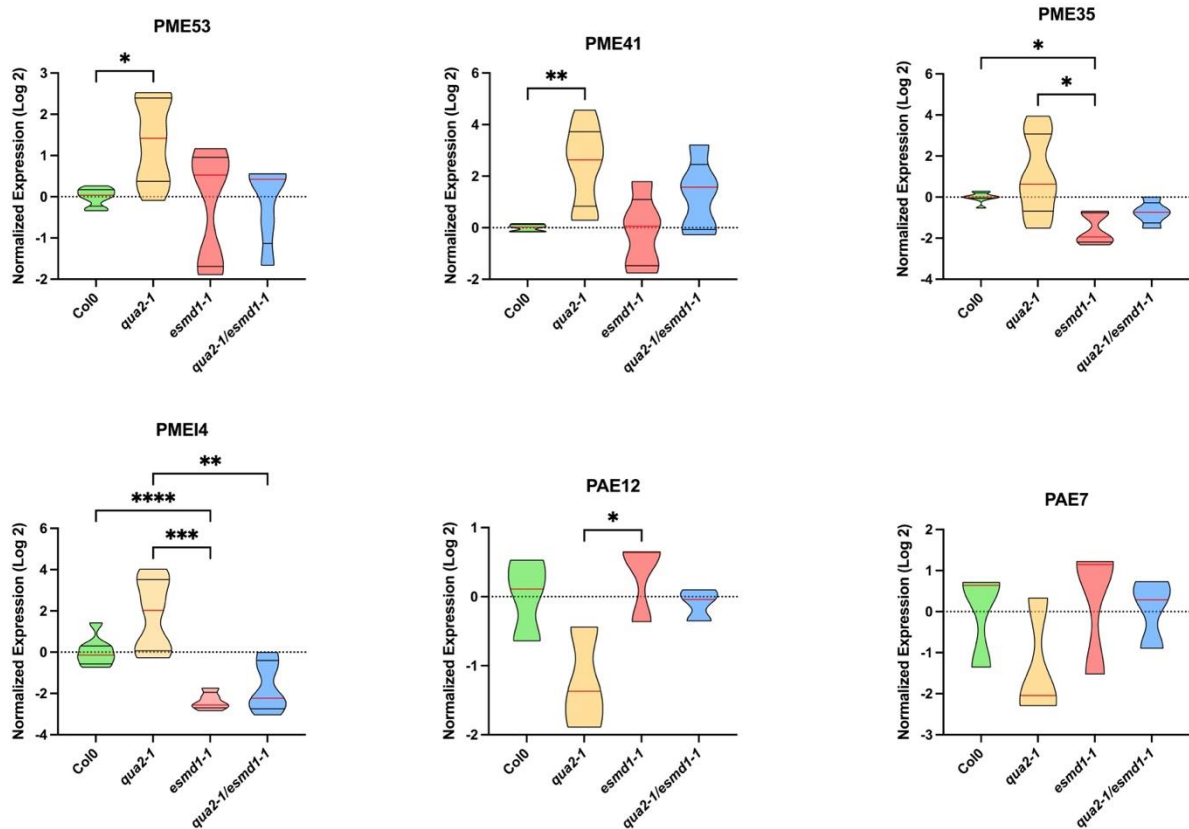
174 (C) Truncated violin plot of sums of the quantity of the classes of HGs fragments released by  
175 *Aspergillus aculeatus* PG digestion of dried cell wall of 4-days-old dark grown seedlings of  
176 Col-0, *qua2-1*, *qua2-1/esmd1-1* and *esmd1-1*. HGs fragments were quantified by LC/MS  
177 according to Voxeur et al., 2019. Red line represents the median, black line the quartiles, ( $n \leq$   
178  $4$  biological replicates per genotype). \*,  $P < 0.05$ , \*\*,  $P < 0.001$ , t-Test.

179

180 **Which factors are responsible for changing the pectin pattern of *qua2*, *qua2/esmd1* and**  
181 ***esmd1*?**

182 To gain a better understanding of how these pectin modifications occur in plants, we performed  
183 RT-qPCR analysis of the *PME*, *PMEI* and *PAE* multigenic families in four days-old dark-grown  
184 seedlings. Among these genes (respectively 66, 76 and 12), only six exhibited significant  
185 expression variations across the genotypes (Figure 3). In *qua2-1*, the expression of *PME53* and  
186 *PME41* were increased compared to the wild type. These overexpressions may explain the  
187 substantial decrease in methyl-esterified HGs and OGs in the *qua2-1* mutant, which probably  
188 leads to the generation of a pectin pattern that is more susceptible to polygalacturonase  
189 degradation. These changes in PME gene expression may contribute to the loss of cell adhesion  
190 in *quasimodo2*. On the other hand, the expression of *PME14* and *PME35* was repressed in the  
191 double mutant and *esmd1-1* compared to *qua2-1* (Figure 3). These underexpressions may play  
192 a role in the control of cell adhesion by the *esmeralda1* mutation, restoring the relative content  
193 of HG methyl-esterified pattern (Figure 2C and Supplementary data 1). Regarding *PAEs*, two  
194 genes exhibited similar expression variations. The expressions of *PAE7* and *PAE12* were  
195 repressed in *qua2-1* whereas in the double mutant, expressions are similar to the wild type  
196 (Figure 3). The restoration of *PAE* expression supports the rebalanced acetyl-esterified and/or  
197 methyl-acetyl-esterified patterns in the double mutant (Figure 2C and Supplementary data 2).  
198 Acetyl-esterification can prevent HG degradation by endo-polygalacturonases (Bonnin et al.,  
199 2003), which may explain the reduction in endogenous OGs in *quasimodo2* (Figure 1).

200 Moreover, the increase in O-acetyl and methyl-O-acetyl-esterified pattern in *qua2-1*, could also  
 201 compensate in some way the disturbance in the gestion of methyl esterified pattern described  
 202 above. The transcriptional regulation of *PAE7* and *PAE12* by *esmd1-1* mutation also possibly  
 203 contributes to the recovery of cell adhesion in *qua2-1*.



204

205 **Figure 3 : PME/PMEI and PAE expression.**

206 Truncated violin plot of Normalized expression levels (log<sub>2</sub>) of *PMEI4*, *PME*, 35, 41, 53 and  
 207 *PAE* 7 and 12, from 4-day-old, etiolated seedlings of Col, *qua2*, *esmd1* and *qua2/esmd1* by RT-  
 208 qPCR. *APT1* & *CLATHRIN* were amplified as reference genes. Normalized expression levels  
 209 (log<sub>2</sub>) were calculated according to a normalization factor obtained from the 2 reference genes  
 210 and calibrated to the expression of the wild type (Taylor et al., 2019). Red line represents the  
 211 median, black line the quartiles ( $n \geq 5$  biological replicates per genotype for *PME* and *PMEI*)  
 212 ( $n = 3$  biological replicates per genotype for *PAE*).

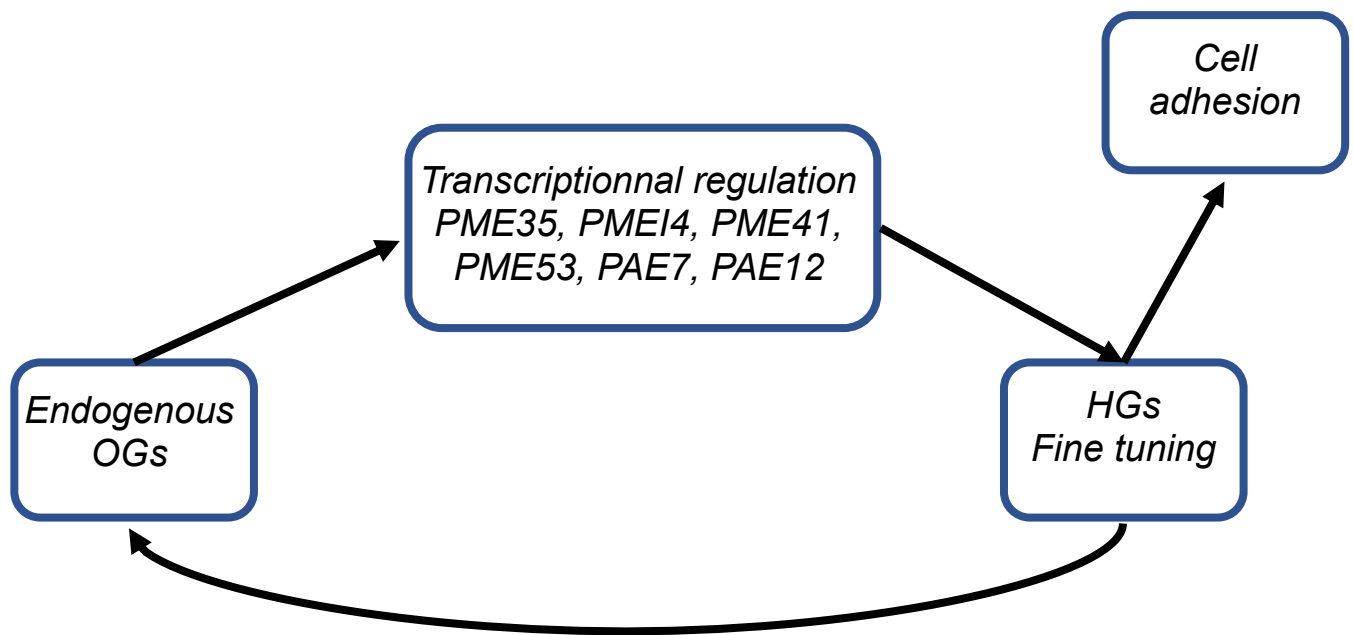
213 \*,  $P < 0.05$  and \*\*,  $P < 0.001$ , \*\*\*,  $P < 0.0001$ , \*\*\*\*,  $P < 0.00001$  Brown-Forsythe and Welch  
 214 ANOVA tests (Dunn's multiple comparisons test).

215

216 Overall, our findings provide evidence of the involvement of PMEs, PMEIs, and PAEs in  
 217 controlling cell adhesion. As these enzymes belong to large multigenic families, classical  
 218 reverse genetic approaches often result in compensatory expression, leading to weak  
 219 phenotypes. However, by focusing on a specific biological phenomenon such as cell adhesion

220 in our case, we were able to better characterize the role of pectin-modifying enzymes.  
221 Therefore, we propose that the restoration of cell adhesion in *quasimodo2* by the *esmeralda1-1*  
222 *1* mutation is a result of a combination of different mechanisms.

223 In summary, our findings suggest that the restoration of cell adhesion in *quasimodo2-1* by the  
224 *esmeralda1-1* mutation involves the production of endogenous OGs, the fine tuning of HGs  
225 pattern and transcriptional modulations of specific pectin-modifying enzymes suggesting a  
226 feedback loop regulation (Figure 4). Further investigation into the role of these enzymes and  
227 the signaling pathways involved in the process will contribute to a better understanding of the  
228 control of cell adhesion in plants.



229

230 **Figure 4 : Cell adhesion-pectins feedback loop**

231

## 232 MATERIALS AND METHODS

### 233 Plant material and growth conditions

234 *Arabidopsis thaliana* seedlings (150 seeds/genotype) were grown in the dark at 21 °C on solid  
235 medium (Agar 0.8 %, Duchefa Biochemie) supplemented with 0.328 g/L Ca(NO<sub>3</sub>)<sub>2</sub> at pH 5.7.  
236 To synchronize germination, the seeds were cold-treated for 48 hours. After exposure to light  
237 for 4 hours, the plates were wrapped in two layers of aluminum foil and cultivated for 92 hours.

238

### 239 **Endogenous OGs extraction**

240 Four biological replicates of 150 four-days-old dark-grown seedlings per genotype were  
241 dissected in a dark room to isolate the hypocotyls. The 150 hypocotyls were splitted into 3 tubes  
242 (50 in each) and directly frozen. Subsequently, 300 µl of 70 % ethanol were added to each tube.  
243 The 3 tubes were then incubated in a thermomixer at 80 °C for one hour. The ethanol-extracted  
244 OGs were pooled in the same tube and dried in a speed vacuum concentrator at room  
245 temperature. The resulting pellet was re-suspended in 100 µL of ultra-pure water, centrifuged,  
246 and the soluble fraction was transferred in a vial and 10 µl was injected for MS analysis.

### 247 **Enzymatic fingerprinting of pectins**

248 Four biological replicates of approximately 300 four-days-old dark-grown seedlings were  
249 harvested in a dark room and placed in tubes containing 1 ml of 96 % ethanol. The tubes were  
250 incubated in a thermomixer at 80 °C for one hour. The previous step with ethanol was repeated  
251 for 20 min. The ethanol was then replaced with 1 ml of acetone, and the tubes were incubated  
252 in a thermomixer at 25 °C for 20 minutes. This acetone wash step was repeated two more times.  
253 The hypocotyls were dried in a speed vacuum concentrator at room temperature overnight, and  
254 their dry weight was measured (approximately 1-2 mg per sample). The samples were digested  
255 with *Aspergillus aculeatus* endo-polygalacturonase M2 (Megazyme) in 50 mM ammonium  
256 acetate buffer pH 5 at 37 °C for 18 hours. After inactivating the enzyme by heating, the digested  
257 fractions were collected for MS analysis.

### 258 **OGs characterization and quantification by LC/HRMS analysis**

259 The endogenous OGs and the OGs released from the digestion were analyzed using High-  
260 performance size-exclusion chromatography (HP-SEC) coupled with MS. The  
261 chromatographic separation was performed on an ACQUITY UPLC Protein BEH SEC Column  
262 (125 Å, 1.7 µm, 4.6 mm x 300 mm) at a flow rate of 400 µl/min and a column oven temperature  
263 of 40 °C. The MS detection was performed in negative mode with the parameters described in  
264 the supplementary Materials and Methods.

265 **RNA extraction and RT-qPCR analysis** Five biological replicates of approximately 150  
266 seedlings grown in the dark for four days were harvested in a dark room. Total RNA was  
267 isolated using the RNeasy Plant Mini Kit (Qiagen) with on-column DNA digestion using Rnase-  
268 Free DNase (Qiagen). Two µg of total RNAs were used to synthesize cDNAs using RevertAid  
269 H minus reverse transcriptase, according to the manufacturer's instructions Thermofisher.

270 Transcript levels were assessed by quantitative RT-PCR using a Light Cycler® 480 System  
 271 (ROCHE), as previously described by (Gutierrez et al., 2009). The expression levels of the  
 272 target genes were normalized to the reference genes *CLATHRIN* (At5g46630) and *APT1*  
 273 (At1g27450), which were selected with GENORM (Vandesompele et al., 2002), according to  
 274 the method described in (Taylor et al., 2019). The primer sequences for RT-qPCR are provided  
 275 in the supplementary Materials and Methods.

276

Gene name	Forward primer sequence	Reverse primer sequence
<b><i>CLATHRIN</i></b> (At5g46630)	5'-GTTTGGGAGAAGAGCGGTTA-3'	5'-CTGATGTCACTGAACCTGAACTG-3'
<b><i>APT1</i></b> (At1g27450)	5'-GAGACATTTTGCCTGGGATT 3'	5'-ATTTTAAGTGGAAACA-3'
<b><i>PME53</i></b> (At5g19730)	5'-TTTGTATCTTGGGAGGGCATGGG-3'	5'-ACTGCCCATAGAACACCGTCATC-3'
<b><i>PME41</i></b> (At4g02330)	5'-TACATCGCCGAACTTCGTTGCC-3'	5'-GCTTCTCTGGTCCAGCGGTATTTC-3'
<b><i>PME35</i></b> (At3g59010)	5'-CCGCCATGGGAGATGGATTTCATAG-3'	5'-AGTTTGGTCCGGCACTGTTTAC-3'
<b><i>PMEi4</i></b> (At4g25250)	5'-AAACGGCATGCAACTCAACAAC-3'	5'-CGGACTTGATGGTGGAGGAATAGG-3'
<b><i>PAE7</i></b> (At4g19410)	5'-ATGCGAGCATTGTCACCGGTTC-3'	5'-CCGACTGCTTTCGCAATTCTCG-3'
<b><i>PAE12</i></b> (At3g05910)	5'-CTTGGTTTGGCGATGATTCT-3'	5'-ACCAAGTTGTGGCAGCTCTT-3'

277 Supplementary Material and Methods : Table 1 List of primers used for qPCR

278 Supplementary Material and Methods :

### 279 **Monosaccharides quantification by GC/MS**

280 The monosaccharides of the cell wall were analyzed with the protocol according to (Clement  
 281 et al., 2018). Four biological replicates of approximately 300 seedlings cultivated in the dark  
 282 over 4 days were harvested in a dark room and placed directly in the 96 ° ethanol for 1 h at 80  
 283 ° C to fix the sample. After removing the ethanol, the first step is repeated for 20 minutes. 1 ml

284 of acetone is added and the tubes are placed in a thermomixer for 20 min at 25 ° C, this step is  
285 repeated twice. The cell walls are then dried in a high vacuum concentrator at room temperature  
286 overnight. 1 mg dry cell wall previously weighed is resuspended in 400 L of TFA (2 M, freshly  
287 prepared), and incubate at 120 ° C for 1 h in a heat block in 1.5 mL screwcap tubes. Then  
288 samples were centrifugated for 10 min. The supernatant was transferred and dried in a speed  
289 vacuum concentrator at room temperature.

290 *Derivatization:* after adding 10 µl of 20 mg/ml methoxamine in pyridine to the samples, the  
291 reaction was performed for 90 min at 28 °C under continuous shaking in an Eppendorf  
292 thermomixer. 50 µl of N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) (Sigma  
293 M7891-10x1 mL) were then added and the reaction continued for 30 min at 37 °C. After  
294 cooling, 45 µl were transferred to an Agilent vial for injection.

295 *Data processing:* Raw Agilent datafiles were converted in NetCDF format and analyzed with  
296 AMDIS <http://chemdata.nist.gov/mass-spc/amdis/>. A home retention indices/ mass spectra  
297 library built from the NIST, Golm, <http://gmd.mpimp-golm.mpg.de/> and Fiehn databases and  
298 standard compounds were used for metabolites identification. Peak areas were also determined  
299 with the Targetlynx software (Waters) after conversion of the NetCDF file in masslynx format.  
300 AMDIS, Target Lynx in splitless and split 30 modes were compiled in one single Excel file for  
301 comparison. After blank mean subtraction, peak areas were normalized to Ribitol and dry  
302 weight.

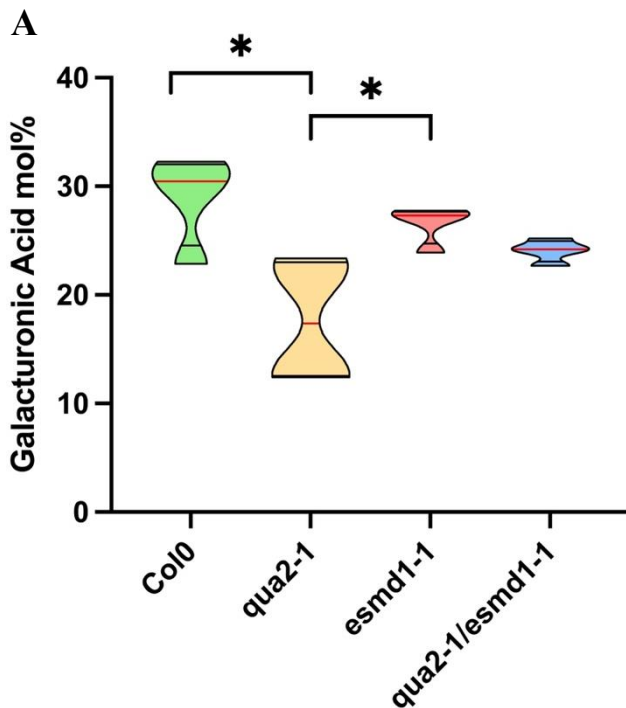
303 *Absolute quantification:* A response coefficient was determined for 4 ng each of a set of  
304 monosaccharides, respectively to the same amount of ribitol. This factor was used to give an  
305 estimation of the absolute concentration of the metabolite in what we may call a “one point  
306 calibration”.

307 High-performance size exclusion chromatography (HP-SEC) coupled with mass spectrometry  
308 analysisChromatographic separation was conducted using an ACQUITY UPLC Protein BEH  
309 SEC Column (125Å, 1.7 µm, 4.6 mm × 300 mm, Waters Corporation, Milford, MA, USA)  
310 coupled with a guard Column BEH SEC Column (125Å, 1.7 µm, 4.6 mm × 30 mm). Elution  
311 was performed with a flow rate of 400 µl/min at a column oven temperature of 40 °C using 50  
312 mM ammonium formate containing 0.1 % formic acid. The injection volume was set to 10 µl.  
313 ESI MS-detection was carried out in negative mode on a Bruker impact II QTOF with the  
314 following parameters: end plate offset set voltage of 500 V, capillary voltage of 4000 V,

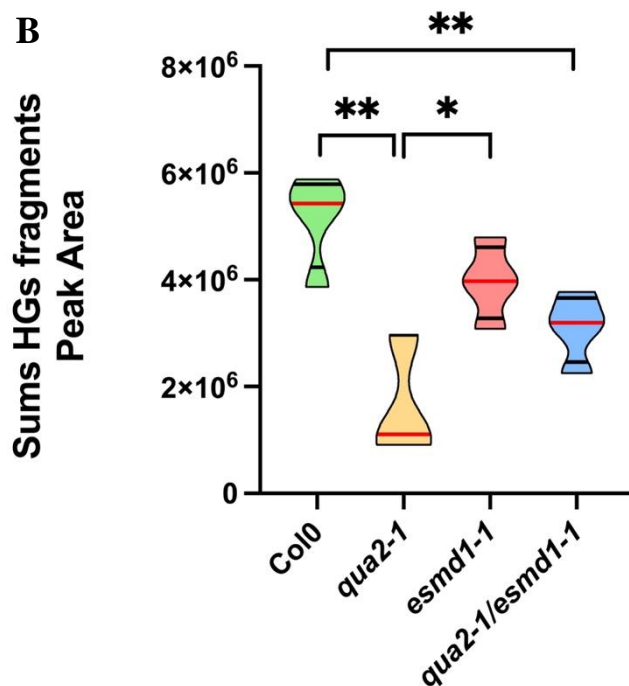
315 Nebulizer at 40 psi, dry gas flow at 8 l/min, and dry temperature at 180 °C. Data acquisition  
316 was performed using Compass 1.8 software (Bruker Daltonics). Data analysis was conducted  
317 using Mzmine 2.53 software, as described by Pluskal et al. (2010). The integration process  
318 involved the following steps: mass detection with a filter noise level set to 500, ADAP  
319 Chromatogram Builder using the parameters range of 6 - 9.60 min, minimum group size of scan  
320 of 10, group intensity threshold of 1500, minimum highest peak of 1000, and m/z tolerance of  
321 0.01 or 10 ppm. The chromatogram peaks were deconvoluted using baseline cut-off with the  
322 baseline level set at 300. Chromatograms were deisotoped with an m/z tolerance of 0.01 or 5  
323 ppm, retention time tolerance of 0.1, maximum charge of 2, and representative isotope as the  
324 most intense. Subsequently, peaks were aligned with an m/z tolerance of 0.01 or 5 ppm,  
325 retention time tolerance of 0.1, and equal weight for m/z and retention time (weight=1). Finally,  
326 a gap-filled peak finder was applied with an m/z tolerance of 0.01 or 5 ppm, retention time  
327 tolerance of 0.1, and intensity tolerance of 20 %. The peak areas were exported at the end.

328

329



330

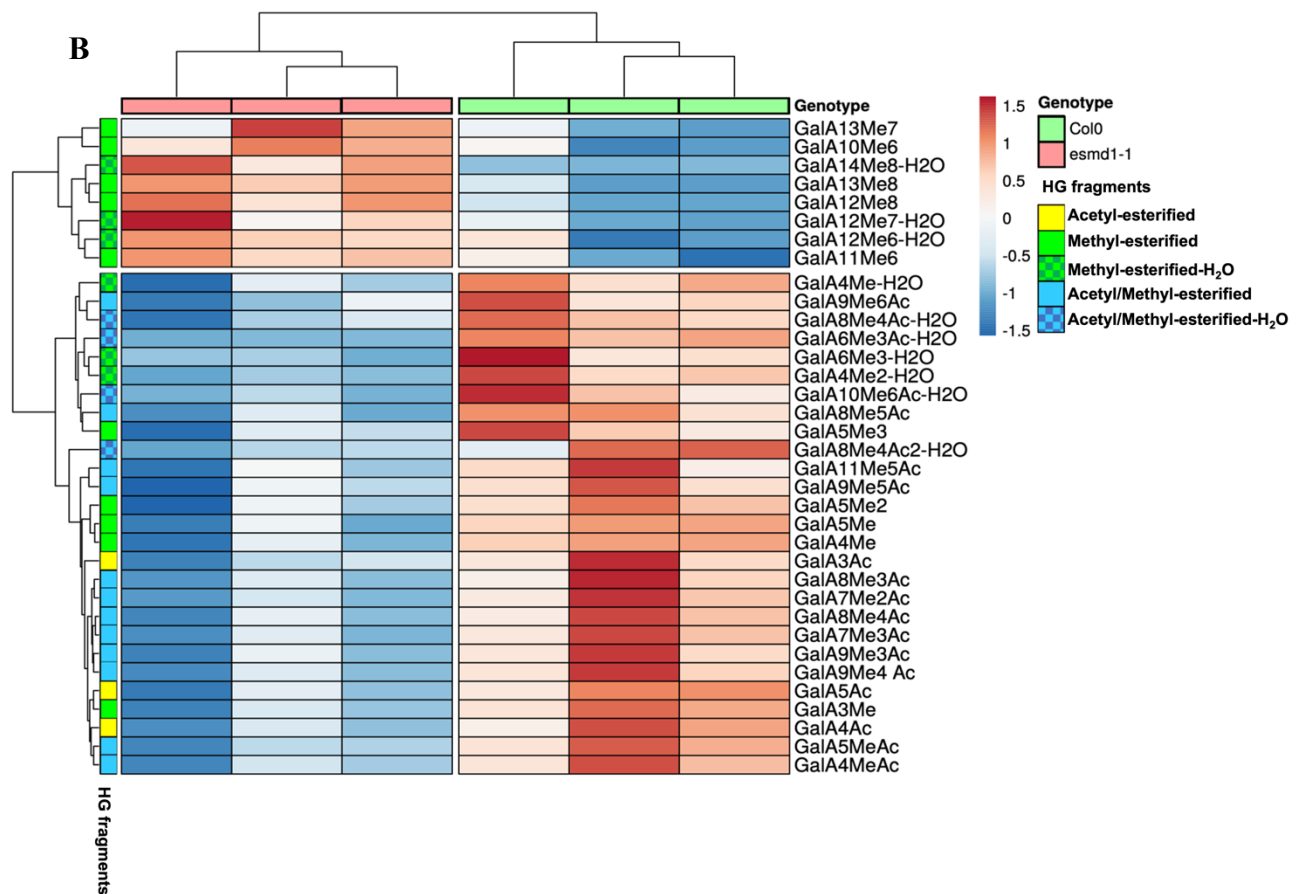
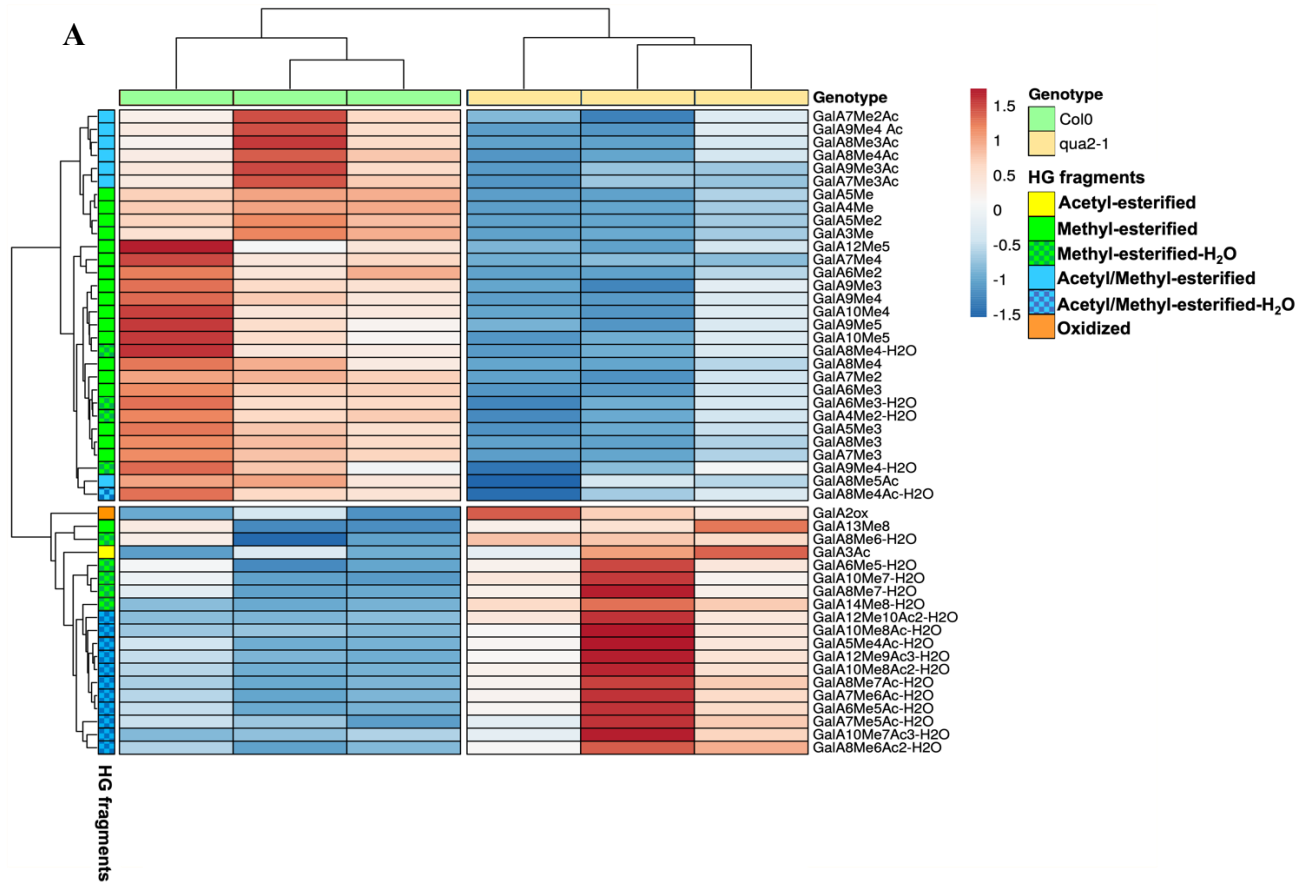


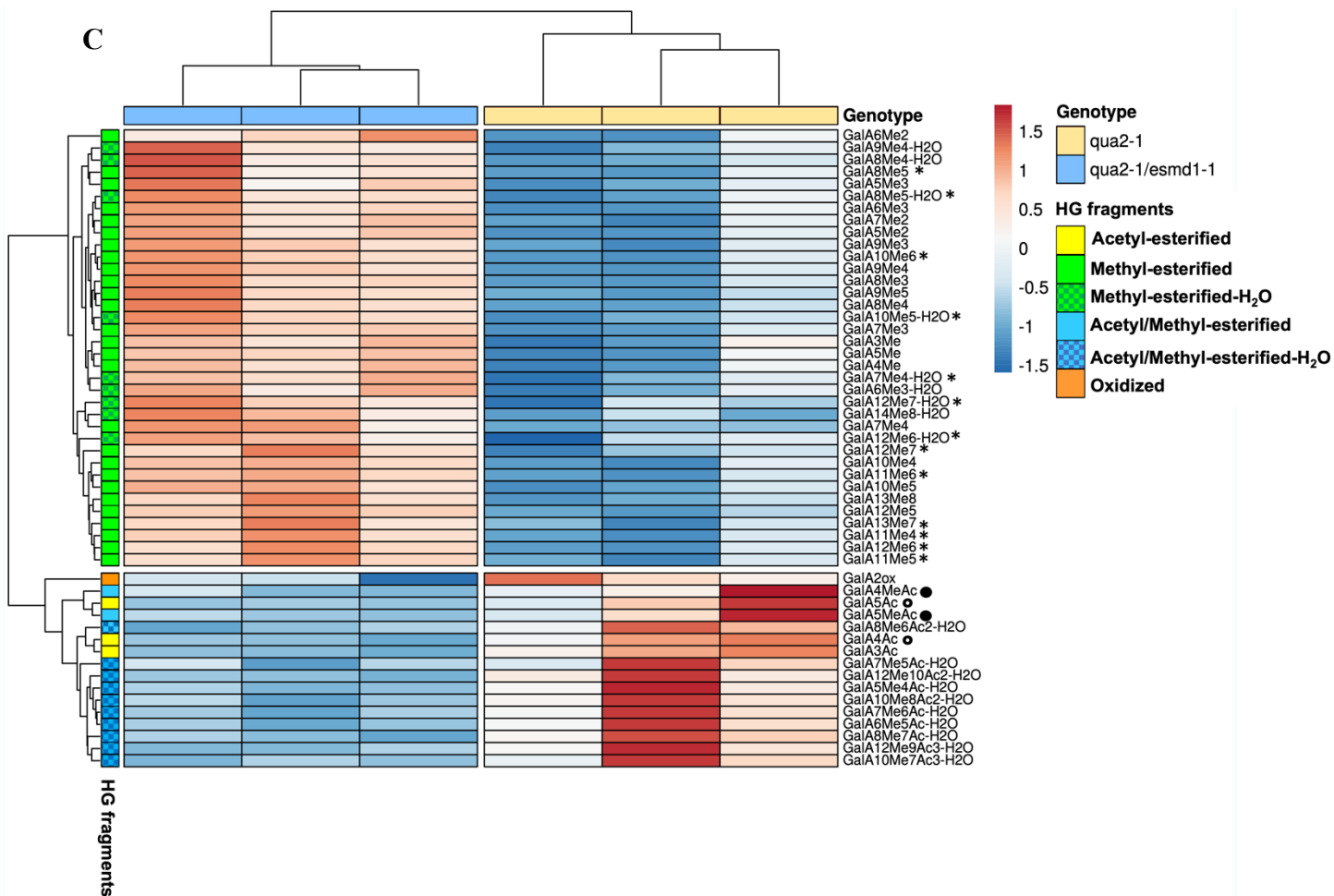
331

332 **Supplemental data 1 : Galacturonic acid content and sums of HGs fragments released**

333 (A) Truncated violin plot of Galacturonic acid content relative to the rhamnose content,  
334 represented in mol% and quantified by GC/MS on dried cell wall seedlings 4 days dark grown  
335 of Col-0, *qua2-1*, *qua2-1/esmd1-1* and *esmd1-1*. Red line represents the median, black line the  
336 quartiles ( $n = 4$  biological replicates per genotype). \*,  $P < 0.05$ , T-Test. (B) Truncated violin  
337 plot of the quantity of HGs fragments & monomers released by PG *Aspergillus aculeatus*  
338 digestion of dried cell wall seedlings 4 days dark grown of Col-0, *qua2-1*, *qua2-1/esmd1-1* and  
339 *esmd1-1*. \*,  $P < 0.05$ , \*\*,  $P < 0.001$ , T-Test







### 343 Supplemental data 2 : HGs fragments released significantly different.

344 (A) Heatmap of all of the HGs fragments released by *PG Aspergillus aculeatus* significantly  
 345 different between Col0 and *qua2-1* (46 rows) for 3 samples (columns). Annotation labels refer  
 346 to HGs fragments structure. Rows are centered; unit variance scaling is applied to rows. Both  
 347 rows and columns are clustered using McQuitty distance and maximum linkage

348 (B) Heatmap of all of the HGs fragments released by *PG Aspergillus aculeatus* significantly  
 349 different between Col0 and *esmd1-1* (35 rows) for 3 samples (columns)

350 (C) Heatmap of all of the HGs fragments released by *PG Aspergillus aculeatus* significantly  
 351 different between *qua2-1* and *qua2-1/esmd1-1* (53 rows) for 3 samples (columns). Methyl-  
 352 esterified (\*) and acetyl-esterified (°) methyl/acetyl-esterified (•) HGs fragments specific to  
 353 *esmd1-1* are highlighted.

354 For (A), (B), and (C) ( $n = 3$  biological replicates per genotype),

355

### 356 Acknowledgements

357 The IJPB benefits from the support of Saclay Plant Sciences-SPS (ANR-17-EUR-0007).

### 358 Competing interests

359 The authors declare no competing or financial interests.

#### 360 **Author contributions**

361 S.B., G.M. and J.P. conceived the research project. S.B., G.M. and C.G. conceived and designed  
362 the experiments. C.G. performed most of the experiments with input from S.C. and A.V. L.G.  
363 and F.J. contribute to transcriptomic experiments. S.B., GM and C.G. wrote the manuscript.

#### 364 **Funding**

365 This research was funded by Région Hauts de France et BAP INRAE.

366

#### 367 **References**

368 **Atakhani, A., Bogdziewicz, L. and Verger, S.** (2022). Characterising the mechanics of cell–  
369 cell adhesion in plants. *Quant. Plant Biol.* **3**,

370 **Bonnin, E., Le Goff, A., Van Alebeek, G. J. W. M., Voragen, A. G. J. and Thibault, J. F.**  
371 (2003). Mode of action of *Fusarium moniliforme* endopolygalacturonase towards  
372 acetylated pectin. *Carbohydr. Polym.* **52**, 381–388.

373 **Daher, F. B. and Braybrook, S. A.** (2015). How to let go: Pectin and plant cell adhesion.  
374 *Front. Plant Sci.* **6**, 1–8.

375 **Du, J., Kirui, A., Huang, S., Wang, L., Barnes, W. J., Kiemle, S. N., Zheng, Y., Rui, Y.,**  
376 **Ruan, M., Qi, S., et al.** (2020). Mutations in the Pectin Methyltransferase  
377 QUASIMODO2 Influence Cellulose Biosynthesis and Wall Integrity in Arabidopsis  
378 [OPEN]. *Plant Cell* **32**, 3576–3597.

379 **Ferrari, S., Savatin, D. V., Sicilia, F., Gramegna, G., Cervone, F. and De Lorenzo, G.**  
380 (2013). Oligogalacturonides: Plant damage-associated molecular patterns and regulators  
381 of growth and development. *Front. Plant Sci.* **4**, 1–9.

382 **Gutierrez, L., Bussell, J. D., Păcurar, D. I., Schwambach, J., Păcurar, M. and Bellini, C.**  
383 (2009). Phenotypic plasticity of adventitious rooting in arabidopsis is controlled by  
384 complex regulation of AUXIN RESPONSE FACTOR transcripts and microRNA  
385 abundance. *Plant Cell* **21**, 3119–3132.

386 **Lin, W., Tang, W., Pan, X., Huang, A., Gao, X., Anderson, C. T. and Yang, Z.** (2022).  
387 Arabidopsis pavement cell morphogenesis requires FERONIA binding to pectin for

388 activation of ROP GTPase signaling. *Curr. Biol.* **32**, 497-507.e4.

389 **Mouille, G., Ralet, M. C., Cavelier, C., Eland, C., Effroy, D., Hématy, K., McCartney,**  
390 **L., Truong, H. N., Gaudon, V., Thibault, J. F., et al.** (2007). Homogalacturonan  
391 synthesis in *Arabidopsis thaliana* requires a Golgi-localized protein with a putative  
392 methyltransferase domain. *Plant J.* **50**, 605–614.

393 **Pelloux, J., Rustérucchi, C. and Mellerowicz, E. J.** (2007). New insights into pectin  
394 methylesterase structure and function. *Trends Plant Sci.* **12**, 267–277.

395 **Philippe, F., Pelloux, J. and Rayon, C.** (2017). Plant pectin acetyesterase structure and  
396 function: New insights from bioinformatic analysis. *BMC Genomics* **18**, 1–18.

397 **Sinclair, S. A., Larue, C., Bonk, L., Khan, A., Castillo-Michel, H., Stein, R. J.,**  
398 **Grolimund, D., Begerow, D., Neumann, U., Haydon, M. J., et al.** (2017). Etiolated  
399 Seedling Development Requires Repression of Photomorphogenesis by a Small Cell-  
400 Wall-Derived Dark Signal. *Curr. Biol.* **27**, 3403-3418.e7.

401 **Somerville, C., Bauer, S., Brininstool, G., Facette, M., Hamann, T., Milne, J., Osborne,**  
402 **E., Paredez, A., Persson, S., Raab, T., et al.** (2004). Toward a Systems Approach  
403 to Understanding Plant Cell Walls. *Science* (80-. ). **306**, 2206–2211.

404 **Stéphane Verger, S., Chabout, S., Gineau, E. and Grégory Mouille, G.** (2016). Cell  
405 adhesion in plants is under the control of putative O-fucosyltransferases.

406 **Taylor, S. C., Nadeau, K., Abbasi, M., Lachance, C., Nguyen, M. and Fenrich, J.** (2019).  
407 The Ultimate qPCR Experiment: Producing Publication Quality, Reproducible Data the  
408 First Time. *Trends Biotechnol.* **37**, 761–774.

409 **Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A. and**  
410 **Speleman, F.** (2002). Accurate normalization of real-time quantitative RT-PCR data by  
411 geometric averaging of multiple internal control genes. *Genome Biol.* **3**,.

412 **Voxeur, A., Habrylo, O., Guénin, S., Miart, F., Soulié, M. C., Rihouey, C., Pau-Roblot,**  
413 **C., Domon, J. M., Gutierrez, L., Pelloux, J., et al.** (2019). Oligogalacturonide  
414 production upon *Arabidopsis thaliana*-*Botrytis cinerea* interaction. *Proc. Natl. Acad. Sci.*  
415 *U. S. A.* **116**, 19743–19752.

416 **Wolf, S.** (2022). Cell Wall Signaling in Plant Development and Defense. *Annu. Rev. Plant*  
417 *Biol.* **73**, 323–353.

