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# Pectin methylesterification modulates cell wall properties to promote neighbour proximity-induced hypocotyl growth

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1 **RESEARCH REPORT**

2

3 **Running title:** Cell wall modulates shade-induced hypocotyl growth

4

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8 **Article title**

9 Pectin methylesterification modulates cell wall properties to promote neighbour proximity-  
10 induced hypocotyl growth

11

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20 **One sentence summary**

21 The degree of methylesterification of pectins modulates global changes in the cell wall and its  
22 mechanical properties that contribute to the neighbour proximity-induced hypocotyl growth in  
23 Arabidopsis

24 **Footnotes**

25 **Author's contribution**

26 **Conceptualization:** CF, DR, FS, SR, EVS. **Formal analysis:** FS, SR, EVS, MT, PS.  
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28 **administration:** CF, DR. **Software:** PS, SR, FS, EVS. **Validation:** FS, SR, EVS, CF, DR.  
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49

50 **Abstract**

51 Plants growing with neighbours compete for light and consequently increase growth of their  
52 vegetative organs to enhance access to sunlight. This response, called shade avoidance  
53 syndrome (SAS), involves photoreceptors such as phytochromes as well as phytochrome  
54 interacting factors (PIFs), which regulate the expression of growth-mediating genes.  
55 Numerous cell wall-related genes belong to the putative targets of PIFs, and the importance of  
56 cell wall modifications for enabling growth was extensively shown in developmental models  
57 such as dark-grown hypocotyl. However, the role of the cell wall in the growth of de-  
58 etiolated seedlings regulated by shade cues remains poorly established. Through analyses of  
59 mechanical and biochemical properties of the cell wall coupled with transcriptomic analysis  
60 of cell wall-related genes, we show the importance of cell wall modifications in neighbour  
61 proximity-induced elongation. Further analysis using loss-of-function mutants impaired in the  
62 synthesis and remodeling of the main cell wall polymers corroborated this. We focused on the  
63 *cgr2cgr3* double mutant that is defective in homogalacturonan (HG) methyltransferase  
64 activity required for methylesterification of HG-type pectins. By following hypocotyl growth  
65 kinetically and spatially and analyzing the mechanical and biochemical properties of cell  
66 walls, we found that methylesterification of HG-type pectins was required to enable global  
67 cell wall modifications. Moreover, HG-class pectin modification was needed for plant  
68 competition-induced hypocotyl growth. Collectively our work suggests that in the hypocotyl  
69 PIFs orchestrate changes in the expression of numerous cell wall genes to enable neighbour  
70 proximity-induced growth.

## 71 **Introduction**

72 Plants growing in dense populations compete for light required for photosynthesis  
73 (Fiorucci and Fankhauser, 2017). Proximity of competitors and shade are perceived as a  
74 change in light intensity and quality, resulting in various developmental adaptations (Galvão  
75 and Fankhauser, 2015). Depending on their response to shade, plants are classified into shade-  
76 tolerant and shade-avoiding species (Gommers et al., 2013). The latter react to shade with  
77 characteristic growth responses in order to reach full sunlight for photosynthesis. This  
78 phenomenon, known as Shade-Avoidance Syndrome (SAS) can be observed in most aerial  
79 organs and involves a range of developmental changes (Pierik and De Wit, 2014). For  
80 example, SAS entails early flowering and inhibition of branching, leaves adopt an upright  
81 position (hyponasty), and petioles, stems and hypocotyls elongate (de Wit et al., 2016). These  
82 changes accelerate the life cycle of plants and warrant survival and propagation under limited  
83 light availability.

84 The phytochrome (phy) type photoreceptors have a central role in SAS with phyB  
85 playing a predominant role in *Arabidopsis thaliana* (Legris et al., 2019). Under high R:FR  
86 conditions, corresponding to full sunlight, active phyB moves into the nucleus, where it  
87 interacts with transcription factors known as Phytochrome Interacting Factors (PIFs) to inhibit  
88 their activities. Low R:FR conditions, on the other hand, cause inactivation of phyB and  
89 derepression of the PIFs, which modulate genes required for shade-induced growth (de Wit et  
90 al., 2016). A central mechanism in shade-induced growth is auxin biosynthesis in cotyledons  
91 and young leaves followed by polar transport and distribution in hypocotyls and stems, which  
92 elongate in response to shade (de Wit et al., 2014). phyB and PIFs also act locally in the  
93 hypocotyl to promote growth through mechanisms that are less clearly established (Fiorucci  
94 and Fankhauser, 2017; Pucciariello et al., 2018). For example PIFs regulate the expression of  
95 genes required for plasma-membrane lipid biogenesis in the hypocotyl (Ince et al., 2022).  
96 Moreover, numerous genes encoding cell wall-modifying proteins are induced by shade and  
97 targeted by PIFs (Kohnen et al., 2016; Pedmale et al., 2016), suggesting role for cell wall  
98 metabolism in the establishment of the shade-regulated growth.

99 The primary cell wall of growing plant organs has seemingly contradictory functions.  
100 On the one hand, it provides mechanical strength to maintain cell shape and plant stature, on  
101 the other hand, it has to remain elastic and plastic to allow cell expansion and plant growth  
102 (Bashline et al., 2014). In dicotyledonous species such as *Arabidopsis*, the primary cell wall  
103 consists of interconnected networks of polysaccharides and structural proteins/glycoproteins  
104 (Cosgrove, 2005; Wolf et al., 2012a; Nguema-Ona et al., 2014). A first network of cellulose  
105 microfibrils cross-linked by hemicelluloses (network 1) is embedded in a second network  
106 made by pectins that have gelling properties (network 2). Pectins are intimately associated  
107 with a third network (network 3), consisting mainly of glycoproteins such as extensins  
108 (EXTs) and arabinogalactan proteins (AGPs) (Hijazi et al., 2014). This complex three-  
109 dimensional mesh resists to internal turgor pressure, and at the same time yields to allow cell  
110 growth. Dynamic adjustment of physical cell wall properties (e.g. stiffness, elasticity,  
111 plasticity) involves cell wall-synthesizing and modifying enzymes that usually belong to

112 multigenic families and constantly modulate cell walls to allow growth (Atmodjo et al., 2013;  
113 Sénéchal et al., 2014b; Pauly and Keegstra, 2016; Showalter and Basu, 2016).

114 Cell wall remodeling plays a central role in the control of seedling development,  
115 however, its contribution to adaptive growth phenomena in response to environmental cues  
116 such as SAS remains poorly understood. Although transcriptomic analyses suggested that cell  
117 wall remodeling may play a central role in adaptive growth processes (Kohnen et al., 2016),  
118 direct functional evidence is scarce. A role for xyloglucans in SAS has been established for  
119 petiole elongation in *Arabidopsis* (Sasidharan et al., 2010; Sasidharan and Pierik, 2010), and  
120 for shade-induced growth in *Stellaria longipes* (Sasidharan et al., 2008). Here, we took a  
121 systematic approach to explore the contribution of cell wall remodeling in SAS of the  
122 *Arabidopsis* hypocotyl. By combining Fourier-transformed infrared (FTIR) analyses of cell  
123 wall constituents with measurements of cell wall biophysics, we show that cell wall  
124 remodeling is triggered at early stages of the growth response induced by low R:FR,  
125 indicative of neighbour proximity that is a form of SAS. Employing systematic transcriptomic  
126 and genetic analysis with mutants affected in various aspects of cell wall biosynthesis and  
127 modification, we establish pectin methylesterification status as a central determinant of cell  
128 wall extensibility in the neighbour proximity-induced hypocotyl growth.

129

## 130 **Results**

### 131 **Low R:FR induces cell elongation mainly in the middle part of the hypocotyl**

132 In order to simulate the proximity of competing neighbours, we subjected Arabidopsis  
133 seedlings to white light supplemented with far red (low R:FR ratio). Within one day, this led  
134 to increased hypocotyl growth compared to control seedlings kept in white light (high R:FR  
135 ratio) (**Fig. 1A**). In order to determine the site of growth, we used the borders of epidermal  
136 cells as marks, since hypocotyl elongation proceeds almost exclusively by cell elongation  
137 without cell division. In order to assess cell dimensions, confocal images of seedlings were  
138 segmented in MorphoGraphX (de Reuille et al., 2015), followed by semiautomated cell size  
139 measurements. Cell length along the length of the hypocotyl was increased by low R:FR  
140 primarily in the middle part of the hypocotyl (**Fig. 1B**).

141

### 142 **Low R:FR changes mechanical properties of hypocotyl cell walls**

143 To assess the mechanical properties of the hypocotyl during growth induced by low  
144 R:FR, we used an automated confocal micro-extensometer (Robinson et al., 2017). This  
145 approach enabled *in vivo* quantification of the elastic properties of cell walls. Hypocotyls of  
146 intact seedlings were abraded by freezing and thawing and subjected to repeated cycles of  
147 application and removal of 5 mN of force. Our analysis revealed increased strain under low  
148 R:FR after one day of treatment, and decreased strain after three days (**Fig. 2A**). Interestingly  
149 this effect was specific to low R:FR conditions, since no such changes were observed under  
150 high R:FR (**Fig. 2A**).

151 To relate growth and mechanical changes induced by low R:FR to cell wall properties,  
152 we applied Fourier-transformed infrared (FTIR) microspectroscopy to cells located in the  
153 middle part of the hypocotyl. The relative absorbance intensities for wavelengths related to  
154 the cell wall (from 830 to 1800  $\text{cm}^{-1}$ ) were selected to assess the composition and status of  
155 various cell wall polysaccharides. FTIR analysis revealed significantly different patterns of  
156 relative absorption induced by low R:FR ratio after three days of treatment (**Fig. 2B and Fig.**  
157 **S1**). The main differences were observed between 1530 and 1200  $\text{cm}^{-1}$  and between 1180 and  
158 1030  $\text{cm}^{-1}$  (**Fig. 2B**). A relative decrease (at 1740 and between 1530-1200  $\text{cm}^{-1}$ ) indicates  
159 lower levels of pectins and xyloglucans, as well as cellulose and lignin. On the other hand, an  
160 increase between 1180 and 1030  $\text{cm}^{-1}$  indicates enrichment of pectins, xyloglucans, cellulose  
161 and arabinogalactan that can relate both to AGPs and rhamnogalacturonan I-type pectins (**Fig.**  
162 **2B**). These results indicate major cell wall remodeling in response to low R:FR ratio. In order  
163 to obtain further insight into the changed cell wall components, we performed hierarchical  
164 clustering for the wavelengths that have previously been assigned to certain cell wall  
165 components (Kakuráková et al., 2000; Wilson et al., 2000; Mouille et al., 2003; Alonso-simón  
166 et al., 2011; Szymanska-Chargot and Zdunek, 2013; Largo-Gosens et al., 2014) (**Fig. 2C**).  
167 This revealed an overall enrichment for pectins with a low degree of methylesterification (de-  
168 esterified pectins), in addition to other pectins, arabinogalactan proteins, cellulose, and  
169 xyloglucans in response to low R:FR, while other wavelengths assigned to pectins, cellulose

170 and xyloglucans showed opposite trends. Depending on the chemical bonds revealed by the  
171 wavelengths, different structural part of the polysaccharides are assessed such as backbone  
172 and side chains. This could explain opposite trends for wavelengths related to the same  
173 polysaccharide, which can be more abundant with reduced side chains for instance. Taken  
174 together, these results revealed major changes in cell wall biosynthesis and remodeling in  
175 response to low R:FR.

176

### 177 **Low R:FR impinges on expression of cell wall-related genes**

178 In order to investigate transcriptional regulation of cell wall properties under low  
179 R:FR, we considered 40 gene families that are thought to be involved in synthesis and/or  
180 remodeling of cell wall components. The expression of a total of 824 genes was analyzed  
181 using an RNA sequencing dataset from a time course experiment under low R:FR conditions  
182 (Kohnen et al., 2016). In this set of genes, 544 were found to be expressed in seedlings grown  
183 under control conditions, among which 224 were regulated by low R:FR in hypocotyls (**Fig.**  
184 **3A**). Only few genes were regulated in cotyledons or in both organs (26 and 16 genes,  
185 respectively) (**Fig. 3A**). Most genes were regulated at later stages (90 or 180 min after onset  
186 of light stimulus), and they were mostly up-regulated, in particular in hypocotyls (**Fig. 3A**).  
187 Modulated genes comprised functions related to (hemi)cellulose (network 1), pectin (network  
188 2), and structural proteins (network 3) (**Fig. 3B**). The few genes that were induced at the early  
189 time points (15 or 45 min. after onset of the light stimulus) in hypocotyls are involved in cell  
190 wall remodelling (At1g49490:EXT, At5g47500:PME, At1g62760:PMEI, At5g02260:EXP,  
191 At5g57560:XTH, At1g02405:EXT, At3g10710:PME, **Tables S1A and S1B**). Considering the  
192 global influence of FR light on cell biosynthetic genes, the highest percentage related to  
193 pectins (network 2) with 96% of the genes expressed in seedlings, and 52% affected by low  
194 R:FR conditions. Taken together, these results indicate that cell wall remodelling is initiated  
195 within the first 15 minutes of low R:FR treatment, followed by general cell wall modifications  
196 involving both synthesis and remodeling of all cell wall constituents, in particular of pectin.

197 SAS is controlled by PIFs, hence, we interrogated previously published ChIP  
198 sequencing data (Kohnen et al., 2016) for interactions of PIF4 and PIF5 with the 224 genes  
199 regulated in the hypocotyl under low R:FR conditions. Indeed, 59 genes showed a direct  
200 interaction with PIF4 and/or PIF5 (**Fig. S2 and Table S1C**), including genes related to all  
201 three polymer networks, as well as to all processes including cell wall synthesis, remodelling,  
202 and signalling.

203

### 204 **Cell wall-related mutants reveal the role of cell wall components in low R:FR-induced** 205 **growth**

206 In order to gain insight into the mechanisms involved in low R:FR-induced growth,  
207 loss-of-function mutants defective in the three networks (cellulose, pectin, glycoproteins)  
208 were investigated for growth phenotypes under low R:FR treatment. We selected *xxt* mutants

209 impaired in xyloglucan biosynthesis (network 1), mutants affected in pectin biosynthesis  
210 (*gaut*, *gat1* and *cgr*) and pectin remodeling (*pme* and *pmei*; network 2), and mutants affected  
211 in AGP biosynthesis (*galt*, *agp10c*, and *fla9*; network 3). All of these mutants were subjected  
212 to low R:FR treatment for three days and their hypocotyl growth response was normalized to  
213 the wild type. None of the single mutants in network 1 had a growth phenotype (**Fig. 4A**),  
214 possibly because of genetic redundancy, but the double mutants *txt1txt2* and *txt2txt5* showed  
215 significantly reduced hypocotyl elongation in response to low R:FR. In network 2 mutants,  
216 only *cgr2*, had a growth defect, which was exacerbated in the *cgr2cgr3* double mutant.  
217 Unexpectedly, mutants affected in network 3 generally grew longer than the wild type (**Fig.**  
218 **4A**). Taken together, these results highlight the importance of xyloglucan and pectin in SAS,  
219 while some proteinaceous component of the cell walls appear to restricts hypocotyl elongation  
220 in the wild type.

221 Based on the induction of several homogalacturonan methyltransferases (*HGMT*),  
222 including the previously characterized *HGMT* genes *CGR2* and *CGR3* (Kim et al., 2015)  
223 (**Fig. S3**), and on the strong growth phenotype of the *cgr2cgr3* double mutant, (**Fig. 4A**), we  
224 investigated cell wall constituents in *cgr2cgr3* by FTIR analyses using wavelengths assigned  
225 to methylesterified ( $1740\text{ cm}^{-1}$ ) and demethylesterified ( $1630\text{ cm}^{-1}$ ) pectins (**Fig. S4**). Using  
226 these wavelengths we estimated the degree of methylesterification (DM), which was  
227 decreased by approximately 40% in *cgr2cgr3* compared to the wild type. In a time course  
228 experiment, *cgr2cgr3* was not affected in hypocotyl growth under control conditions (high  
229 R:FR), however, under low R:FR conditions, growth was reduced (**Fig. 4B**). Notably,  
230 *cgr2cgr3* reacted slower (4d+1d) and weaker (4d+3d) than the wild type (**Fig. 4B**). The  
231 growth defect of *cgr2cgr3* was particularly pronounced in the middle part of the hypocotyl  
232 that normally shows the strongest growth response (**Fig. S5**). This indicates that *cgr2cgr3*  
233 mutants have a defect in low R:FR-induced epidermal cell elongation.

234 We next assessed the mechanical properties of *cgr2cgr3* hypocotyls in response to low  
235 R:FR (**Fig. 4C**). Wild type hypocotyls had shown an increase in cell wall strain after 1d, and a  
236 decrease after 3d of FR treatment (**Fig. 2A**). In contrast, *cgr2cgr3* did not show a change in  
237 strain at either time point, indicating that cell wall remodeling is defective in the double  
238 mutant. To further address this aspect, we investigated cell wall composition of *cgr2cgr3* by  
239 FTIR microspectroscopy as in the wild type (**Fig. 2B,C**), in the range of wavelengths from  
240  $830$  to  $1800\text{ cm}^{-1}$  to obtain a cell wall fingerprint. This analysis was performed in the middle  
241 part of hypocotyls which shows the strongest growth increment under low R:FR (**Fig. 1B**,  
242 **Fig. S6**). Relative absorbances for Col-0 and *cgr2cgr3* did not show significant changes in  
243 response to low R:FR after the first day (**Fig. 5A**; **Figs. S6A,B upper panels**). However, 3  
244 days after transfer, both wild type and *cgr2cgr3* showed significant changes of relative  
245 absorbances in response to low R:FR conditions (**Fig. 5A**; **Figs. S6A,B, lower panels**).  
246 Overall, the pattern of the significantly affected wavelengths were similar for both genotypes,  
247 but the differences were more pronounced in the wild type than *cgr2cgr3* (**Fig. 5B**).

248 The main differences between the genotypes were observed in the range of  
249 wavelengths from  $1630$  to  $1500\text{ cm}^{-1}$  with a decrease in the wild type but not in *cgr2cgr3*  
250 (**Fig. 5B**), and between  $1180$  to  $1030\text{ cm}^{-1}$ , where the wild type showed a stronger increase

251 than the double mutant. These results suggest that a change in pectin methylesterification in  
252 *cgr2cgr3* results in secondary changes in cell wall composition (**Fig. 5B**).

## 253 Discussion

254 While growth is simple to quantify, in particular in an organ like the hypocotyl that  
255 grows essentially in only one dimension, the underlying molecular mechanisms are extremely  
256 complex because they involve multiple regulatory levels such as hormonal and metabolic  
257 regulation, transcriptional activation of growth-related genes, and ultimately remodeling of  
258 the complex three-dimensional cell wall polymer network. Thanks to the ease of mutational  
259 analysis of growth, many of the upstream regulatory components in SAS have been identified  
260 (Procko et al., 2014; Ballaré and Pierik, 2017; Fiorucci and Fankhauser, 2017). A reduced  
261 R:FR indicative of neighbour proximity is primarily perceived by phyB in cotyledons and  
262 young leaves (Ballaré and Pierik, 2017; Fiorucci and Fankhauser, 2017). This leads to PIF-  
263 mediated induction of auxin production, which following transport to the hypocotyl promotes  
264 elongation (Procko et al., 2014; Ballaré and Pierik, 2017; Fiorucci and Fankhauser, 2017).  
265 PIFs can also directly regulate expression of hypocotyl specific genes as shown for genes  
266 encoding enzymes involved in plasma-membrane biogenesis (Ince et al., 2022). Our data  
267 suggests low R:FR induces extensive hypocotyl-specific regulation of genes encoding  
268 enzymes involved in cell wall biosynthesis and remodeling (**Fig. 3**), that ultimately results in  
269 in alteration of cell wall properties and induction of growth. However, due to the  
270 interdependency of cell wall components, it has been difficult to disentangle the role of  
271 individual cell wall components in growth. Here, we identified and characterized pectin  
272 methylesterification status as a central element in the growth phenomenon of Arabidopsis  
273 seedlings in the shade avoidance response.

274 Pectin consists mainly of homogalacturonan (HG) which represents a linear polymer  
275 of galacturonic acid (GalA) synthesized by galacturonosyltransferases (GAUTs) and GAUT-  
276 like (GATL) enzymes in the Golgi apparatus (Atmodjo et al., 2013). HG is subsequently  
277 methylesterified by HGMTs before secretion into the cell wall, where it can be selectively  
278 demethylesterified by pectin methylesterases (PMEs) (Sénéchal et al., 2014b). Thus, PME  
279 activity adjusts the degree of methylesterification (DM), which in turn modulates cell wall  
280 mechanical properties (Peaucelle et al., 2011; Wang et al., 2020). Furthermore, PME-  
281 mediated demethylesterification can expose HG to pectin-degrading enzymes such as  
282 polygalacturonases (PGs) and pectate lyases-like (PLLs) (Sénéchal et al., 2014b). Pectin  
283 degradation by these enzymes can contribute to cell wall loosening. There is evidence for a  
284 functional role of pectin metabolism in the regulation of growth (Bouton, 2002; Mouille et al.,  
285 2007) (Pelletier et al., 2010; Guénin et al., 2011; Wolf et al., 2012b; Sénéchal et al., 2014a)  
286 (Wang et al., 2010; Xiao et al., 2014; Rui et al., 2017), however, the high degree of  
287 redundancy in cell-wall remodeling enzymes has complicated the analysis. For example, there  
288 are 66 and 76 genes, respectively, that encode PMEs and PME inhibitors (PMEIs), allowing  
289 for extensive compensatory responses upon genetic or pharmacological interference.

290 A mechanistic understanding of growth phenomena requires the combined use of  
291 genetic, analytic, and biomechanical methods to identify the causal elements in cell growth.  
292 Using FTIR analysis, we document changes in cell wall composition in response to changes in  
293 the R:FR ratio, which correlate with accelerated growth. We identified two  
294 homogalacturonan-methyltransferase (HGMT) genes (*CGR2* and *CGR3*) that are required for

295 neighbour proximity-induced growth. Biomechanical assays showed that *CGR2* and *CGR3*  
296 contribute to cell wall extensibility at the onset of growth. Previous studies used atomic force  
297 microscopy to analyze cell walls, which measures properties perpendicular to the direction  
298 of growth, to detect differences in mechanical properties in plants with modified pectin that  
299 correlated with growth (Peaucelle et al., 2008; Peaucelle et al., 2011; Braybrook and  
300 Peaucelle, 2013; Peaucelle et al., 2015). In this study we were able to measure differences in  
301 mechanical properties using an extensometer which measures properties in the direction of  
302 growth. Both approaches show a correlation between modifying pectin chemistry, changes in  
303 cell wall mechanical properties and growth, supporting a role of pectin in growth regulation.  
304 Further work is required to understand the nature of this regulation and how it relates to the  
305 other cell wall components (Coen and Cosgrove, 2023).  
306

307 **Materials and Methods**

308 Information on the biological material and methods is available in the supplemental data

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320

## 321 **Figure legends**

322 **Figure 1. Low R:FR induces fast growth in the middle part of the hypocotyl. (A)** Length  
323 of the hypocotyls in response to high and low R:FR treatments. Col-0 seedlings were grown  
324 for 4 days in high R:FR before being transferred under low R:FR (blue bars) or kept in high  
325 R:FR (yellow bars) for 3 additional days. The bars show means in mm  $\pm$  confidence intervals  
326 measured at 4 (4d), 5 (4d+1d) and 7 (4d+3d) days. Significant differences (indicated with  
327 letters) were determined according to one-way Anova followed by a multiple comparisons  
328 with Tukey's test. **(B)** Length of the hypocotyl epidermis cells in response to high and low  
329 R:FR treatments. After 4 days under high R:FR (blue curve), Col-0 seedlings were grown for  
330 1 day under high R:FR (red curve) or low R:FR (green curve). The length of the epidermis  
331 cells were plotted from the top to the bottom part of the hypocotyl. The curves show means in  
332  $\mu\text{m} \pm$  confidence intervals (shaded areas).

333 **Figure 2. Low R:FR induces changes in mechanical and cell wall properties of the**  
334 **hypocotyl. (A)** Elastic properties of hypocotyl assessed under high and low R:FR treatment.  
335 Hypocotyls were frozen and thawed then subjected to cycles of application and removal of 5  
336 mN of force using an automated confocal micro extensometer (see methods). The average  
337 magnitude of strain incurred by seedlings grown in a high R:FR (yellow bars) or low R:FR  
338 (blue bars) light regime after 1 (4d+1d) and 3 days (4d+3d) is shown. Bright-field images  
339 were collected every 645 ms and strain was computed from regions that were tracked in the  
340 images using the ACME tracker software. The bars show means in %  $\pm$  SD ( $n > 10$   
341 independent seedlings, at least five oscillations were made). Pairwise comparisons were made  
342 using Welch t-test brackets indicated statistical tests that were made with significance  $p < 0.1^*$ ,  
343  $p < 0.05^{**}$  and  $p < 0.01^{***}$ . **(B,C)** Cell wall properties in the middle part of the hypocotyl under  
344 high and low R:FR treatments. Cell wall chemical bounds were analyzed by Fourier-  
345 Transformed InfraRed (FTIR) microspectroscopy. For each hypocotyl, 6 spectra were  
346 collected in the middle part, avoiding the central cylinder, for at least 5 independent  
347 hypocotyls per condition. Baseline correction and data normalization were made for the  
348 absorbances between  $1810$  and  $830\text{ cm}^{-1}$  (corresponding to the cell wall fingerprint, see  
349 **Supplemental Figure S1**). Pairwise comparison between high and low R:FR was made after  
350 1 and 3 days treatments and significant differences were identified using Student's t-test for  
351 each wavelength. **(B)** All Student's t-values were plotted against wavelengths with horizontal  
352 lines referring to significant threshold for  $p < 0.05$ . Student's t-values above  $+2$  or below  $-2$   
353 indicate respectively an enrichment or an impoverishment of cell wall components in low  
354 compared to high R:FR. **(C)** Student's t-values for wavelengths assigned to cell wall  
355 components were used to build the heatmap with negative and positive t-values respectively  
356 represented by a range of colors from blue to orange.

357 **Figure 3. Low R:FR triggers changes in the expression of cell wall-related genes. (A)**  
358 Number of cell wall-related genes identified as expressed in seedling and regulated by low  
359 R:FR in hypocotyl, cotyledon or both. From cell wall-selected genes and RNA sequencing  
360 data (Kohnen *et al.* 2016), Venn diagrams highlight cell wall-related genes expressed in  
361 seedling and that are regulated by low R:FR in hypocotyl, cotyledon or both. For each,  
362 number of up and down-regulated genes are shown along the kinetic of low R:FR treatment.

363 Percentages of the low R:FR-regulated genes were determined according to the total of cell  
364 wall-related genes expressed in seedling. **(B)** Number of cell wall-related genes expressed in  
365 seedling and regulated by low R:FR classified according to their putative function in cell wall  
366 synthesis, remodeling and signaling as well as their related networks for synthesis and  
367 remodeling. Percentages were determined according to the total of cell wall-related genes  
368 classified for each condition (values between brackets). Network 1: cellulose and  
369 hemicelluloses ; Network 2: pectins ; Network 3: structural proteins ; CW: Cell Wall.

370 **Figure 4. Analysis of mutants impaired in cell wall metabolism reveals importance of**  
371 **CGR2 and CGR3 in the regulation of the hypocotyl growth and the elastic properties**  
372 **under low R:FR. (A)** Length of the hypocotyls in response to low R:FR treatment. Seedlings  
373 were grown for 4 days in high R:FR and then for 3 additional days in low R:FR. Data of  
374 growth induced by low R:FR during the 3 days were normalized against the wild-type and  
375 expressed in log<sub>2</sub>FC. Significant differences ( $p < 0.05^*$ ,  $p < 0.001^{***}$ ) were determined  
376 according to Student's t-test. **(B)** Length of the hypocotyls in response to high and low R:FR  
377 treatments in Col-0 and *cgr2cgr3*. Seedlings were grown for 4 days in high R:FR before being  
378 transferred under low R:FR (blue bars) or kept in high R:FR (yellow bars) for 3 additional  
379 days. The bars show means in mm  $\pm$  confidence intervals measured at 4 (4d), 5 (4d+1d) and 7  
380 (4d+3d) days. Significant differences (indicated with letters) were determined according to  
381 one-way Anova followed by a multiple comparisons with Tukey's test. **(C)** Elastic properties  
382 of hypocotyl assessed under high and low R:FR treatment for Col-0 and *cgr2cgr3*.  
383 Hypocotyls were frozen and thawed then subjected to cyclic loading at 5 mN of force and the  
384 strain compared to the data obtained for the wild-type seedlings in Figure 2. The average  
385 magnitude of strain incurred by seedlings grown in a high R:FR or low R:FR light regime  
386 after one (4d+1d) and three days (4d+3d) is shown. The bars show means in %  $\pm$  SD ( $n > 10$   
387 independent seedlings for Col-0 and  $n > 5$  independent seedlings for *cgr2cgr3*, at least five  
388 oscillations were made per seedling). Pairwise comparisons were made between the mutant  
389 and the wild-type using Welch t-test brackets indicated statistical tests that were made with  
390 significance  $p < 0.1^*$ ,  $p < 0.05^{**}$  and  $p < 0.01^{***}$ .

391 **Figure 5. Changes of cell wall properties that occur in response to low R:FR are reduced**  
392 **in *cgr2cgr3***

393 **(A,B)** Cell wall properties in the middle part of the hypocotyl under high and low R:FR  
394 treatments for Col-0 and *cgr2cgr3*. Cell wall chemical bounds were analyzed by Fourier-  
395 Transformed InfraRed (FTIR) microspectroscopy. For each hypocotyl, 6 spectra were  
396 collected in the middle part, avoiding the central cylinder, for at least 5 independent  
397 hypocotyls per condition. Baseline correction and data normalization were made for the  
398 absorbances between 1810 and 830  $\text{cm}^{-1}$  (corresponding to the cell wall fingerprint, see  
399 **Supplemental Figure S6**). Pairwise comparison between high and low R:FR was made after  
400 1 and 3 days treatments for Col-0 and *cgr2cgr3* and significant differences were identified  
401 using Student's t-test for each wavelength. **(A)** All Student's t-values were plotted against  
402 wavelengths with horizontal lines referring to significant threshold for  $p < 0.05$  for Col-0 (left  
403 panel) and *cgr2cgr3* (right panel). Student's t-values above +2 or below -2 indicate  
404 respectively an enrichment or an impoverishment of cell wall components in low compared to

405 high R:FR. **(B)** Student's t-values for wavelengths assigned to cell wall components were  
406 used to build the heatmap with negative and positive t-values respectively represented by a  
407 range of colors from blue to orange.

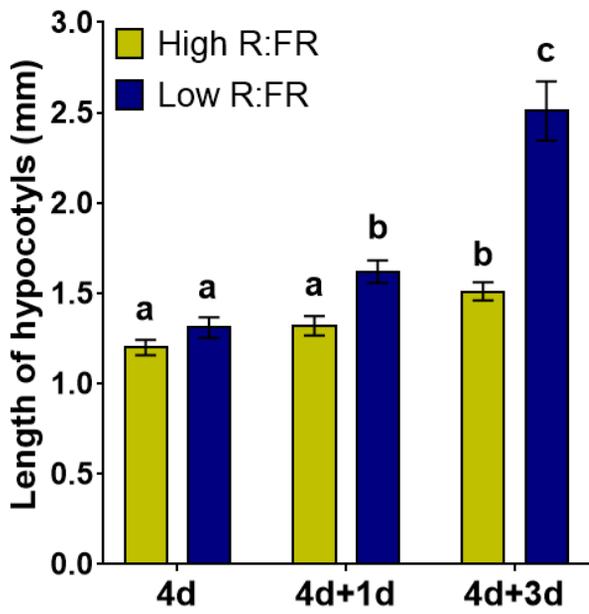
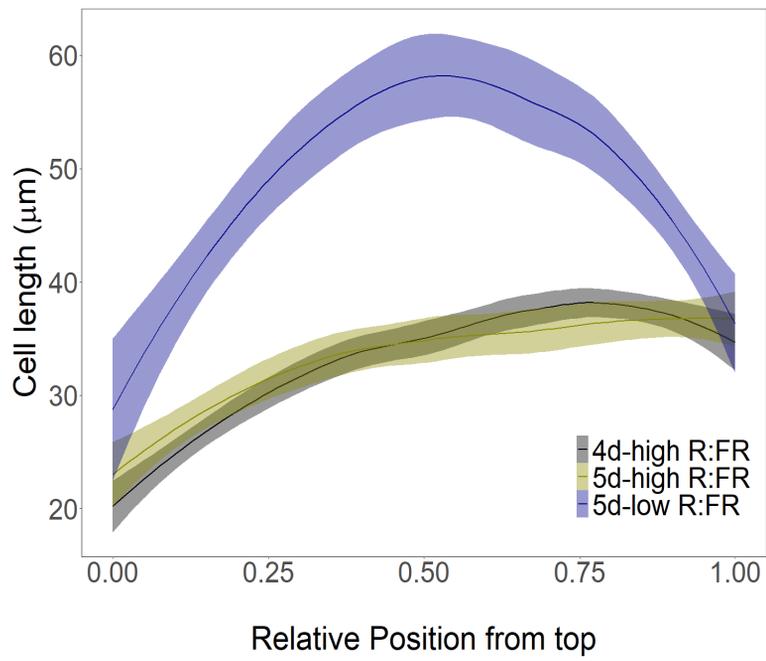
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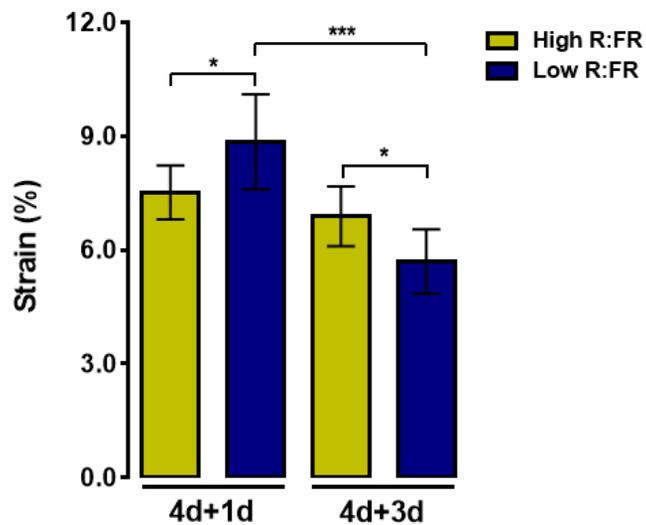
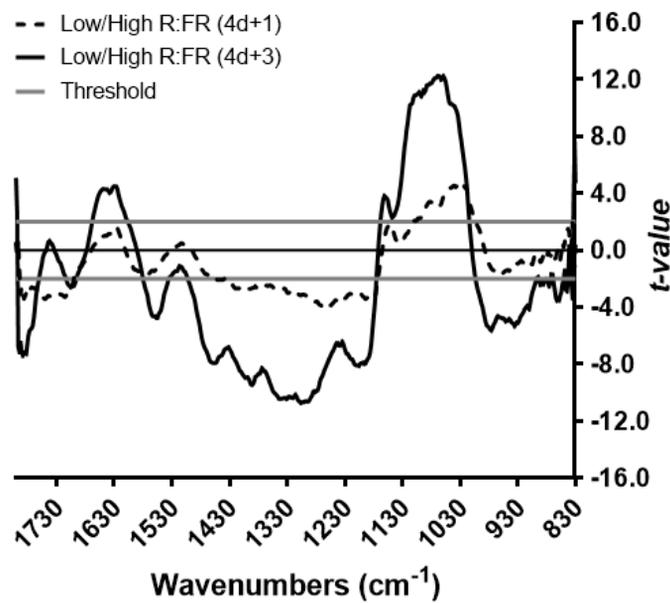
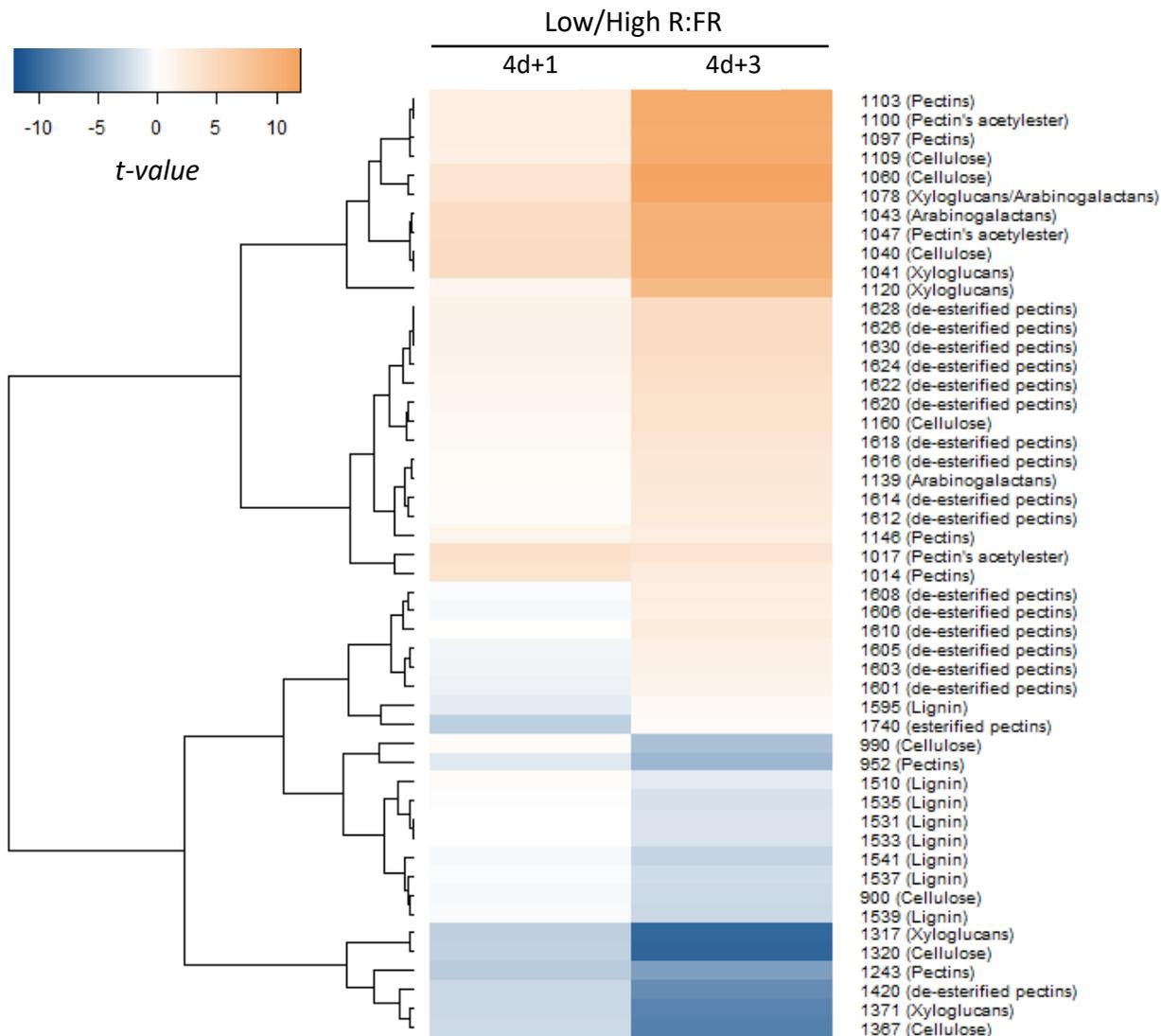
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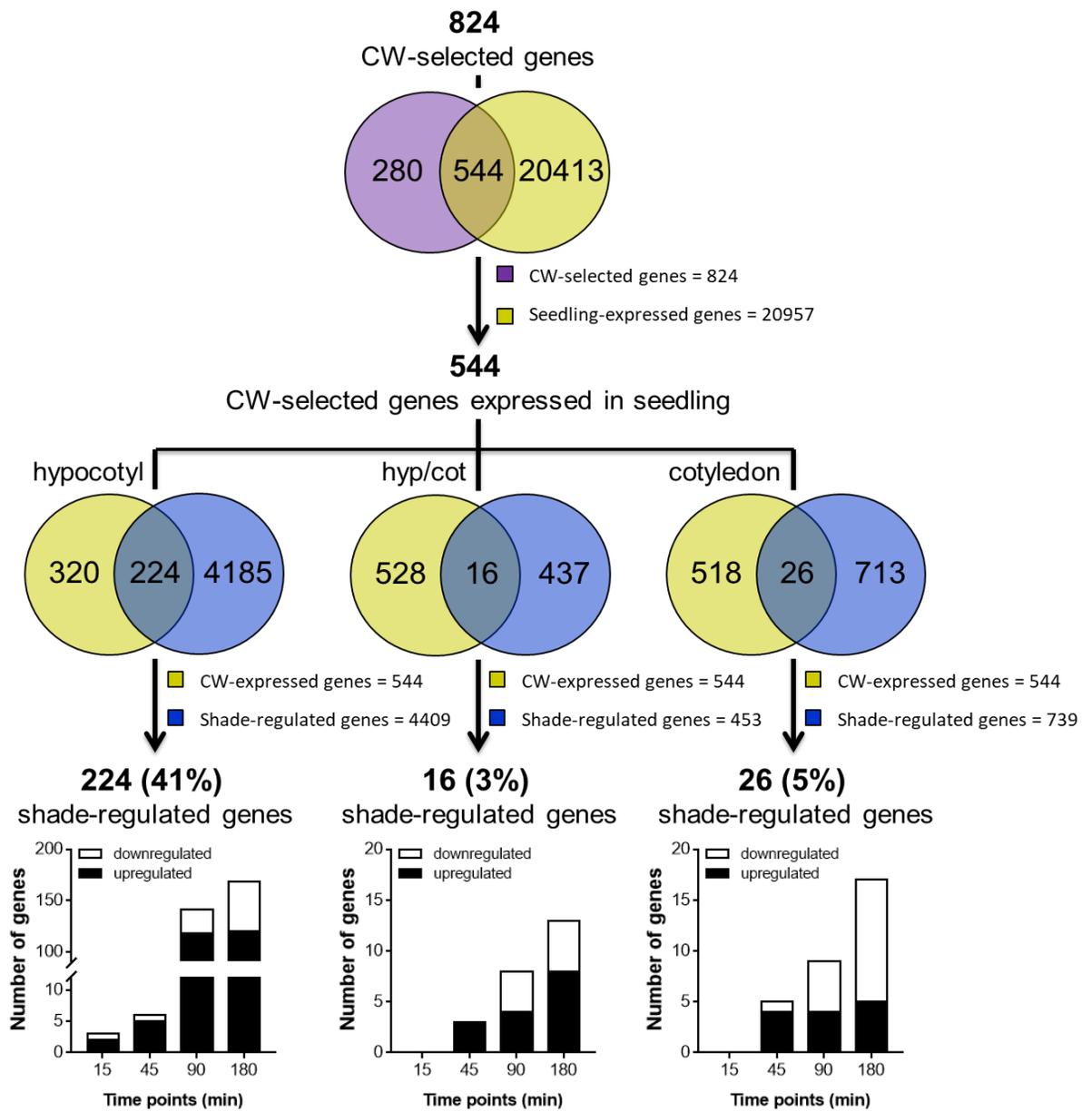
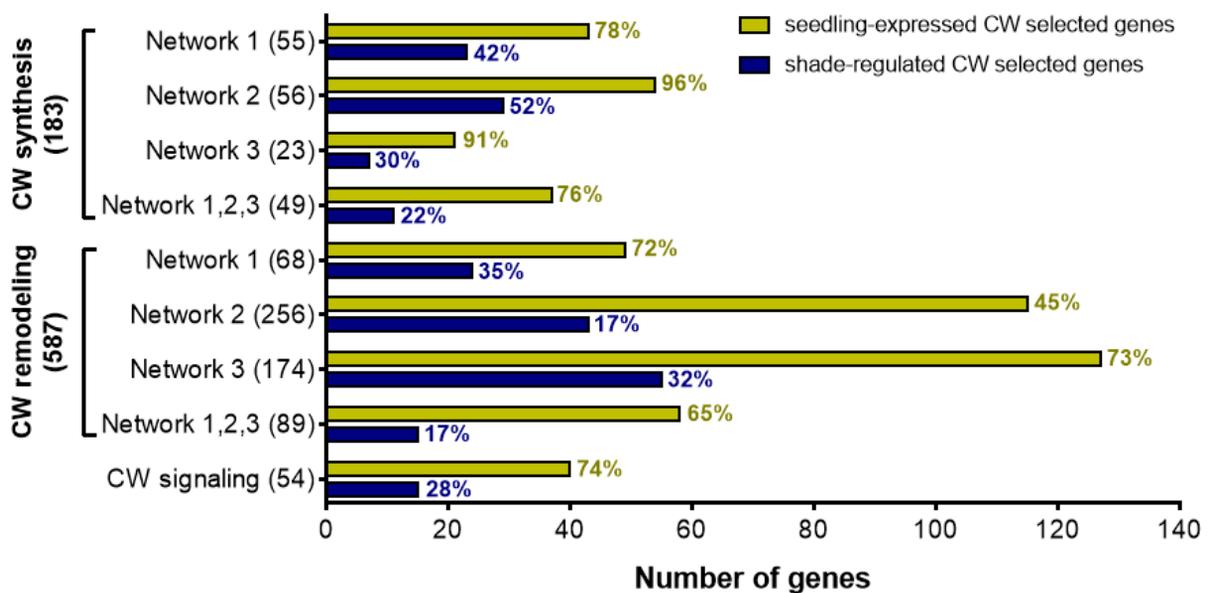
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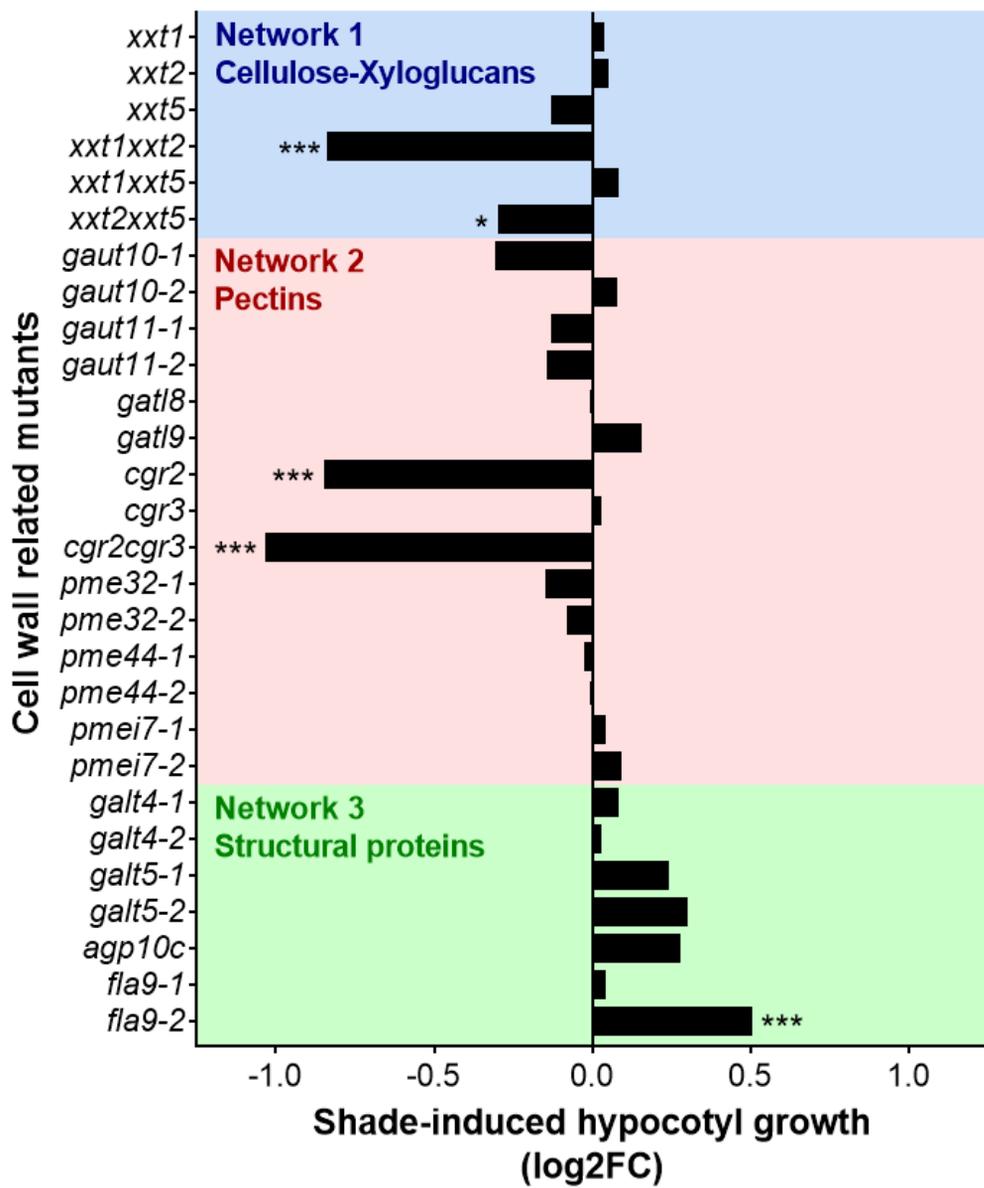
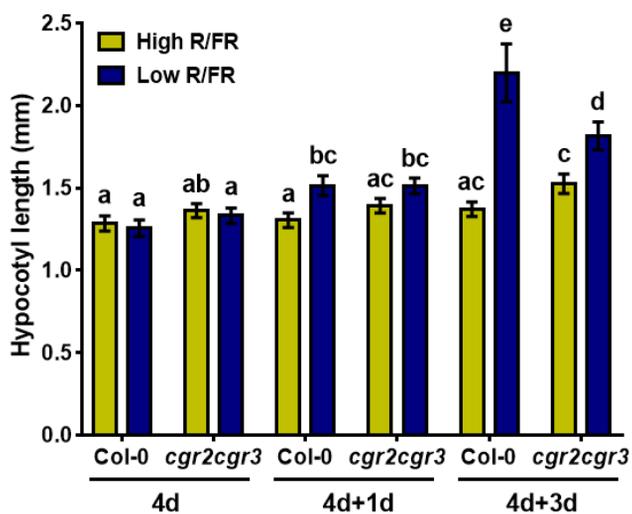
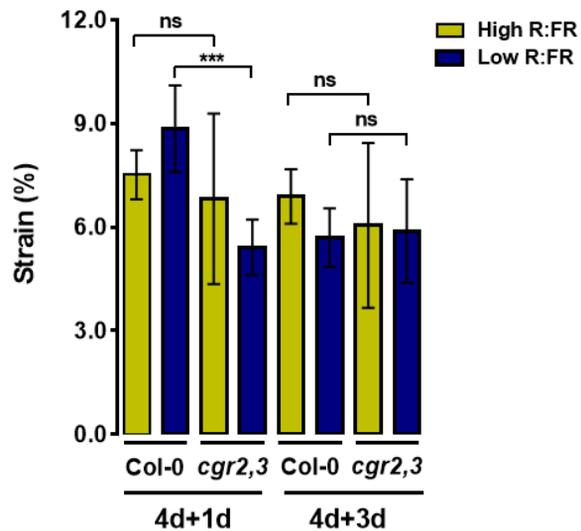
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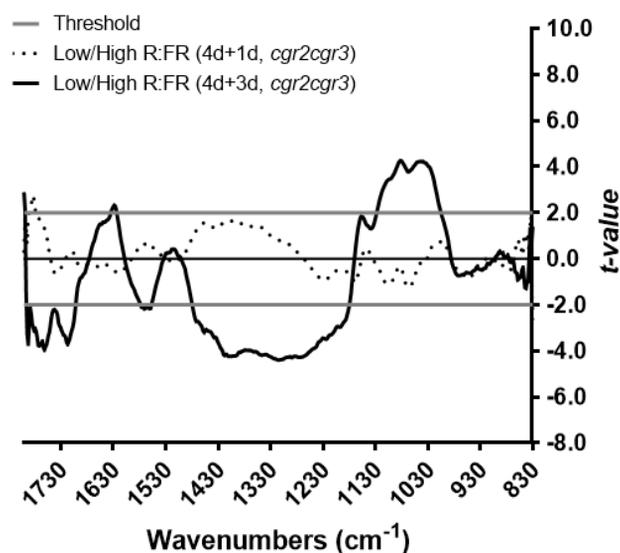
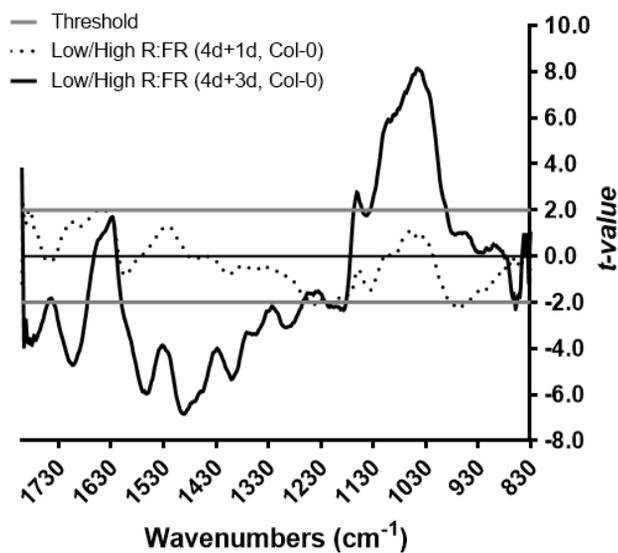
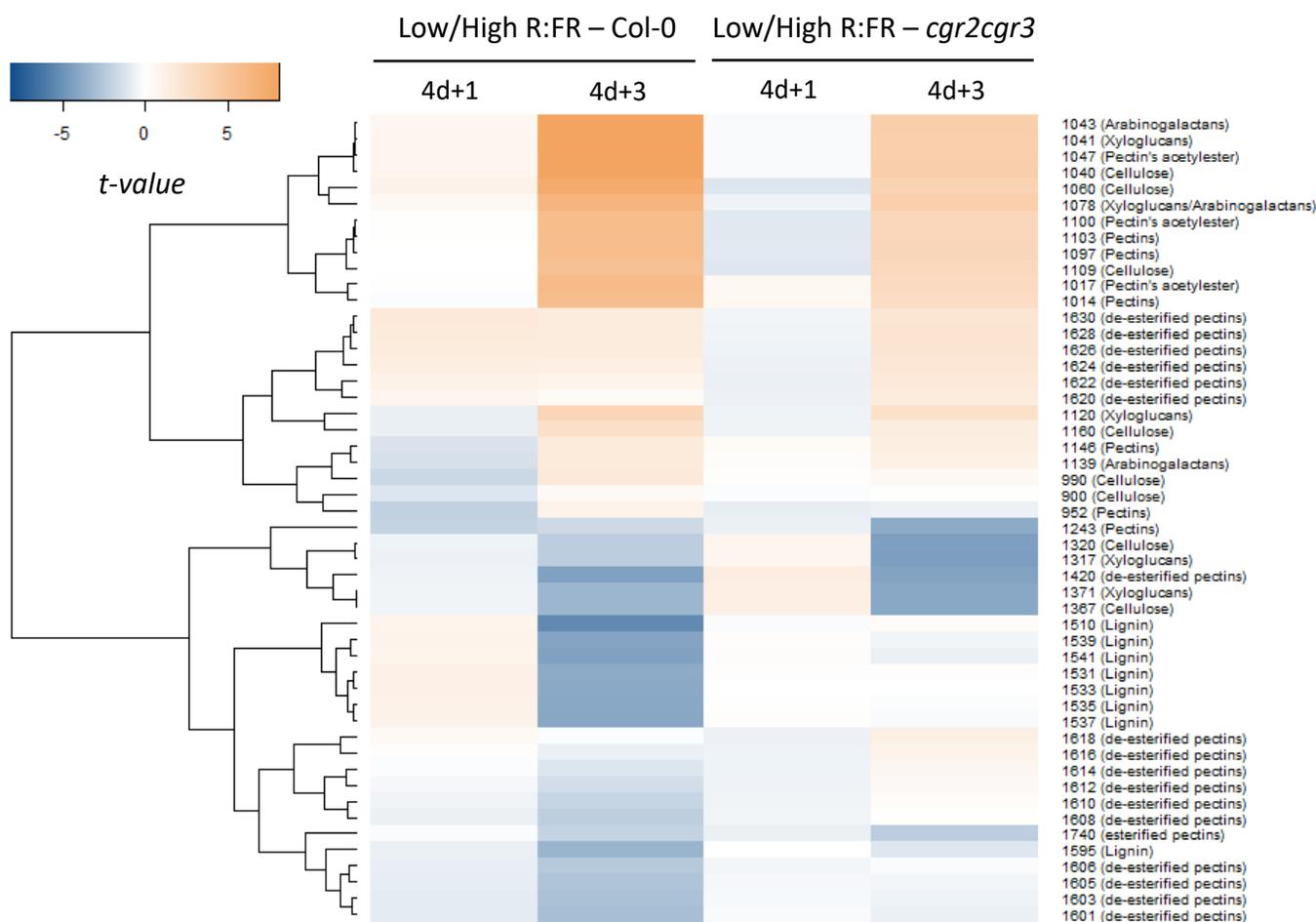
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**A****B****Figure 1**

**A****B****C****Figure 2**

**A****B****Figure 3**

**A****B****C****Figure 4**

**A****B****Figure 5**

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