

# Integrating roots into a whole plant network of flowering time genes in *Arabidopsis thaliana*

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Running title:

**Rooting the flowering process.**

## 1 **ABSTRACT**

2 Molecular data concerning the involvement of the roots in the genetic pathways regulating floral  
3 transition are lacking. In this study, we performed global analyses of root transcriptome in  
4 Arabidopsis in order to identify flowering time genes that are expressed in the roots and genes  
5 that are differentially expressed in the roots during the induction of flowering. Data mining of  
6 public microarray experiments uncovered that about 200 genes whose mutation was reported to  
7 alter flowering time are expressed in the roots but only few flowering integrators were found.  
8 Transcriptomic analysis of the roots during synchronized induction of flowering by a single 22-h  
9 long day revealed that 595 genes were differentially expressed. A delay in clock gene expression  
10 was observed upon extension of the photoperiod. Enrichment analyses of differentially  
11 expressed genes in root tissues, gene ontology categories and cis-regulatory elements converged  
12 towards sugar signaling. We inferred that roots are integrated in systemic signaling whereby  
13 carbon supply coordinates growth at the whole plant level during the induction of flowering.  
14

## 15 INTRODUCTION

16 Flowering is a crucial step of plant development that must be precisely timed to occur when  
17 external conditions are favourable for successful reproduction. Floral induction is therefore  
18 controlled by several environmental and endogenous cues, whose inputs are integrated into  
19 finely-tuned regulatory gene networks. In *Arabidopsis thaliana*, genetic analyses unveiled a  
20 number of flowering pathways that are activated in response to photoperiod, temperature, sugars,  
21 hormones and plant aging, or eventually occurs autonomously (Bouché et al., 2016). These  
22 pathways are not restricted to the shoot apical meristem where flowers are initiated but also  
23 involve the leaves at least, supporting the fact that flowering, as shown previously at the  
24 physiological level, is a systemic process. Clearest evidence came from the photoperiodic  
25 pathway that accelerates flowering in response to increasing daylength to ensure bolting in  
26 spring (Song et al., 2015). A key actor in this pathway is the transcription factor CONSTANS  
27 (CO) whose expression follows a circadian pattern but is degraded in the dark (Suarez-Lopez et  
28 al., 2001; Valverde et al., 2004). Light must therefore coincide with CO synthesis to stabilize the  
29 protein and enable activation of its targets (Valverde et al., 2004). This occurs during long days  
30 in the companion cells of phloem, where CO activates *FLOWERING LOCUS T (FT)* (Samach et  
31 al., 2000). The FT protein then moves systemically (Corbesier et al., 2007) and in the shoot  
32 apical meristem interacts with the transcription factor FD via 14-3-3 proteins (Abe et al., 2005;  
33 Wigge, 2005; Taoka et al., 2011). This flowering activation complex triggers the expression of  
34 genes that are responsible for the conversion of the vegetative shoot apical meristem into an  
35 inflorescence meristem and for the promotion of floral fate in lateral primordia (Ó'Maoiléidigh et  
36 al., 2014).

37 The prominent role of the FT protein in the systemic signaling operating at floral transition  
38 opens questions concerning the role of side molecules that are co-transported from leaf sources  
39 in the phloem and the pleiotropic effects of FT and putative co-transported signals in different  
40 sinks. Sugar loading is the first step of long-distance mass-flow movement in phloem and hence  
41 carbohydrates might influence flowering signals delivery (Dinant and Suarez-Lopez, 2012).  
42 Several reports however indicate that sugars act as flowering signals themselves, at two sites in

43 the plant. In the leaves, photosynthesis and activity of TREHALOSE-6-PHOSPHATE  
44 SYNTHASE 1 (TPS1), which catalyzes the formation of trehalose-6-phosphate (T6P) involved  
45 in sugar sensing, are required for the induction of the *FT* gene, even under inductive photoperiod  
46 (King et al., 2008; Wahl et al., 2013). The plant so integrates an environmental signal (the  
47 activation of *FT* by CO in response to increasing day length) with a physiological signal (the  
48 presence of high carbohydrate levels, as indicated by T6P) (Wahl et al., 2013). Interestingly, CO  
49 regulates the expression of GRANULE-BOUND STARCH SYNTHASE (GBSS), an enzyme  
50 controlling the synthesis of amylose in starch granules, and could thereby mediate modification  
51 of transitory starch composition to increase the sugar mobilization at floral transition (Ortiz-  
52 Marchena et al., 2014). Using starchless mutant, Corbesier et al. (1998) concluded that starch  
53 mobilization was critical for floral induction in conditions which did not involve an increased  
54 photosynthetic activity. All those results build evidence for sugar contribution to the florigenic  
55 signaling. In the shoot apex, sucrose content increases when *Arabidopsis* plant flowers in  
56 response to a photosynthetic long day (King et al., 2008; Corbesier et al., 1998) or eventually in  
57 short days (Eriksson et al., 2006). Sugars can induce the expression of flowering genes in the  
58 meristem, e.g. via the T6P pathway, independently of *FT* (Wahl et al., 2013). Beside sugars, the  
59 phloem sap of *Arabidopsis* is also enriched in amino acids and hormones of the cytokinin family  
60 when flowering is induced by a photoperiodic change (Corbesier et al., 1998; 2003). Cytokinins  
61 can promote flowering by inducing the paralogue of *FT*, *TWIN SISTER OF FT (TSF)*, in the  
62 leaves and downstream flowering genes in the shoot apical meristem (D'Aloia et al., 2011).

63 If we can infer from the previous section that multiple flowering signals including FT, sugars  
64 and hormones are transported in phloem, the signaling route appears as simplified to one way  
65 from leaves to the shoot apical meristem. Roots are ignored. At the physiological level though, a  
66 shoot-to-root-to-shoot loop has been described to drive sugar and cytokinin fluxes at floral  
67 transition in the *Arabidopsis* relative white mustard (Havelange et al., 2000). More directly,  
68 tagging of the FT protein with GFP in *Arabidopsis* allowed to detect movement of the fusion  
69 protein from overexpressor scion to *ft* mutant rootstock, indicating that it is not restricted to  
70 aerial parts of the plant (Corbesier et al., 2007). In other species, FT-like proteins exported from  
71 the leaves can induce belowground processes such as tuberization in potato (Navarro et al.,

72 2011) or bulb formation in onion (Lee et al., 2013). These reports indicate that developmental  
73 signals from leaf origin reach the underground organs.

74 Little information is available about the expression of flowering time genes in the roots. In a few  
75 cases only, analysis of expression patterns or phenotyping of mutants included careful  
76 examination of the roots and were followed by complementation tests (Bernier and Périlleux,  
77 2005). This rationale was used for *FT*, which is not expressed in the roots but whose partner *FD*  
78 is (Abe et al., 2005), raising the possibility of a role for the flowering activation complex in the  
79 roots. However, the root-specific expression of *FT* did not rescue the phenotype of *ft* single  
80 mutant, indicating that the expression of *FT* in root tissues is not sufficient - albeit it might  
81 contribute - to flowering (Abe et al., 2005). Other flowering time mutants such as *fca*, several  
82 *squamosa-promoter binding protein like (spl3, spl9 and spl10)* or *terminal flower 1 (tfl1)* show  
83 root architecture phenotypes (Macknight et al., 2002; Lachowiec et al., 2015; Yu et al., 2015).  
84 Major flowering QTL in *Arabidopsis* were also found to be associated with root xylem  
85 secondary growth (Sibout et al., 2008). However, whether those traits indicate root-specific  
86 functions or indirect effects of flowering time genes remains to be demonstrated.

87 The aim of this study was to clarify the role of roots in the flowering process. Two  
88 complementary approaches were used. First, data mining of public microarray databases was  
89 performed to obtain a global view of flowering-time genes expressed in the roots. Second, the  
90 transcriptome of the roots was analysed during the induction of flowering. The set of differential  
91 by expressed genes was crossed with publicly available datasets obtained in different contexts  
92 for discovering potential regulatory networks.

93

## 94 RESULTS

### 95 A majority of flowering-time genes are expressed in roots

96 Data mining was performed using transcriptomic analyses of roots that are available in the  
97 ArrayExpress repository (Kolesnikov et al., 2015) (Figure 1). The whole set of selected  
98 experiments contained 1,673 Arabidopsis ATH1 Genome arrays (Supplemental Table 1). For  
99 each array, we performed an Affymetrix present/absent call to identify root-expressed genes.  
100 Genes were considered as being expressed when transcripts were detected ( $p < 0.01$ ) in at least  
101 50% of the 1,673 arrays. We crossed the results of this filter with a comprehensive list of 306  
102 flowering-time genes that we established in the FLOR-ID database (Bouché et al., 2016). These  
103 genes are allocated among different pathways whereby flowering occurs in response to  
104 photoperiod, vernalization, aging, ambient temperature, hormones or sugar; an “autonomous  
105 pathway” leads to flowering independently of these signals and involves regulators of general  
106 processes such as chromatin remodeling, transcriptional machinery or proteasome activity. Eight  
107 genes under the control of several converging pathways are defined as “flowering-time  
108 integrators”: *FT*, *TSF*, *SUPPRESSOR OF OVEREXPRESSION OF CO1 (SOC1)*, *AGAMOUS-*  
109 *LIKE 24 (AGL24)*, *FRUITFULL (FUL)*, *FLOWERING LOCUS C (FLC)*, *SHORT VEGETATIVE*  
110 *PHASE (SVP)* and *LEAFY (LFY)*. Given the design of ATH1 microarrays, 37 flowering-time  
111 genes including 11 genes encoding microRNAs could not be included in our survey because  
112 they are not represented in the probe set. Out of the 269 represented flowering time genes, 183  
113 (68%) were expressed in roots in more than half of the analyzed arrays (Figure 1A;  
114 Supplemental Table 2). Some flowering pathways were more enriched than others (Figure 1B),  
115 e.g. the photoperiodic pathway with 70% of its genes being expressed in the roots or the sugar  
116 pathway with 7 genes out of 9 being active in the roots, including *TPSI*. As expected, genes  
117 controlling autonomous flowering via general regulatory processes were widely detected in roots  
118 (80%). A side category of circadian clock genes was also highlighted in the analysis. By  
119 contrast, a low proportion of genes from the hormones and aging pathways could be detected.

120 If we analysed one-by-one the data and focused on master flowering-time genes that are  
121 highlighted in flowering snapshots (Bouché et al., 2016), we found that most of them were  
122 actually not expressed in the roots or at least did not pass the filter setting of being detected in at  
123 least 50% of the available root transcriptomes (Figure 1C). In the photoperiod pathway, *CO* and  
124 *FT* were not at all detected in the dataset; only *GIGANTEA (GI)* was, which mediates between  
125 the clock and *CO* regulation (Mishra and Panigrahi, 2015). The flowering activation complex  
126 component *FD* and its paralogue *FDP* were not hit on the analysis either (detected in 5% of the  
127 arrays only). In the aging pathway, *MIRNA* genes were not analysed on ATH1 arrays, but their  
128 *SPL* targets involved in flowering were not found in the majority of root microarrays. In the  
129 vernalization pathway, *FLC* was detected in 11% of the arrays only. As could be expected,  
130 flower meristem identity genes *LFY* and *APETALA1 (API)* were not detected at all but the  
131 upstream MADS box gene *SOCI* was expressed in 42% of the array.

132 The only pathways whose key regulators are expressed in the roots are the sugar pathway, as  
133 *TPSI* was detected in 81% of the arrays, and the ambient temperature pathway, with *SVP* and  
134 *FLOWERING LOCUS M (FLM)* coming up in 73% and 51% of the arrays analysed,  
135 respectively. This finding makes sense since all plant parts undoubtedly sense sugars and  
136 surrounding temperature, including the roots.

## 137 **Root transcriptome changes during the induction of flowering**

138 To identify new candidate genes expressed in the roots and potentially involved in flowering, we  
139 analysed root transcriptome during the induction of flowering (Figure 2). Plants were grown in  
140 hydroponics for 7 weeks under 8-h short days (8-h SD) and then induced to flower by a single  
141 22-h long day (22-h LD), as described in Tocquin et al. (2003). We harvested roots 16 and 22 h  
142 after the beginning of the 22-h LD and at the same times in control 8-h SD. We chose these  
143 timing points to target early signaling events of floral induction. Two weeks after the  
144 experiment, we dissected the remaining intact plants to check that those exposed to the 22-h LD  
145 had entered floral transition whereas the 8-h SD controls were still vegetative (Figure 2A). Three  
146 independent experiments were performed and used for a transcriptome analysis with Arabidopsis  
147 ATH1 genome arrays; the raw results had been included in the data mining reported above. A

148 total of 10,508 AGI loci passed filtering criteria (see Material and Methods) and thus were  
149 considered as being expressed in the roots in our experimental system. These 10,508 loci  
150 included 168 flowering-time genes, among which 152 were common with the subset revealed by  
151 the global data mining shown in Figure 1, somehow confirming these results. Sixteen additional  
152 flowering-time genes were then expressed in our experimental set-up, and hence may be  
153 regulated by plant age or growing conditions (Supplemental Table 3). Among them, we found  
154 the floral integrator *SOCI* and two flowering-time genes involved in the control of meristem  
155 determinacy: *TERMINAL FLOWER 1 (TFL1)*, a gene of the same family as *FT* but repressing  
156 floral transition in the shoot apical meristem (Kobayashi et al., 1999) and *XAANTAL2 (XAL2)*,  
157 also named *AGL14*, a gene involved in shoot and root development (Garay-Arroyo et al., 2013;  
158 Pérez-Ruiz et al., 2015).

159 The root transcriptome was found to undergo numerous changes during the inductive LD. At  
160 h16, *i.e.* 8 hours from the extension of the photoperiod, 86 differentially expressed genes were  
161 identified in the roots and at h22, the number had increased to 583 (Figure 2C). We considered  
162 genes as being differentially expressed when the adjusted p-value was  $\leq 0.01$  and fold-change  $\geq$   
163 2. The heatmap shows that most changes occurring at h16 were actually amplified at h22 (74 of  
164 the 86 differentially expressed genes) (Figure 2B) indicating that the experimental design  
165 targeted early events. In total, 595 differentially expressed genes were identified in the roots  
166 (Supplemental Table 3) among which 18 known flowering time genes allocated to the  
167 photoperiod pathway, the circadian clock and the sugar pathway (Figure 2D). This number thus  
168 represented about 10% of all the flowering time genes detected in the roots by data mining.

169 Members of the photoperiodic pathway included negative regulators of CO: *CYCLING DOF*  
170 *FACTOR2* and 3 (*CDF2/3*), *B-BOX DOMAIN PROTEIN 19 (BBX19)* and *SUPPRESSOR OF*  
171 *PHYA-105 1 (SPA1)* but whereas *CDF2/3* and *BBX19* were down-regulated in LD, *SPA1* was  
172 upregulated. Two positive regulators of CO were also up-regulated: *GI* and the blue-light  
173 photoreceptor gene *CRYPTOCHROME1 (CRY1)*. Two CO-like genes - *CONSTANS-LIKE5*  
174 (*COL5*) and *SALT TOLERANCE (STO)* - were down-regulated at h22 in LD, as well as the gene  
175 encoding the phytochrome B-interacting protein *VASCULAR PLANT ONE ZINC FINGER*  
176 *PROTEIN 2 (VOZ2)*.

177 Among clock components, several morning genes - *CIRCADIAN CLOCK ASSOCIATED 1*  
178 (*CCA1*), *LATE ELONGATED HYPOCOTYL (LHY)*, *NIGHT LIGHT-INDUCIBLE AND*  
179 *CLOCK-REGULATED 2 (LNK2)*, and *REVEILLE 2 (RVE2)* - were repressed at h22 in LD. On  
180 the opposite, two evening genes were upregulated: *GI* and *EARLY FLOWERING 4 (ELF4)*.

181 The increase in photoperiod also induced the expression of two sugar metabolism-related genes:  
182 *TPS1* and *ADP GLUCOSE PYROPHOSPHORYLASE 1 (ADG1)*, encoding a subunit of ADP-  
183 glucose pyrophosphorylase (AGPase). Finally, we found that the expression of two genes  
184 involved in the control of meristem fate was also altered: *TFL1* was upregulated in LD whereas  
185 *XAL2* was repressed at h22.

## 186 **Differentially expressed genes are enriched in phloem tissue**

187 The list of 595 differentially expressed genes was thereafter submitted to different tests to see  
188 whether particular networks emerged. We performed three different searches based on (i) tissue  
189 enrichment, (ii) gene ontology and (iii) promoter sequences (Figure 3).

190 First, to know in which tissues the differentially expressed genes were enriched, we crossed their  
191 list with the tissue-specific root transcriptome dataset published by Brady et al. (2007). As a  
192 reference, we used the whole set of 10,508 genes expressed in the roots in our experimental  
193 system. We found that while the genes expressed in the roots are mostly detected in xylem and  
194 hair cells, this distribution was notably modified in the subset of differentially expressed genes  
195 with phloem and lateral root tissues hosting a significant part of the observed changes (Figure  
196 3A).

197 Second, we performed a gene ontology enrichment test and found that 'Photoperiodism' was the  
198 most significantly enriched term in differentially expressed genes (Figure 3B), followed by  
199 'Pyrimidine ribonucleotide biosynthesis', 'Trehalose metabolic processes', 'Response to  
200 disaccharide' and 'Circadian rhythm'.

201 Third, we searched for enriched cis-elements in the promoters of differentially expressed genes  
202 by using the tools of the MEME suite software (Figure 3C). Differentially expressed genes were

203 distributed among four subsets corresponding to the expression patterns illustrated in Figure 2B:  
204 up or down in LD, at h16 or h22. A *de novo* motif search was then performed with MEME  
205 (motif length between 8 and 15 nucleotides) and DREME (motif length  $\leq 8$ ) to find the most  
206 represented motifs in the promoters of each of the four gene subsets. Based on Korkuc et al.  
207 (Korkuc et al., 2014) study, we scanned the regions spanning -500 to +50 nt from the  
208 transcription start site of the genes. Among the resulting motifs, we found several close matches  
209 to five known cis-elements: the telo-box (AAACCC[TA]), the site II element (A[AG]GCCCA),  
210 the I-Box, the TATCCA element, and the G-box (CACGTG). To determine which of these  
211 motifs were specifically associated with the four expression patterns, we tested for the  
212 enrichment of each motif in the four subsets of differentially expressed genes with the AME  
213 tool. We found that both telo-box and site II elements were significantly enriched in upregulated  
214 differentially expressed genes, I-Box and TATCCA were rather associated with repressed  
215 differentially expressed genes. The G-box was not significantly enriched in any of the subsets  
216 (Figure 3C).

## 217 **The change in photoperiod affects the root circadian clock**

218 RT-qPCR analyses were performed on selected differentially expressed genes in order to  
219 confirm their differential expression (Figure 4). Since several clock genes appeared on the list,  
220 we performed time-course experiments to evaluate in more detail to which extent circadian-  
221 regulated processes were affected by the photoperiodic treatment. Roots were therefore  
222 harvested every 4 h during the inductive 22-h LD and in control 8-h SD.

223 We analysed the expression of *GI*, *CCA1* and *PSEUDO-RESPONSE REGULATOR 7 (PRR7)* as  
224 representative clock genes (Hsu and Harmer, 2014). The 22-h LD caused a 4-h delay in the  
225 expression patterns of these three genes, suggesting a phase shift of the circadian clock (Figure  
226 4A, left panel). Since such an effect could globally impact clock outputs, we attempted to  
227 evaluate the proportion of clock-regulated genes among the 595 differentially expressed genes.  
228 We therefore crossed the list with datasets from transcriptomic analyses of circadian clock-  
229 regulated genes in lateral roots (Voß et al., 2015) and shoot (Covington et al., 2008). A large

230 overlap of 78% and 63% was found with these datasets, respectively, revealing that the majority  
231 of the differentially expressed genes were indeed regulated by the circadian clock (Figure 4B).

232 Our analysis also included candidate genes involved in sugar sensing and cytokinin biosynthesis  
233 (Figure 4A, right panel). Most interestingly, *TPSI* whose activity is required for flowering in the  
234 leaves and in the shoot apical meristem (Wahl et al., 2013) was up-regulated in the roots during  
235 the 22-h LD. Our analysis also showed upregulation in LD of two  
236 *ISOPENTENYLTRANSFERASE* encoding genes (*IPT3* and *IPT7*) whereas a third one (*IPT5*) did  
237 not vary. These results confirmed the microarray data and clearly suggested that sugar signaling  
238 and cytokinin biosynthesis were stimulated in the roots in response to the photoperiodic  
239 treatment.

## 240 **Reverse genetic analysis of differentially expressed genes did not** 241 **reveal strong phenotypes.**

242 We selected a subset of 30 differentially expressed genes for functional analyses, following a  
243 number of criteria such as their expression fold change in the microarray analysis, their root-  
244 specific expression pattern (inferred from Covington et al. (2008)'s dataset), their putative  
245 function or their novelty (Supplemental Table 4). The corresponding mutants available were  
246 characterized for two traits: flowering-time and root architecture (Figure 5). Flowering time was  
247 quantified as the total number of leaves below the first flower. Surprisingly, only 5 mutants  
248 showed an altered flowering time phenotype in LD (Figure 5A). Some of these mutants had been  
249 previously characterized such as *gi-2* which, as expected, was very late flowering (Koornneef et  
250 al., 1991) and *glycine-rich RNA-binding protein 7* (*grp7*, also called *ccr2*) which was only  
251 slightly delayed (Streitner et al., 2008). The cytokinin biosynthesis mutants *ipt3* and *ipt3;5;7*  
252 showed an early flowering phenotype but the latter was highly pleiotropic and displayed  
253 abnormal growth (Miyawaki et al., 2006). Finally, the mutant for the *AT3G03870* gene of  
254 unknown function showed the earliest flowering phenotype, producing 4 fewer leaves than Col-0  
255 WT.

256 In order to select the genotypes whose root system significantly differed from WT, we performed  
257 a Principal Component Analysis (PCA) using the length of the primary root, the length of the  
258 apical unbranched zone, the lateral root density, the lateral root number, the total lateral root  
259 length and the lateral root angle. The first two Principal Components (PC1 and PC2) were  
260 compared using Student tests with a threshold at  $p < 0.01$ . The selected genotypes were then  
261 compared to WT for each variable (t-test,  $p < 0.01$ ) (Figure 5B). The first principal component  
262 (PC1), which explained about 45 % of the variability of the dataset, reflects mostly the number of  
263 lateral roots, the length of the primary root, the length of the lateral roots, as well as the length of  
264 the apical unbranched zone of the primary root (Figure 5C). The PC2 mainly reveals lateral root-  
265 related changes, such as their length, their insertion angle on the primary root as well as their  
266 density. The *tps1* mutant was affected in PC1 only, showing reduced length of the apical  
267 unbranched zone as well as shorter primary and lateral roots. The pleiotropic *ipt3;5;7* triple  
268 mutant showed a statistically different PC1, displaying an increased number and density of lateral  
269 roots (Chang et al., 2013). The *ipt3* single mutant also displayed a different PC1, albeit with a  
270 weaker lateral-root phenotype.

271

## 272 DISCUSSION

273 Molecular data concerning the involvement of the roots in the process of flowering are lacking.  
274 In this study, transcriptome analyses showed that about 200 genes whose mutation had been  
275 shown to alter flowering time are expressed in the roots: 183 were identified in public resources  
276 as being expressed in more than 50% of the arrays and 16 additional genes popped-up in our  
277 experimental design aiming at analysing root transcriptome at floral transition. This data-  
278 crossing relies on an hand-curated database of flowering-time genes that we established recently  
279 (Bouché et al., 2016).

280 The small discrepancy in flowering-time gene numbers found in the two analyses is informative  
281 on the fact that some of these genes might be developmentally regulated in the roots. Indeed,  
282 most arrays deposited in databases were obtained from a few-day old seedlings whereas we  
283 studied mature 7-week old plants. Among the 16 genes expressed in hydroponics but not  
284 reaching the 50% threshold in the data mining survey, we found genes regulating meristem  
285 determinacy in the shoot: *XAL2* and *TFL1*. Most interestingly, *XAL2* is a direct regulator of  
286 *TFL1* expression in the shoot apical meristem but both genes have opposite effects on flowering  
287 time (Shannon and Meeks-Wagner, 1991; Pérez-Ruiz et al., 2015). Both genes also have  
288 opposite effects on root growth: *XAL2* is necessary for normal patterning of root meristem, at  
289 least partly through auxin transport (Garay-Arroyo et al., 2013), whereas *TFL1* was recently  
290 identified as a repressor of root growth (Lachowiec et al., 2015). We observed that the two genes  
291 were differentially expressed in the roots during the 22-h LD, but again in opposite ways: *XAL2*  
292 was down-regulated and *TFL1* was up-regulated, a situation that in the shoot would delay  
293 flowering and in the root would repress growth. The upregulation of *TFL1* in the root is thus  
294 intriguingly similar to what is observed in the shoot meristem where activation of *TFL1* at floral  
295 transition is important to counterbalance incoming flowering signals (Jaeger et al., 2013) but  
296 whether this is relevant in the root requires further investigation.

297 In both the global and experimental microarray analyses, the photoperiodic pathway was found  
298 to be enriched in the roots and several regulators of *CO* were differentially expressed during the  
299 induction of flowering by one LD. Among them we found *CDFs* and *SPA1*, involved in the

300 proteolysis of the CO protein. These results are striking since *CO* itself was not detected in the  
301 roots, confirming the very low level reported in other microarray studies (e.g. Birnbaum et  
302 al.(2003)). In Takada and Goto (2003), several *CO::GUS* reporter lines showed an expression in  
303 the roots while others did not, suggesting that the genomic region in which the transgene is  
304 inserted may alter the function of *CO* promoter, which would therefore not reflect its actual  
305 expression pattern in roots. Regulators of *CO* thus have other putative targets in the roots, which  
306 remain to be discovered. Interestingly, two *CO*-like genes (*COL5* and *STO*) were found to be  
307 downregulated during the inductive LD but whether they share regulatory mechanisms with *CO*  
308 is currently unknown.

309 Some genes of the photoperiodic pathway that are expressed in the roots encode photoreceptors,  
310 such as *PHYTOCHROME A-B-C*, and *CRYPTOCHROME1-2* (Supplemental Table 2). Direct  
311 light effects on root growth are well documented and several reports therefore recommend to  
312 conduct experiments with roots kept in darkness (Yokawa et al., 2013; 2014; Silva-Navas et al.,  
313 2015). However, numbers of studies on root architecture in Arabidopsis are performed *in vitro*  
314 and routine protocols consist in growing seedlings in transparent Petri dishes with all parts being  
315 illuminated. The majority of the root microarrays used for the data mining were obtained from  
316 material harvested in these conditions (1,040 out of 1,673 arrays, Supplemental Table 1). We can  
317 then speculate that root illumination introduced a bias in the assembled dataset. By contrast, in our  
318 hydroponic device, roots were completely in darkness and hence we can assume that any light  
319 effect would be indirect. We tested this hypothesis by crossing our dataset with a transcriptomic  
320 analysis of seedling roots grown in the dark and exposed to 1-h red light (Molas et al., 2006).  
321 After aligning the filter settings of Molas et al.'s analysis with ours, only 55 genes that were  
322 differentially expressed after the 1-h red light treatment were detected in the roots in our  
323 hydroponics device. Out of them, 11 were differentially expressed during the 22-h LD, including  
324 two flowering-time genes: *STO* and *ELF4* (Supplemental Table 5). It is noteworthy however that  
325 if both genes are indeed induced by light and interact with different components of light signaling  
326 (Khanna et al., 2003; Indorf et al., 2007), they also exhibit circadian expression pattern (Doyle et  
327 al., 2002; Indorf et al., 2007), which is the most likely reason why they were differentially  
328 expressed in LD.

329

330

331 We indeed estimated that around 70% of the genes that are differentially expressed in the roots  
332 during the 22-h LD are regulated by the circadian clock. This proportion is probably  
333 overestimated since it was calculated by crossing our dataset with public databases filtered with  
334 low stringency tools to retrieve rhythmic gene expression patterns (see Materials and Methods).  
335 The clock mechanism was shown in *Arabidopsis* to rely on three interlocking feedback loops  
336 (Hsu and Harmer, 2014). The morning-phased loop comprises *PPR7* and *PPR9* and is activated  
337 by *CCA1* and *LHY*; the evening-loop includes *EARLY FLOWERING 3* (*ELF3*), *ELF4* and  
338 *LUX ARRHYTHMO*, which act together in an evening complex, and other evening genes  
339 including *GI* and *TIMING OF CAB EXPRESSION 1* (*TOC1*). The central loop makes the link  
340 between the two others since *TOC1* activates *CCA1* and *LHY* whereas *CCA1* and *LHY* proteins  
341 repress *TOC1* (Harmer et al., 2000; Alabadi et al., 2001).

342 Interestingly, we found that members of the evening loop - *GI* and *ELF4* - were upregulated  
343 whereas morning genes such as *CCA1* and *LHY* were downregulated in 22-h LD as compared to  
344 8-h SD. These differential expression levels were recorded at the two time points (h16 and h22)  
345 and were probably due to a delay in the expression patterns of these circadian genes upon  
346 extension of the photoperiod, as indicated by the time-course analyses (Figure 5) and also  
347 reported in other studies (de Montaigu et al., 2015). Such changes might reflect the fact that the  
348 circadian clock in plants is entrained to light:dark cycles by photosynthetic inputs. It is known  
349 indeed that sugars derived from photosynthesis entrain the circadian clock through morning genes  
350 in the shoot (Haydon et al., 2013) and that a shoot-derived photosynthesis product is necessary for  
351 the oscillation of the evening genes in the roots (James et al., 2008). Moreover, the circadian  
352 clock orchestrates the coordinate adjustment of carbon partitioning and growth rate that occurs in  
353 response to photoperiod (Yazdanbakhsh et al., 2011). Consistently, we observed the differential  
354 expression of *ADGI*, encoding a subunit of AGPase involved in starch synthesis, and of *TPSI* that  
355 catalyses formation of T6P, during the 22-h LD. T6P was found to mediate the sugar-dependent  
356 post-translational activation of AGPase (Geigenberger, 2011) and hence upregulation of *ADGI*  
357 and of *TPSI* might cooperatively stimulate starch synthesis in the roots during the extension of the  
358 photoperiod. Moreover, T6P was found to be positively correlated with rosette growth rate

359 (Sulpice et al., 2014) and to be required in the leaves and the shoot apical meristem at flowering  
360 (Wahl et al., 2013). All together, our results suggest that roots are integrated in systemic signaling  
361 whereby carbon supply coordinates growth at the whole plant level during the induction of  
362 flowering. This coordination possibly involves sugar input to the circadian clock and T6P  
363 pathway.

364 This inference is further supported by our *de novo* analysis of the promoters of genes  
365 upregulated at h16 and h22 during the 22-h LD. Both time points revealed an enrichment of the  
366 telo-box motif, which is present in the promoter of genes expressed in dividing cells of root  
367 meristems and is known to mediate the upregulation of glucose-responsive genes (Rook et al.,  
368 2006). The telo-box, which would be part of a midnight regulatory module (Michael et al.,  
369 2008), is frequently found associated with other motifs, such as the site II element  
370 (Trémousaygue et al., 2003; Zografidis et al., 2014) that we also found in our analysis. The  
371 functional relevance of the association between these elements has been demonstrated for the  
372 SKIP-mediated control of root elongation (Zhang et al., 2012). Conversely, the promoters of  
373 genes downregulated during the 22-h LD were found to be enriched in both I-boxes, which are  
374 known to be part of a light regulatory module (López-Ochoa et al., 2007), and in the sugar- and  
375 gibberellin-responsive element TATCCA, which is bound by MYB factors (Lu et al., 2002).  
376 TATCCA element and G-box were also found to be core components of the sugar response  
377 sequence (SRS) in the promoter of a sugar starvation-inducible rice  $\alpha$ -amylase gene (Amy3, Lu  
378 et al. (1998)). These results support a prominent role for sugars in the control of gene expression  
379 during the 22-h LD.

380 Another coincidence is the enrichment of differentially expressed genes in the phloem tissue of  
381 the roots, which is the arrival route of sugars transported from the shoot. For example *IPT3* and  
382 *IPT7*, two cytokinin-biosynthesis genes expressed in the root vasculature and the endodermis  
383 (Hirose et al., 2008), were differentially expressed during the 22-h LD whereas *IPT5*, which is  
384 expressed in the root cap, was not. An increased transport of cytokinins from the roots to the  
385 aerial part of the plant would establish a feedforward loop promoting flowering since these  
386 hormones are known to activate promoters of flowering in the shoot, such as *TSF* in the leaves  
387 and *SOCI* in the shoot apical meristem (D'Aloia et al., 2011). These mechanisms provide a

388 molecular basis to the physiological shoot-to-root-to-shoot loop disclosed in the mustard *Sinapis*  
389 *alba* where sucrose arriving from the shoot induces cytokinin export from the roots to stimulate  
390 floral transition (Havelange et al., 2000).

## 391 MATERIAL AND METHODS

### 392 Plant growth

393 All experiments were performed with *Arabidopsis thaliana* Col-0 accession. The *ipt3* single and  
394 *ipt3;5;7* triple mutants were provided by Prof. Tatsuo Kakimoto (Osaka University, Japan) and  
395 the *gi* mutant was given by Prof. George Coupland (Max Planck Institute for Plant Breeding  
396 Research, Köln, Germany). Other mutants were obtained from the Nottingham Arabidopsis  
397 Stock Center (<http://www.arabidopsis.info>). Accession numbers are provided in the  
398 Supplemental Table 4. All seeds, including Col-0 WT, were bulked at the same time to reduce  
399 variability. Plants were grown in hydroponic device made of black containers and accessories  
400 (<http://www.araponics.com>). Nutrient solution was a mix of commercial stocks (0.5 ml l<sup>-1</sup>  
401 FloraMicro, FloraGro and FloraBloom; <http://www.generallyhydroponics.com>). Light was  
402 provided by fluorescent white tubes at 60  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  PPFD; temperature was 20°C (day/night)  
403 and air relative humidity 70%. For transcriptomic analyses in WT plants, flowering was induced  
404 by a single 22-h LD after 7 weeks of growth in 8-h SD and the flowering response was scored as  
405 the % of plants having initiated floral buds two weeks after the LD (Tocquin et al., 2003). For  
406 mutant phenotyping, plants were cultivated in 16-h LD and duration of vegetative growth was  
407 scored as the total number of leaves below the first flower (rosette + cauline leaves) to estimate  
408 flowering time.

### 409 Microarray analysis

410 Roots of 18 individual plants were harvested 16h and 22h after the beginning of the inductive  
411 LD and pooled. Sampling at the same times in 8-h SD happened during the dark period and were  
412 performed under dim green light. Roots were stored at -80°C until used. Tissues were ground in  
413 liquid nitrogen and RNA was extracted with TRizol according to manufacturer's instructions

414 ([www.lifetechnologies.com](http://www.lifetechnologies.com)). Before processing further with the RNA samples, we assessed  
415 RNA integrity with the Experion<sup>tm</sup> automated electrophoresis system ([www.bio-rad.com](http://www.bio-rad.com)). All  
416 the samples used for microarray analysis had maximum RNA quality indicator (RQI) values of  
417 10. The RNA samples were labeled using 3' IVT Expressed kit according to the manufacturer's  
418 instructions (Affymetrix, [www.affymetrix.com](http://www.affymetrix.com)). Three biological replicates obtained from  
419 independent experiments were hybridized on ATH1 Genome arrays (Affymetrix). We analyzed  
420 raw data using the limma package (Ritchie et al., 2015). Data were GCRMA-normalized and  
421 probeset were filtered for an absolute expression level of at least 100 in  $\geq 20\%$  of the arrays. We  
422 fitted the data to a linear model using the `lmfit()` function, analyzed the variance with the  
423 `ebayes()` function, and corrected the p-value for multiple testing using Benjamini and  
424 Hochberg's method (Benjamini and Hochberg, 1995). We considered genes as being  
425 differentially expressed when the adjusted p-value was  $\leq 0.01$  and fold-change  $\geq 2$ .

## 426 ***In silico* analysis**

427 Data mining - *In silico* transcriptomic analyses were performed on Arabidopsis Affymetrix  
428 ATH1 raw data retrieved from the ArrayExpress database (<http://www.ebi.ac.uk/arrayexpress/>)  
429 using the query "roots". The resulting list was manually sorted to remove experiments lacking  
430 comprehensive methodological information. Each experiment was manually curated to select  
431 only root-specific raw files. The list of experiments included in the survey is available in  
432 supplemental material (Supplemental Table 1). The subsequent data analysis was performed  
433 using the R programming language (R Core Team). The "simpleaffy" Bioconductor package  
434 V.2.44.0 (Huber et al., 2015; Wilson and Miller, 2005) was used to read the raw data and  
435 perform the present/absent call on individual arrays using the `detection.p.val()` function. Genes  
436 were considered as being expressed when p-value  $< 0.01$ . Within each experiment, we computed  
437 the proportion of arrays in which expression of the gene of interest could be detected.

438 Experimental microarray analyses - The analysis of tissue enrichment was performed using the  
439 dataset published in Brady et al. (2007). Each gene represented in the ATH1 arrays was  
440 associated with the tissue where its expression level was maximal in Brady's study. The  
441 resulting map was used to localize the genes identified in our study and to calculate their

442 distribution among the different tissues of the roots. This exercise was performed on our  
443 microarray analysis with the list of all root-expressed genes (expression level of at least 100 in  $\geq$   
444 20% of the arrays) or the genes differentially expressed during the photoperiodic induction of  
445 flowering (adjusted p-value  $\leq 0.01$ ). Using the resulting data, we performed a Fisher's exact test  
446 to determine whether tissues were over- or under-represented in the differentially expressed  
447 genes list; the tissues in which the number of differentially expressed genes was higher than the  
448 expected value was tested for over-representation while tissues in which the number of  
449 differentially expressed genes was lower than the the expect number was tested for under-  
450 representation (p-value  $\leq 0.01$ ).

451 The Gene Ontology Enrichment analysis was performed using the topGO package V2.20.0  
452 (Alexa and Rahnenfuhrer, 2010) with the annotation of the ATH1 array from ath1121501.db  
453 package V3.1.4. We performed a Biological Process (BP) enrichment analysis using the classic  
454 Fisher's exact test (p<0.001). Redundant GO terms were removed. The expected numbers of  
455 differentially expressed genes were computed based both on the total number of root-expressed  
456 genes (see above) and the number of differentially expressed genes in our microarray analysis.

457 The analysis of circadian clock-regulated genes exploited datasets obtained in studies of the  
458 circadian clock in shoots (Covington et al., 2008) and lateral roots (Voß et al., 2015). To identify  
459 the shoot circadian clock-regulated genes, Covington and colleagues analyzed different  
460 publically available circadian microarray datasets. We used the list containing the highest  
461 number of circadian clock-entrained genes. In Voß's study, the authors identified highly-  
462 probable circadian clock-regulated genes in the roots using three different analysis tools. The list  
463 we selected was based on the less stringent parameters, as we included the genes predicted to be  
464 clock-regulated by at least one of those tools. When we crossed our experimental list of  
465 differentially expressed genes with these datasets, we found that some differentially expressed  
466 genes were not represented in Covington's or Voß's arrays and hence we excluded them for the  
467 comparison.

## 468 RT-qPCR analysis

469 Roots of 18 individual plants were harvested every 4h during the 22-h LD and at the same times  
470 in control 8-h SD. Roots were stored at -80°C until used. Tissues were ground in liquid nitrogen  
471 and RNA was extracted with TRizol according to manufacturer's instructions  
472 ([www.lifetechnologies.com](http://www.lifetechnologies.com)). RNA samples were treated with DNase (0.2 U DNase  $\mu\text{g}^{-1}$ ). We  
473 synthesized first-strand cDNA from 1.5  $\mu\text{g}$  RNA using MMLV reverse transcriptase and  
474 oligo(dT)15 according to manufacturer's instructions (<http://www.promega.com>). Quantitative  
475 PCR (qPCR) reactions were performed in triplicates using SYBR-Green I  
476 (<http://www.eurogentec.com>) in 96-well plates with an iCycler IQ5 (<http://www.bio-rad.com>).  
477 We extracted quantification cycle (Cq) values using the instrument software and imported the data  
478 in qbase<sup>PLUS</sup> 2.0 (<http://www.biogazelle.com>). A GeNorm analysis (Vandesompele et al., 2002)  
479 was performed in a preliminary experiment to identify suitable reference genes. We selected  
480 *ACTIN2* (*ACT2*) and *TUBULIN2* (*TUB2*) as reference genes for root kinetic expression analysis  
481 (geNorm M value <0.2). The computed geometric mean of their Cq values was used to calculate  
482 the normalization factor, as described in (Vandesompele et al., 2002). The list of primers is  
483 available in Supplemental Table 6.

## 484 Root phenotyping

485 Plants grown for root architecture analysis were sown in vitro on 0.5x MS supplemented with  
486 1% sucrose. Square Petri dishes were used and placed vertically, under 100  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  PPFD, in  
487 16h-LD. Root pictures were taken every three days using a CCD camera (Canon EOS 1100D  
488 with a Canon Lense EF 50mm 1:1.8) and analyzed using the ImageJ plugin "SmartRoot" (Lobet  
489 et al., 2011). The resulting root tracing were exported and analysed in R (R Core Team). In order  
490 to select the genotypes whose root system significantly differed from WT, we performed a  
491 Principal Component Analysis (PCA) using the length of the primary root, the length of the  
492 apical unbranched zone, the lateral root density, the lateral root number, the total lateral root  
493 length and the lateral root angle. The resulting PC's were compared using Student tests with a  
494 threshold at  $p < 0.01$ . The selected genotypes were then compared to WT for each variable (t-test,  
495  $p < 0.01$ ). Data visualization was performed using ggplot2 package (Wickham, 2009).

## 496 **Cis-elements analysis**

497 For each subsets of similarly controlled genes, we prepared a fasta formatted file containing the  
498 promoter sequences (-500, +50) obtained from the TAIR10 ftp repository (Lamesch et al., 2012).  
499 The analyses were performed using the command line version of the MEME-Suite (Bailey et al.  
500 (2015), <http://meme-suite.org>, version 4.10.0). The parameters for MEME were set as default  
501 values, except for: maximum width of each motif: 15 bp; maximum number of motifs to find:  
502 10; background sequences: all TAIR10 promoters (-500, +50). The parameters for DREME and  
503 AME were set as default values, with the background sequences being the promoters of the  
504 10,508 genes found expressed in roots in this study.

## 505 **Accession numbers**

506 Microarray data are available in the ArrayExpress database (<http://www.ebi.uk/arrayexpress>)  
507 with the accession numbers E-MTAB-4129 and E-MTAB-4130.

## 508 **Supplemental data**

509 **Supplemental Table 1:** List of root micro-arrays used for the data-mining analysis.

510 **Supplemental Table 2:** Flowering-time genes in roots.

511 **Supplemental Table 3:** List of genes differentially expressed in the roots during a 22h LD.

512 **Supplemental Table 4:** List of mutants characterized in the present work.

513 **Supplemental Table 5:** List of genes differentially expressed in the roots during a 22h LD and in  
514 Molas et al. (2006).

515 **Supplemental Table 6:** List of primers used for the RT-qPCR analysis

516

## 517 **ACKNOWLEDGMENTS**

518 The authors would like to thank Kévin Mistiaen for his participation to the setup of microarray  
519 analysis pipeline. FB and GL are grateful to the F.R.S.-FNRS for the award of a Ph.D.  
520 fellowship (FC 87200) and a postdoctoral research grant (1.B.237.15F) respectively. This  
521 research was funded by the Interuniversity Attraction Poles Programme initiated by the Belgian  
522 Science Policy Office, P7/29. We thank Prof. Tatsuo Kakimoto (Osaka University, Japan) and  
523 Prof. George Coupland (Max Planck Institute for Plant Breeding Research, Köln, Germany) who  
524 kindly provided us with *ipt3 / ipt3;5;7* and *gi* seeds, respectively.

## 525 **AUTHOR CONTRIBUTIONS**

526 FB, MD and CP designed the experiments. FB, MD, and ND performed the experiments. FB did  
527 the microarray analysis. PT did the promoter analysis. FB and GL did the data analysis and  
528 figures. FB, CP, PT and GL participated to the writing of the manuscript. All co-authors read  
529 and approved the final version of the manuscript.

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## 770 FIGURES LEGENDS

### 771 **Figure 1. Flowering-time genes expressed in the roots of *Arabidopsis*** 772 ***thaliana*.**

773 Root-expressed genes were identified by a present/absent call on 1,673 root ATH1 arrays  
774 retrieved from ArrayExpress repository (<https://www.ebi.ac.uk/arrayexpress/>). Flowering-time  
775 genes were extracted from FLOR-ID. **A.** All 306 flowering genes. **B.** Pie charts showing the  
776 same set of genes classified into flowering time pathways, circadian clock and flower  
777 development. Some genes are involved in more than one pathway. Pie chart area is proportional  
778 to gene number. **C.** The snapshot of flowering pathways was extracted and adapted from FLOR-  
779 ID. Genes highlighted in green boxes were detected in  $\geq 50\%$  of root arrays. Genes in blue boxes  
780 were detected in  $< 50\%$  arrays. Genes and compounds not analyzed in ATH1 arrays are in grey.

### 781 **Figure 2. Root transcriptome changes during the induction of** 782 **flowering by a single 22-h LD.**

783 **A.** Experimental design. The proportions of plants having initiated flower buds two weeks after  
784 the experiment are shown on the right. **B.** Heatmap of the differentially expressed genes  
785 (adjusted p-value  $\leq 0.01$ ; fold-change  $\geq 2$ ) showing three independent biological replicates per  
786 condition. Low expression levels in red, high expression levels in green. Relative expression  
787 values are scaled per transcript (lines). **C.** Venn diagram of differentially expressed genes at both  
788 sampling time points. **D.** List of differentially expressed flowering-time genes.

789

790 **Figure 3. Enrichment analyses of the 595 genes differentially**  
791 **expressed in the roots during an inductive 22-h LD.**

792 **A.** Tissue enrichment. For each gene, expression was localized in the tissue where Brady et al.  
793 (2007) found highest transcript level. In each tissue, the number of differentially expressed genes  
794 is indicated in bold whereas the number of genes that would be expected for this dataset is  
795 enclosed within brackets. Shaded area shows p-values > 0.01. Over- and under-represented  
796 genes are separated by the horizontal dashed red line. **B.** Gene ontology term enrichment in the  
797 list of 595 differentially expressed genes. The number of differentially expressed genes  
798 experimentally associated with each term is indicated in bold, whereas the number of genes  
799 associated with the GO term that would be expected by chance for this dataset is enclosed within  
800 brackets. Bars indicate the  $-\log_{10}(\text{p-value})$  for each term (Fisher's exact test). **C.** Motif  
801 enrichment analysis in the -500 to +50 nt region of the genes that were down- or up-regulated at  
802 h16 or at h22 in LD. Numbers are the p-values of motifs that were identified as enriched by  
803 AME at  $p < 0.05$  in any of the 4 differentially expressed gene subsets. / indicates non-enriched  
804 motif.

805 **Figure 4. Temporal aspects of transcriptomic changes.**

806 **A.** Time-course analyses of candidate gene expression. Relative transcript levels were analysed  
807 by RT-qPCR during an 8-h SD (closed symbols) or a single 22-h LD (open symbols). Boxes in  
808 the bottom show light (white) and dark (black) periods. Data were normalized using *ACT2* and  
809 *UBQ10* genes. Error bars indicate the standard error of the mean for three experimental  
810 replicates. Data are from one representative experiment. **B.** Estimate of circadian clock-regulated  
811 differentially expressed genes. Venn diagrams showing the overlap between the differentially  
812 expressed genes identified in this study and the circadian clock-regulated genes expressed in  
813 lateral roots [left; Dataset from Voß et al. (2015)] or in the shoot [right; Dataset from Covington  
814 et al. (2008)].

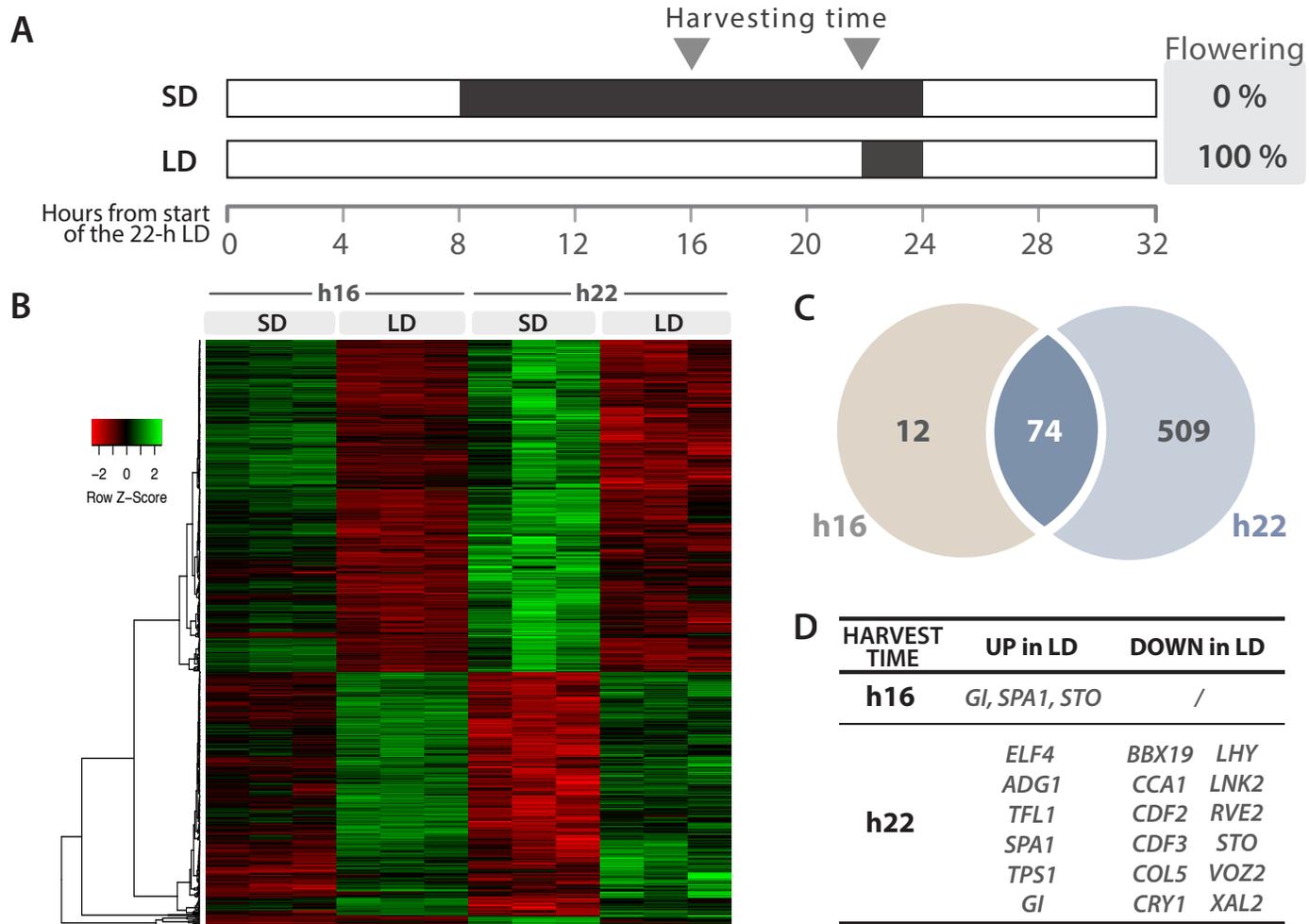
815

816 **Figure 5. Flowering-time and root architecture phenotypes of selected**  
817 **mutants in 16-h LD.**

818 **A.** Total number of leaves below the first flower (n=15). \* indicates a significant difference with  
819 WT Col-0 (Tukey's HSD test,  $p < 0.05$ ). \*\*\* indicates a highly significant difference with WT  
820 (Tukey's HSD test,  $p < 0.01$ ). WT is shown in blue. **B.** Plot of the first two components of the  
821 Principal Component Analysis performed on root system architecture features. **C.** Biplot of the  
822 two first components of the PCA. Orange color indicates significant differences with the WT  
823 Col-0.

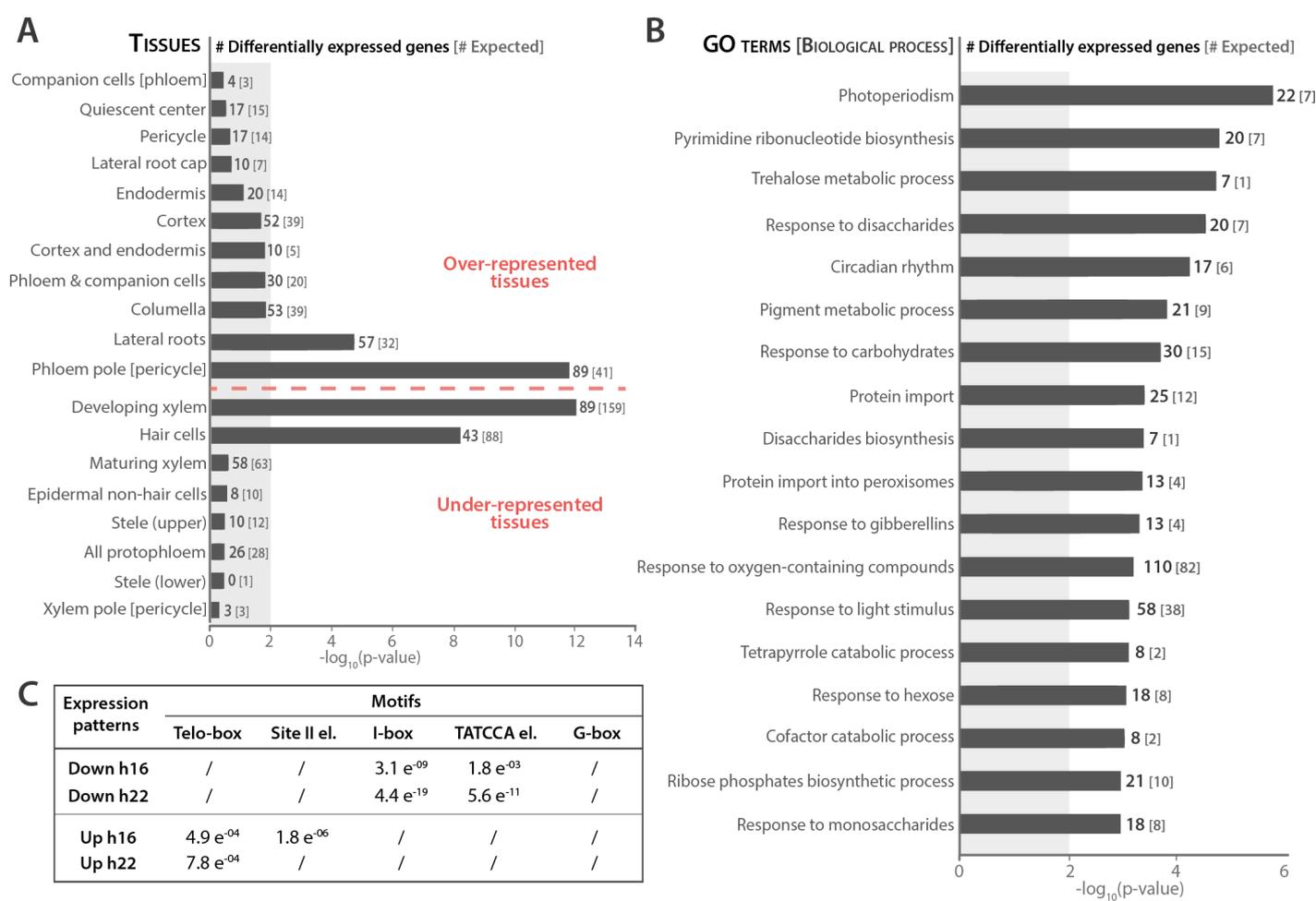
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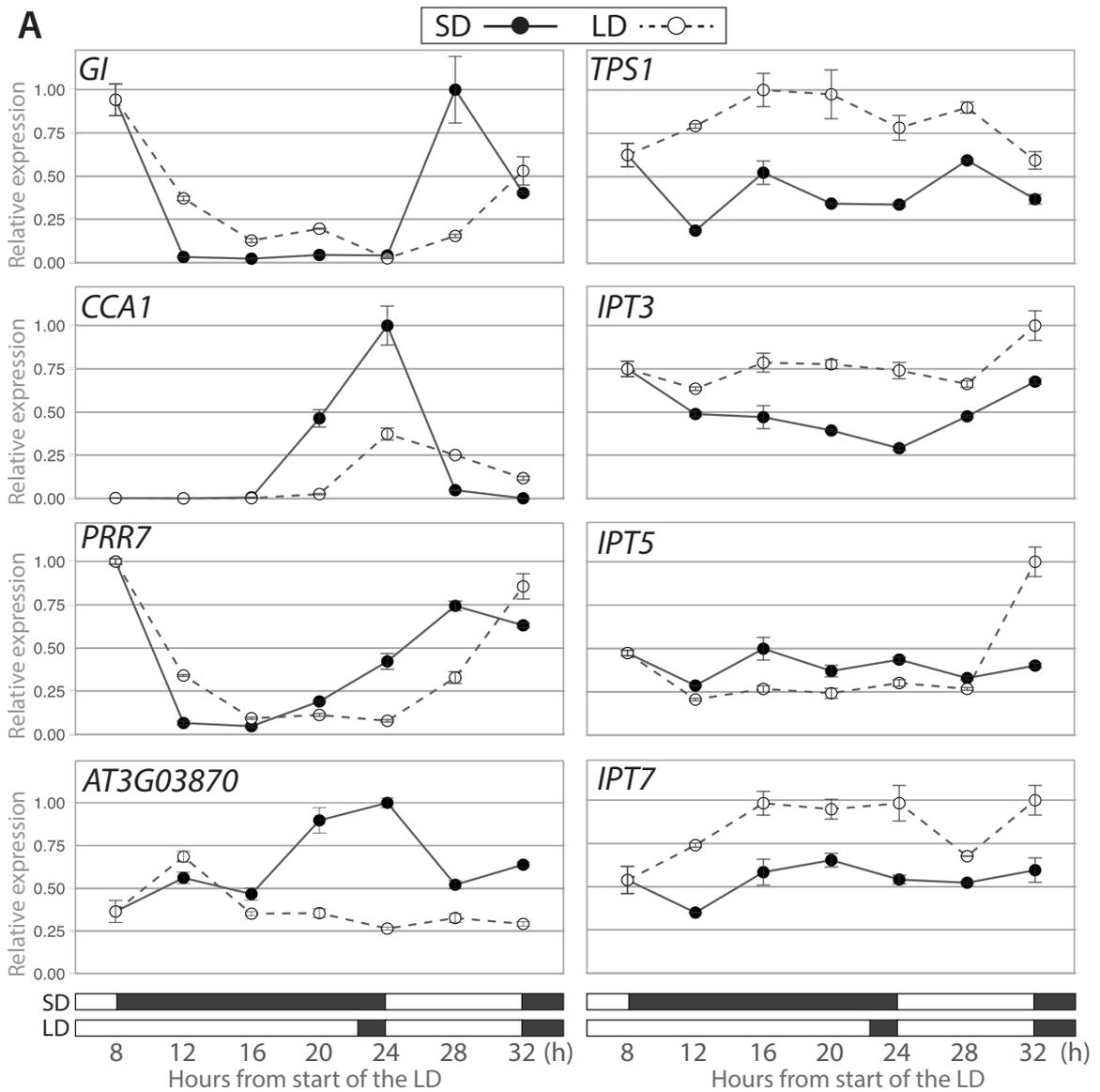


**Figure 2. Root transcriptome changes during the induction of flowering by a single 22-h LD. A.**

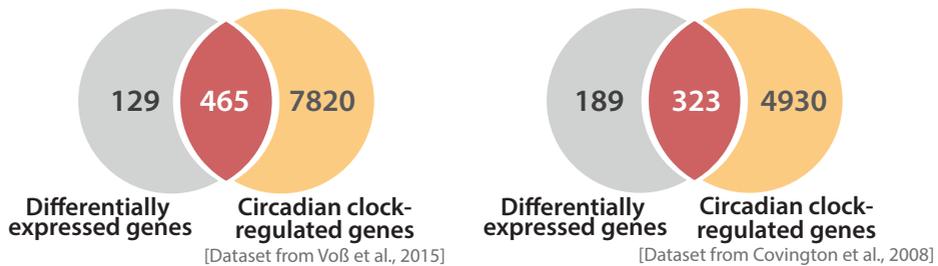
Experimental design. The proportions of plants having initiated flower buds two weeks after the experiment are shown on the right. **B.** Heatmap of the differentially expressed genes (adjusted p-value  $\leq 0.01$ ; fold-change  $\geq 2$ ) showing three independent biological replicates per condition. Low expression levels in red, high expression levels in green. Relative expression values are scaled per transcript (lines). **C.** Venn diagram of differentially expressed genes at both sampling time points. **D.** List of differentially expressed flowering-time genes.



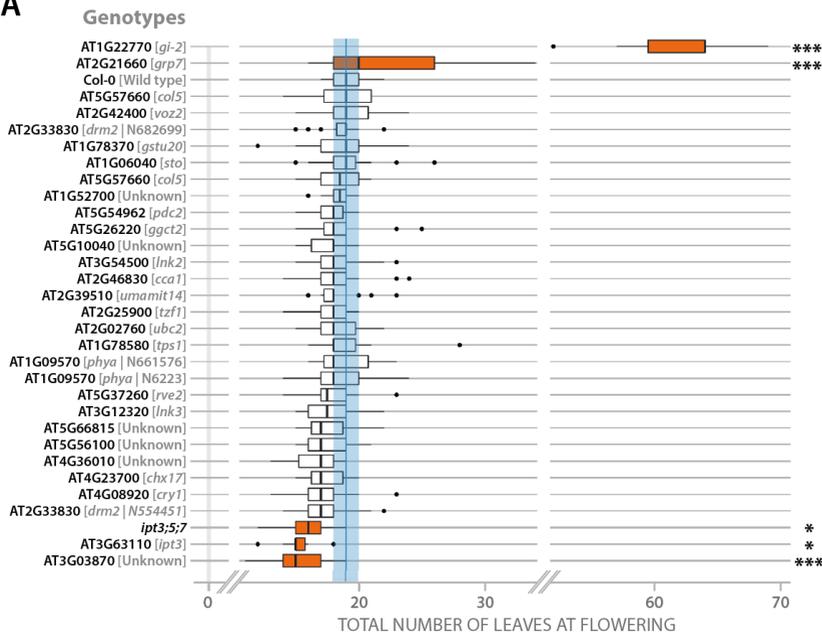
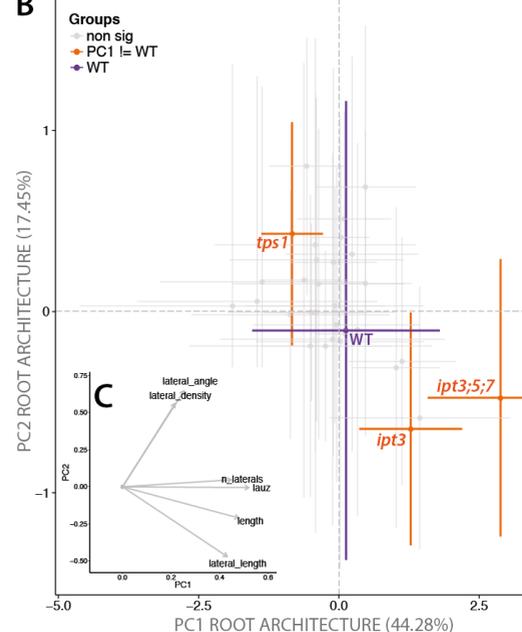
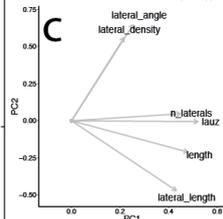
**Figure 3. Enrichment analyses of the 595 genes differentially expressed in the roots during an inductive 22-h LD.** **A.** Tissue enrichment. For each gene, expression was localized in the tissue where Brady et al. (2007) found highest transcript level. In each tissue, the number of differentially expressed genes is indicated in bold whereas the number of genes that would be expected for this dataset is enclosed within brackets. Shaded area shows p-values > 0.01. Over- and under-represented genes are separated by the horizontal dashed red line. **B.** Gene ontology term enrichment in the list of 595 differentially expressed genes. The number of differentially expressed genes experimentally associated with each term is indicated in bold, whereas the number of genes associated with the GO term that would be expected by chance for this dataset is enclosed within brackets. Bars indicate the  $-\log_{10}(\text{p-value})$  for each term (Fisher's exact test). **C.** Motif enrichment analysis in the -500 to +50 nt region of the genes that were down- or up-regulated at h16 or at h22 in LD. Numbers are the p-values of motifs that were identified as enriched by AME at  $p < 0.05$  in any of the 4 differentially expressed gene subsets. / indicates non-enriched motif.



**B**



**Figure 4. Temporal aspects of transcriptomic changes.** **A.** Time-course analyses of candidate gene expression. Relative transcript levels were analysed by RT-qPCR during an 8-h SD (closed symbols) or a single 22-h LD (open symbols). Boxes in the bottom show light (white) and dark (black) periods. Data were normalized using *ACT2* and *UBQ10* genes. Error bars indicate the standard error of the mean for three experimental replicates. Data are from one representative experiment. **B.** Estimate of circadian clock-regulated differentially expressed genes. Venn diagrams showing the overlap between the differentially expressed genes identified in this study and the circadian clock-regulated genes expressed in lateral roots [left; Dataset from (Voss et al., 2015)] or in the shoot [right; Dataset from (Covington et al., 2008)].

**A****B****C**

**Figure 5. Flowering-time and root architecture phenotypes of selected mutants in 16-h LD. A.**

Total number of leaves below the first flower (n=15). \* indicates a significant difference with WT Col-0 (Tukey's HSD test,  $p < 0.05$ ). \*\*\* indicates a highly significant difference with WT (Tukey's HSD test,  $p < 0.01$ ). WT is shown in blue. **B.** Plot of the first two components of the Principal Component Analysis performed on root system architecture features. **C.** Biplot of the two first components of the PCA.

Orange color indicates significant differences with the WT Col-0.