FLOWERING GOES UNDERGROUND

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Flowering is a crucial step in plant life cycle and is therefore tightly controlled by both environmental and endogenous cues. The involvement of the aerial organs of the plant in the molecular mechanisms controlling floral transition has been extensively documented while the participation of the roots remains poorly investigated. However, the induction of flowering by photoperiod involves systemic signals that move in the phloem from leaves to sinks, and hence presumably reach the roots. We therefore performed a transcriptomic analysis of the roots during the induction of flowering in Arabidopsis thaliana and indeed identified a large number of differentially expressed genes. A reverse genetic approach further confirmed the pleiotropic effects of flowering time genes on root architecture.

FLOWERING TIME GENES ARE EXPRESSED IN ROOTS

The genetic control of flowering is complex : deepest knowledge has been accumulated on *Arabidopsis* where we identified 278 flowering time genes from a litterature survey. We then crossed the list with transcriptomic data obtained from roots and found that 98 flowering time genes were expressed in the roots of *Arabidopsis* plants grown in hydroponics, on soil, or *in vitro* (fig. 1a,b). Among them, genes involved in photoperiodism or light perception were well represented whereas others – e.g. genes involved in flower development - were not (fig. 1c).

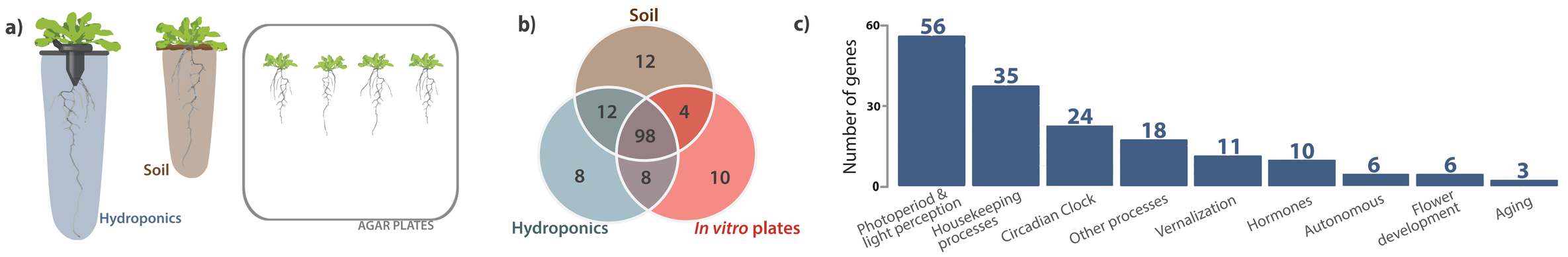

Figure 1. Genes controlling flowering time in Arabidopsis are expressed in the roots. (a) Root transcriptomic data were obtained from plants grown in hydroponics, on soil and *in vitro*. Plants grown in hydroponics and on soil were harvested after 6 weeks of growth in 10-h short days. The *in vitro* analysis (arrayexpress database E-GEOD-5631) was performed with 15-day old seedlings grown on 1x MS medium, 0.5 % sucrose, in 16-h long days. All transcriptomic analyses were performed in triplicate using ATH1 arrays (Affymetrix). **(b)** Venn diagram of flowering time genes expressed in roots of plants grown on different substrates. Genes were considered as expressed if their transcripts could be detected in 1/3 of the arrays at least ($p < 0.01$). **(c)** Number of flowering time genes expressed in roots and classified according to the pathway to which they belong.

FLORAL INDUCTION IMPACTS THE ROOT TRANSCRIPTOME

Because many flowering time genes were found to be expressed in the roots, we analyzed the changes in gene expression occurring in the roots during the induction of flowering. To do so, 7-week old vegetative plants grown in short days (SD) were induced to flower by exposure to a single 22-h long day (LD). Roots were harvested during the photoperiod extension for transcriptomic analysis. We found that hundreds of genes were differentially expressed in the roots in LD vs SD (fig. 2a). The biological significance of these changes was addressed by recording whether loss of function mutants had a flowering phenotype. Interestingly the cytokinin-biosynthesis genes *IPT3, 5 & 7* (*IPT; ISOPENTENYL TRANSFERASE*) were all up-regulated in the roots during the during the induction of flowering (fig. 2b). According to the root map published by Brady et al. (2007), the expression of *IPT3* and *IPT5* is restricted to some tissues of the roots (fig. 2c). We also looked at the expression pattern of well-characterized flowering time genes detected in roots (fig. 1b,c). Among them, we observed that *SHORT VEGETATIVE PHASE* (*SVP*) is expressed throughout the roots (fig. 2c).

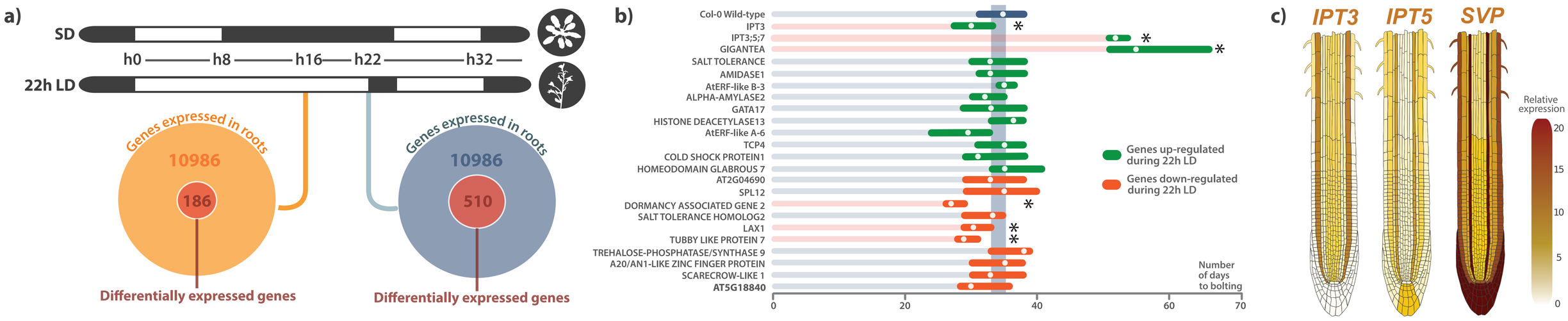

Figure 2. Microarray analysis of root transcriptome during the induction of flowering. (a) Plants of *Arabidopsis thaliana* were grown in hydroponics (Araponics), in 8-h short days (SD) for 7 weeks before receiving the flowering inductive 22-h long day (LD). Roots were harvested 16h and 22h after start of the LD and used for transcriptome analysis by cRNA hybridization of ATH1 arrays (Affymetrix). Raw data were normalized using GCRMA method and subsequently analysed with R. Data were obtained from biological triplicates for h16 and duplicates for h22. Red areas represent the proportion of genes that were differentially expressed at h16 and h22 (non-adjusted p-value < 0.001 ; fold change > 2). **(b)** Flowering time of selected mutants. Selected T-DNA lines were grown in hydroponics in 16-h LD and flowering time was determined by macroscopic appearance of floral buds. Data shown are from a representative experiment with 20 plants per genotype. Blue boxes indicate WT flowering time; green and red boxes show T-DNA mutants for genes that were up- or down-regulated in the roots at h16 of the inductive LD, respectively. Asterisks indicate statistically significant results (Tukey's test ; p-value < 0.05). **(c)** Tissue localisation of root expression of selected genes. Data were extracted from Brady et al. (2007) and mapped on a custom scheme using R.

FLOWERING TIME MUTANTS DISPLAY ROOT PHENOTYPES

In order to assess the relationship between flowering time control and roots, flowering time mutants and mutants isolated from the reverse genetic approach presented above were characterized for root architecture (fig. 3a). Unexpectedly, we observed that the late flowering *constans* (*co*) mutant had a striking root phenotype (fig. 3b) although we did not detect expression of the *CO* gene in the root (data not shown). By contrast, the *svp* mutant did not display any root phenotype albeit the gene was highly expressed throughout the roots (fig. 2c). The *dormancy associated2* mutant had a significant phenotype, including longer primary and secondary roots and an increased lateral root density. The same observations stand for the *ipt3;5;7* triple mutant but not for the *ipt3* single mutant.

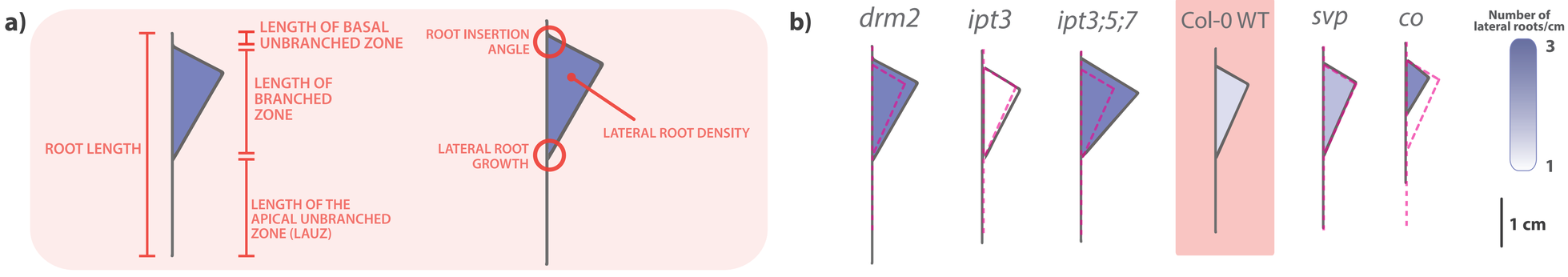

Figure 3. Several flowering time mutants display alterations in root architectures. (a) Root architecture was measured using SmartRoot (Lobet et al., 2011) and schematized as shown in figure 3a. **(b)** Root architecture of selected *Arabidopsis thaliana* genotypes grown *in vitro* in 16h-LD. Plants were grown for 13 days on 0.5 x MS, 1 % sucrose medium.

In conclusion, complementary approaches based on published expression data, transcriptomic experiments and genetic analyses indicate that genes involved in flowering time control are expressed in the roots and/or affect root architecture. Further experiments are on the way to downregulate flowering time genes in the roots by overexpressing artificial microRNAs in an organ-targeted manner.

References : Brady, S.M. et al., 2007. A high-resolution root spatiotemporal map reveals dominant expression patterns. Science, 318 (5851), 801-6.
Lobet, G. et al., 2011. Novel Image Analysis Toolbox Enabling Quantitative Analysis of Root System Architecture. Plant Physiology,157, 29-39.

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