Development 139, 2198-2209 (2012) doi:10.1242/dev.077164

Spatially distinct regulatory roles for gibberellins in the promotion of flowering of *Arabidopsis* under long photoperiods

Aimone Porri, Stefano Torti*, Maida Romera-Branchat and George Coupland‡

SUMMARY

The plant growth regulator gibberellin (GA) contributes to many developmental processes, including the transition to flowering. In *Arabidopsis*, GA promotes this transition most strongly under environmental conditions such as short days (SDs) when other regulatory pathways that promote flowering are not active. Under SDs, GAs activate transcription of *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) and *LEAFY* (*LFY*) at the shoot meristem, two genes encoding transcription factors involved in flowering. Here, the tissues in which GAs act to promote flowering were tested under different environmental conditions. The enzyme GIBBERELLIN 2 OXIDASE 7 (GA2ox7), which catabolizes active GAs, was overexpressed in most tissues from the viral *CaMV 35S* promoter, specifically in the vascular tissue from the *SUCROSE TRANSPORTER 2* (*SUC2*) promoter or in the shoot apical meristem from the *KNAT1* promoter. We find that under inductive long days (LDs), GAs are required in the vascular tissue to increase the levels of *FLOWERING LOCUS T* (*FT*) and *TWIN SISTER OF FT* (*TSF*) mRNAs, which encode a systemic signal transported from the leaves to the meristem during floral induction. Similarly, impairing GA signalling in the vascular tissue reduces *FT* and *TSF* mRNA levels and delays flowering. In the meristem under inductive LDs, GAs are not required to activate *SOC1*, as reported under SDs, but for subsequent steps in floral induction, including transcription of genes encoding SQUAMOSA PROMOTER BINDING PROMOTER LIKE (SPL) transcription factors. Thus, GA has important roles in promoting transcription of *FT*, *TSF* and *SPL* genes during floral induction in response to LDs, and these functions are spatially separated between the leaves and shoot meristem.

KEY WORDS: *Arabidopsis*, Flowering, Gibberellins

INTRODUCTION

Flowering occurs when the shoot apical meristem (SAM), from which all aerial tissues are derived, undergoes a developmental transition that allows the production of flowers instead of leaves. In *Arabidopis thaliana*, this transition is controlled by several pathways that are regulated by endogenous developmental signals or by external environmental cues (Fornara et al., 2010). These pathways include the photoperiodic pathway that promotes flowering in response to long days (LDs) characteristic of summer, and the response pathway to the growth regulator gibberellin, which has its strongest effect under short days (SDs).

In the photoperiodic pathway, transcription of the *FLOWERING LOCUS T* (*FT*) and *TWIN SISTER OF FT* (*TSF*) genes is activated specifically under LDs (Kobayashi and Weigel, 2007; Turck et al., 2008). These genes encode small proteins that are members of the CEN1, TFL1, FT (CETS) family related to phosphatidyl-ethanolamine-binding proteins (Kardailsky et al., 1999; Kobayashi et al., 1999; Pnueli et al., 2001). FT has been demonstrated to move through the phloem system to the SAM (Corbesier et al., 2007; Jaeger and Wigge, 2007; Mathieu et al., 2007). FT and TSF interact with the bZIP transcription factor FD, which is expressed at the shoot apical meristem (Abe et al., 2005; Wigge et al., 2005). Genetic analysis demonstrated that FT, TSF and FD all contribute to characteristic changes in gene expression at the SAM during floral transition, including induction of transcription of *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS* (*SOC1*) and *FRUITFULL* (FUL), which encode related MADS box transcription factors and are among the first genes to be activated after exposure of plants to LDs (Abe et al., 2005; Jang et al., 2009; Samach et al., 2000; Searle et al., 2006; Wang et al., 2009; Wigge et al., 2005). After induction of *SOC1*, expression of many flowering genes is rapidly induced in the meristem. These include members of the family of genes encoding the SQUAMOSA PROMOTER BINDING PROTEIN LIKE (SPLs) transcription factors. Three members of this family, *SPL3*, *SPL4* and *SPL5*, are direct targets of SOC1 and FD (Jung et al., 2011), while transcriptome profiling and in situ hybridization demonstrate that their expression also requires *FT TSF* and *SOC1 FUL* function (Schmid et al., 2003; Torti et al., 2012). Ectopic expression of *SPL3* accelerates flowering, supporting the idea that they are part of the floral inductive process (Cardon et al., 1997; Wang et al., 2009; Yamaguchi et al., 2009). Similarly, suppression of the function of many *SPL* genes through overexpression of *miR156*, which targets *SPL* mRNAs, delays floral transition (Schwab et al., 2005; Schwarz et al., 2008; Wu and Poethig, 2006). In turn, the floral meristem identity genes *APETALA 1* (*AP1*) and *LEAFY* (*LFY*), as well as the flowering-time gene *FRUITFULL* (*FUL*) are directly activated by SPL3 (Wang et al., 2009; Yamaguchi et al., 2009), whereas AP1 and LFY confer floral identity on developing primordia (Bowman et al., 1993). Thus, a series of direct interactions in the shoot meristem linking SOC1, SPLs and floral meristem identity genes reveals one route from floral induction by LDs to floral development.

Max Planck Institute for Plant Breeding Research, Carl von Linne Weg 10, D-50829 Cologne, Germany.

*Present address: Nomad Bioscience, Weinbergweg 22, D-06120 Halle/Saale, Germany
‡Author for correspondence (coupland@mpiz-koeln.mpg.de)

Accepted 5 April 2012

Genetic analysis suggests that gibberellins have their most important function in flowering under SD. The *ga1-3* mutant, which is impaired in GA biosynthesis, fails to flower in SD but shows a relatively weak late-flowering phenotype under LD (Wilson et al., 1992). The stronger effect of GA under SDs, is probably due to the photoperiodic pathway masking the effect of loss of GA signalling under LDs (Reeves and Coupland, 2001). A mechanistic basis for the interaction between the photoperiodic and GA pathways is suggested by the convergence of both pathways on the promotion of *SOC1* transcription in the meristem (Achard et al., 2004; Moon et al., 2003; Searle et al., 2006). Furthermore, flowering of *soc1* mutants shows reduced sensitivity to GA treatments (Moon et al., 2003). Previous reports demonstrated that GA activates later events in the meristem during flowering, such as the activation of *LFY* transcription (Blazquez et al., 1998), although it is now unclear whether these are an indirect consequence of increased *SOC1* expression. In addition, GA has been reported to affect flowering by other mechanisms, but these are not yet clearly integrated into the flowering network. GA increases expression of miR159 and of its target mRNA encoding the MYB transcription factor MYB33 (Achard et al., 2004), which has been proposed to regulate *LFY* expression (Achard et al., 2004; Gocal et al., 2001; Woodger et al., 2003). In addition, the *GATA NITRATE INDUCIBLE CARBON-METABOLISM INVOLVED* (*GNC*) and *GNC-LIKE* (*GNL*) genes encode GATA factors that inhibit flowering, and are repressed by GAs (Richter et al., 2010). Finally, *FT* transcript is reduced in the strong GA biosynthetic mutant *ga1-3* after transfer from SD to far-red enriched LD (Hisamatsu and King, 2008). The relevance of this observation to floral induction under standard white light LD conditions has not yet been demonstrated. Overall, GA may regulate flowering of *Arabidopsis* by different mechanisms that are not clearly distinguished.

Bioactive GAs, particularly GA1, GA4 and GA3, are synthesized through a complex pathway (Yamaguchi, 2008). Genes encoding the enzyme GA20 oxidase, which is required to synthesize bioactive GA, are widely expressed in the plant, suggesting that GA is synthesized in most tissues (Rieu et al., 2008b). In addition, GA4 content increases 100-fold in the *Arabidopsis* shoot apex during the transition to flowering, although this could not be correlated with increased expression of biosynthetic enzymes (Eriksson et al., 2006). The levels of active GAs are also reduced by 2-β hydroxylation catalyzed by GA2 oxidases (GA2oxs) (Rieu et al., 2008a; Schomburg et al., 2003). In *Arabidopsis*, two classes of GA2oxs have been identified. Class I and II GA2oxs act directly on bioactive GA1 and GA4 to generate inactive hydroxylated forms. By contrast, Class III GA2oxs act earlier in the biosynthetic pathway to reduce the abundance of precursors of bioactive GAs. Overexpression of either class of GA2ox from the viral *CaMV 35S* promoter reduces the levels of bioactive GAs in vivo and causes phenotypes associated with GA depletion (Rieu et al., 2008a; Schomburg et al., 2003).

GAs regulate gene expression through a relatively short signal transduction pathway (Harberd et al., 2009). This pathway influences gene expression by promoting the degradation of DELLA proteins (Dill et al., 2004; Fu et al., 2004; McGinnis et al., 2003; Nakajima et al., 2006; Willige et al., 2007). This removal of DELLA proteins releases transcription factors that are otherwise prevented from binding DNA by DELLAs, including PHYTOCHROME INTERACTING FACTOR 4 (PIF4) and PIF5 (de Lucas et al., 2008; Feng et al., 2008).

Here, we assess the effect on flowering of overexpressing GA2ox and thereby depleting GA in specific tissues and demonstrate spatially distinct functions in the promotion of flowering under LDs.

MATERIALS AND METHODS

Growth conditions and plant materials

Plants were grown on soil under controlled conditions of LDs (16 hours light/8 hours dark) and SDs (8 hours light/16 hours dark) at 20°C. The level of photosynthetic active radiation was 60 μmol m^{-2} s^{-1} under both conditions. For quantitative PCR, leaves of 12-day-old seedlings were collected every 3 hours in a 24 hour cycle under LDs, and mRNA was extracted. For in situ hybridization, plants were grown for 3 weeks in SD, then shifted to LD, and apices were collected 8 hours after dawn before transfer, and after 3, 5 and 9 LDs. These analyses were performed in three biological replicates.

GAS1:FT SUC2:GA2ox7, *SUC2:GA2ox7 KNAT1:GA2ox7* were obtained by crossing both progenitors. For these crosses, *SUC2:GA2ox7* (3) and *KNAT1:GA2ox7* (4) were used.

GA treatment

GA_4 (Sigma) was stored in ethanol 100% with final concentration of 1 mM. Two solutions were then prepared: (1) GA_4 10 μM, Tween 0.1%; and (2) pure ethanol 1%, Tween 0.1%. GA treatment was carried out by brushing leaves, apices or seedlings of 10 individual plants with solution 1, while solution 2 was applied to the mock plants.

Flowering time determination

Flowering time was determined by counting the number of cauline and rosette leaves of at least 10 individual plants. Data are reported as mean leaf number ±s.d. and were measured from homozygous lines. Four independent transformants were used for each overexpressor plant

Plasmid construction, plant transformation and transformant selection

The full-length *GA2ox7* and *gai* cDNAs were amplified by PCR and used to generate an entry clone via BP reaction (Invitrogen, http://www.invitrogen.com). The entry clones were used to generate an expression clone via the LR reaction. The plasmids were then introduced into Agrobacterium strain GV3101 (pMP90RK) and transformed into WT Columbia by floral dip.

Determination of chlorophyll concentration

Chlorophyll concentration was estimated by using SPAD-502 leaf chlorophyll meter (Markwell et al., 1995).

In situ hybridization and GUS staining

In situ hybridization was performed according to a method already described (Torti et al., 2012) to measure *SOC1* (Searle et al., 2006), *SPL3* and *SPL9* (Wang et al., 2009; Wu et al., 2009) and *SPL5* (Cardon et al., 1999) expression. Primers to generate *GA2ox7*, *SPL4* probe are in supplementary material Table S1. GUS staining was performed as previously described (Blazquez et al., 1997).

RNA extraction and quantitative real-time PCR

Total RNA was isolated from plant tissues by using RNAeasy extraction kit (Qiagen). Transcript levels were quantified by quantitative PCR (Roche) and *PEX4* (At5G25760) was used as a control. Reactions were performed using the primers described in supplementary material Table S2. Total RNA, including small RNAs, was extracted by using miRNeasy Mini Kit (Qiagen). After DNAse treatment (Ambion), the mature form of miRNA156 was then amplified as previously described (Yang et al., 2009) (P. Huijser, unpublished). All quantitative real-time PCRs were performed with at least three independent RNA samples.

RESULTS

Misexpression of *GA2ox7* in different tissues causes GA deficiency phenotypes

Overexpression of *GA2ox7* mRNA from the *CaMV 35S* promoter reduces levels of bioactive GAs (Schomburg et al., 2003). To test the effect of reducing GA levels in specific tissues, *GA2ox7* cDNA was fused to promoters with specific expression patterns that have been used previously to misexpress regulatory proteins (An et al.,

2004; Ranjan et al., 2011). The *KNAT1* promoter, which is active in the shoot apical meristem, and the *SUC2* promoter, which is specific to the companion cells of the phloem, were used. The *CaMV 35S* promoter acted as a control to overexpress *GA2ox7* in most tissues. The three gene fusions were introduced into wild-type Columbia plants, and independent transformants were selected.

Four independent transformants expressing *GA2ox7* transcript at differing levels were identified for each construct. The abundance of *GA2ox7* mRNA was measured by qRT-PCR in seedlings of *35S:GA2ox7* (Fig. 1A), in leaves of *SUC2:GA2ox7* (Fig. 1B) and in apices of *KNAT1:GA2ox7* (Fig. 1C), and was present in each transformant at much higher levels than in wild type. To determine

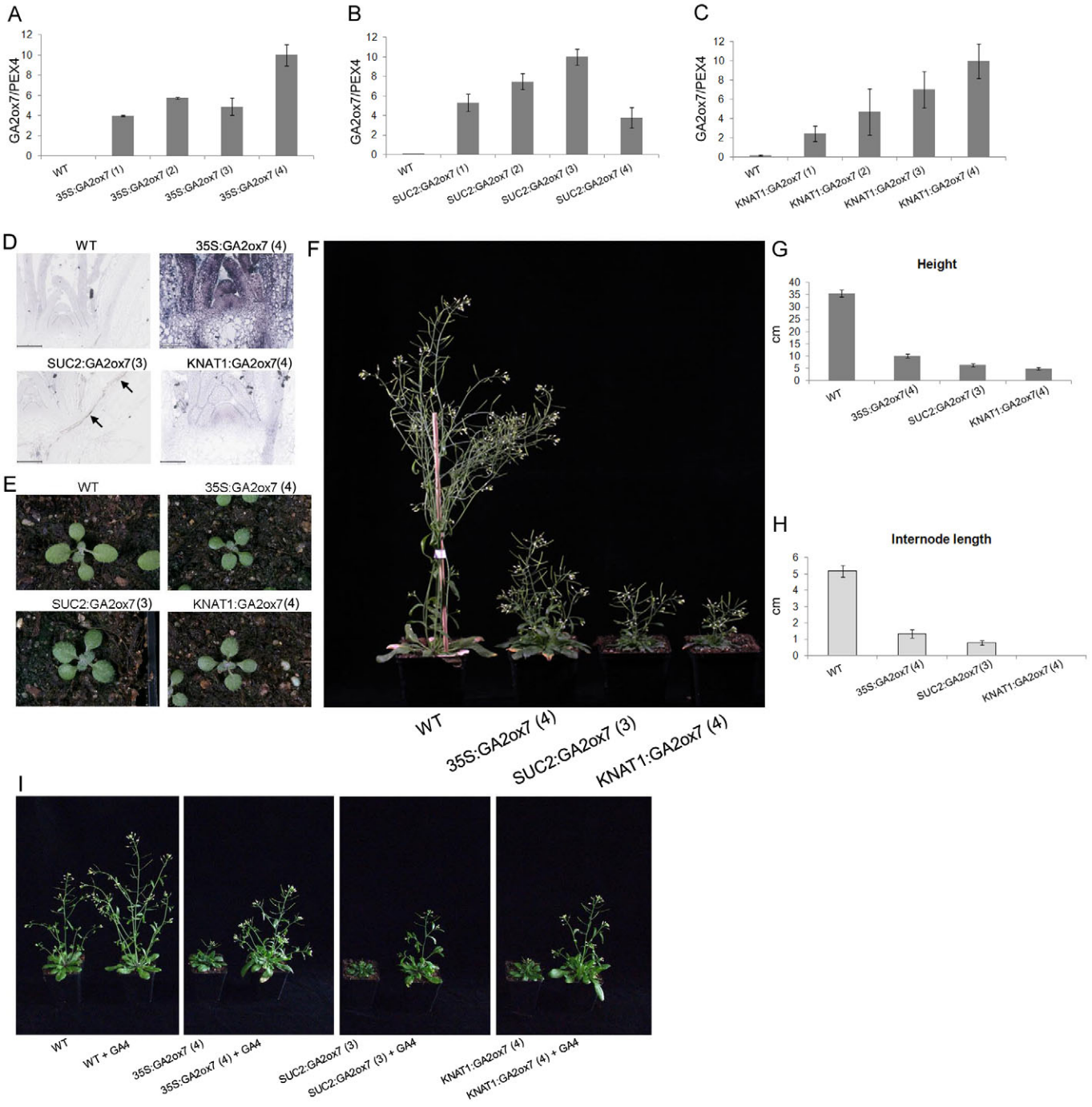

Fig. 1. Phenotypic characterization of *GA2ox7* overexpressor plants. (**A-C**) *GA2ox7* transcript levels in seedlings of *35S:GA2ox7* (A), in leaves of *SUC2:GA2ox7* (B) and in apices of *KNAT1:GA2ox7* (C). Samples were harvested from 12-day-old plants growing under LDs. Data are mean±s.d. (**D**) In situ hybridization of *GA2ox7* spatial expression pattern in transgenic plants. Apices of 14-day-old plants grown in SDs were harvested. Black arrows indicate detection of *GA2ox7* mRNA. Scale bars: 75 μm (left); 50 μm (right). (**E**) Phenotypes of young transgenic lines grown in LDs. (**F**) Phenotypes of adult transgenic lines grown in LDs. (**G**,**H**) Determination of height (G) and internode length (H) of transgenic lines compared with Col wild type. Data are mean±s.d. of at least ten plants. (**I**) Effect of GA4 treatment (10 μM) on phenotype of the transgenic lines grown in LDs: GA4 was applied on seedlings of *35S:GA2ox7*, in leaves of *SUC2:GA2ox7* and in apices of *KNAT1:GA2ox7*. All tests were performed with four independent transformants for each construct and Col wild type was used as control.

the spatial expression pattern of *GA2ox7* in transformants carrying each transgene, in situ hybridization was performed (Fig. 1D). In wild-type plants, no signal was detected, consistent with the very low level of expression of *GA2ox7* mRNA detected by qRT-PCR (Fig. 1A-C). *35S:GA2ox7* plants showed abundant *GA2ox7* mRNA in most tissues, including leaves, vasculature and shoot apical meristem (SAM). By contrast, in *SUC2:GA2ox7*, *GA2ox7* mRNA was detected only in the vasculature, whereas, in *KNAT1:GA2ox7*, it was found only in the shoot meristem (Fig. 1D). Thus, the heterologous promoters *CaMV 35S*, *KNAT1* and *SUC2* misexpress *GA2ox7* mRNA in the expected patterns.

The transgenic lines were analyzed for height, internode length, leaf radius and chlorophyll content, phenotypes that are strongly impaired in GA-deficient plants (Rieu et al., 2008b). Young transgenic seedlings were darker green and smaller than wild-type plants (Fig. 1E). Misexpression of *GA2ox7* from all three heterologous promoters greatly reduced plant height, as measured by the length of the main shoot before senescence (Fig. 1F,G) or the length of the internode between the last rosette and first cauline leaf (Fig. 1H). *KNAT1:GA2ox7* had the strongest effect on plant height, demonstrating that depleting GA from the SAM impairs stem elongation.

The leaf radius of each of the transgenic plants was significantly shorter than that of wild-type (Table 1; supplementary material Fig. S1A). The leaves of the transgenic lines also appeared darker green (supplementary material Fig. S1A), and therefore their chlorophyll levels were measured (Table 1). In the leaves of *35S:GA2ox7* and *SUC2:GA2ox7*, these were ~50% higher than wild type, whereas no significant differences were observed in the *KNAT1:GA2ox7*. Thus, GA is required to promote leaf growth in the vasculature and at the SAM, but in the regulation of chlorophyll levels an effect was detected only in the leaf vasculature.

KNAT1:GA2ox7 acts at the SAM to deplete GA, so the reduction of leaf size observed in these plants was unexpected. To test whether low level expression of *KNAT1:GA2ox7* in leaves could contribute to this phenotype, *GA2ox7* mRNA level was measured directly by qRT-PCR. However, *GA2ox7* transcript levels were not significantly different in leaves of *KNAT1:GA2ox7* plants compared with wild type (supplementary material Fig. S1B). In addition, *GA20ox1* transcript levels were also measured in these samples to assess whether GA levels were likely to be changed in the leaves of *KNAT1:GA2ox7* plants. This gene is under GA-negative feedback regulation and its mRNA level is therefore increased in tissues in which GA content is reduced (Phillips et al., 1995; Xu et al., 1995). In *35S:GA2ox7* plants, *GA20ox1* mRNA levels were more abundant compared with wild type, indicating that as expected these plants contained lower GA (supplementary material Fig. S1C). By contrast, in leaves of *KNAT1:GA2ox7*, *GA20ox1* mRNA levels did not differ significantly compared with wild type (supplementary material Fig. S1D). In addition, *GA20ox1* expression was tested in apices of *KNAT1:GA2ox7* plants where expression of the transgene is expected to reduce GA levels. In contrast to what was observed in leaves, the level of *GA20ox1* transcript was much higher in apices of *KNAT1:GA2ox7* compared with wild-type plants (supplementary material Fig. S1E). The above experiment indicated that the leaf phenotypes of *KNAT1:GA2ox7* plants cannot be explained by increased expression of *GA2ox7* nor by reduced levels of GA in mature leaves.

Table 1. Rosette leaf radius length and chlorophyll concentration of the transgenic lines

Genotype	Rosette radius (mm)	Chlorophyll (μmoles m^{-2})
Wild type	30.6±2.1	241±7.5
35S:GA2ox7 (4)	16.7±2.28	376±28
SUC2:GA2ox7 (3)	17±1.61	371±12
KNAT1:GA2ox7 (4)	13.5±1.56	248±9.6

Rosette leaf radius measurements were carried out in 10 individual plants at the end of the vegetative phase prior to bolting.
Chlorophyll concentration was estimated in three individual plants. The measurements are the mean±s.d. Col wild type was used as control.

Taken together, the phenotypic characterization data suggest that ectopic expression of *GA2ox7* from tissue-specific promoters causes phenotypes associated with GA deficiency. To test this further, the transgenic plants were treated with exogenous GA4. The severity of the GA deficiency phenotypes of the transgenic lines was greatly reduced by the GA applications, supporting the conclusion that reduced levels of bioactive GA are the basis of the phenotypes observed (Fig. 1I).

SUC2:GA2ox7 and *KNAT1:GA2ox7* plants show different flowering-time behaviours under SDs

Mutations impairing GA biosynthesis or signalling delay flowering of *Arabidopsis* most strongly under SDs (Wilson et al., 1992). Therefore, the flowering times of all transgenic lines were measured under SDs and compared with wild type.

35S:GA2ox7 plants flowered much later than wild-type plants under SD (Fig. 2A,D), as previously shown (Schomburg et al., 2003). Under our conditions, the transgenic plants flowered with around 40 rosette leaves more than wild-type plants.

To assess whether reducing GA levels in the phloem and at the shoot apical meristem alters flowering time in non inductive SDs, flowering of *KNAT1:GA2ox7* and *SUC2:GA2ox7* transgenic plants were also scored. *KNAT1:GA2ox7* did not flower during the course of the experiment (Fig. 2B,E), although they had produced around 100 rosette leaves compared with 60 for the wild type at flowering. Conversely, *SUC2:GA2ox7* plants flowered only slightly later than Columbia (Fig. 2C,F), producing around 70 rosette leaves compared with 60 of wild type. However, under these conditions, wild-type plants produced several cauline leaves more than *SUC2:GA2ox7* plants, so that the total leaf number at flowering was similar for wild-type and transgenic plants (Fig. 2C).

Taken together, these data suggest that the floral promotive effect of GA under SDs is mainly located at the shoot apical meristem, where depletion of GA largely prevents flowering.

35S:GA2ox7, *KNAT1:GA2ox7* and *SUC2:GA2ox7* show delayed flowering under long days

Although impairment of GA synthesis or signalling most strongly delays flowering under SDs, a weaker effect is also detected under LDs (Wilson et al., 1992). *35S:GA2ox7* also showed delayed flowering under LDs (Fig. 2G,J), as observed previously (Schomburg et al., 2003). Similarly, *KNAT1:GA2ox7* and *SUC2:GA2ox7* were late flowering, forming around 20 leaves compared with 15 for wild type (Fig. 2H,K,I,L). Thus, ectopic expression of *GA2ox7* in either the vascular tissue or the shoot meristem delays flowering under LDs, but the strongest effect is observed when *GA2ox7* is expressed generally from the *CaMV 35S* promoter.

The severity of the late-flowering phenotype of individual lines was significantly correlated ($P<0.001$) to the level of *GA2ox7* mRNA (supplementary material Fig. S1F), so that the lines that expressed *GA2ox7* mRNA most strongly were the latest flowering. This observation suggests that the effect of *GA2ox7* on flowering is dose dependent.

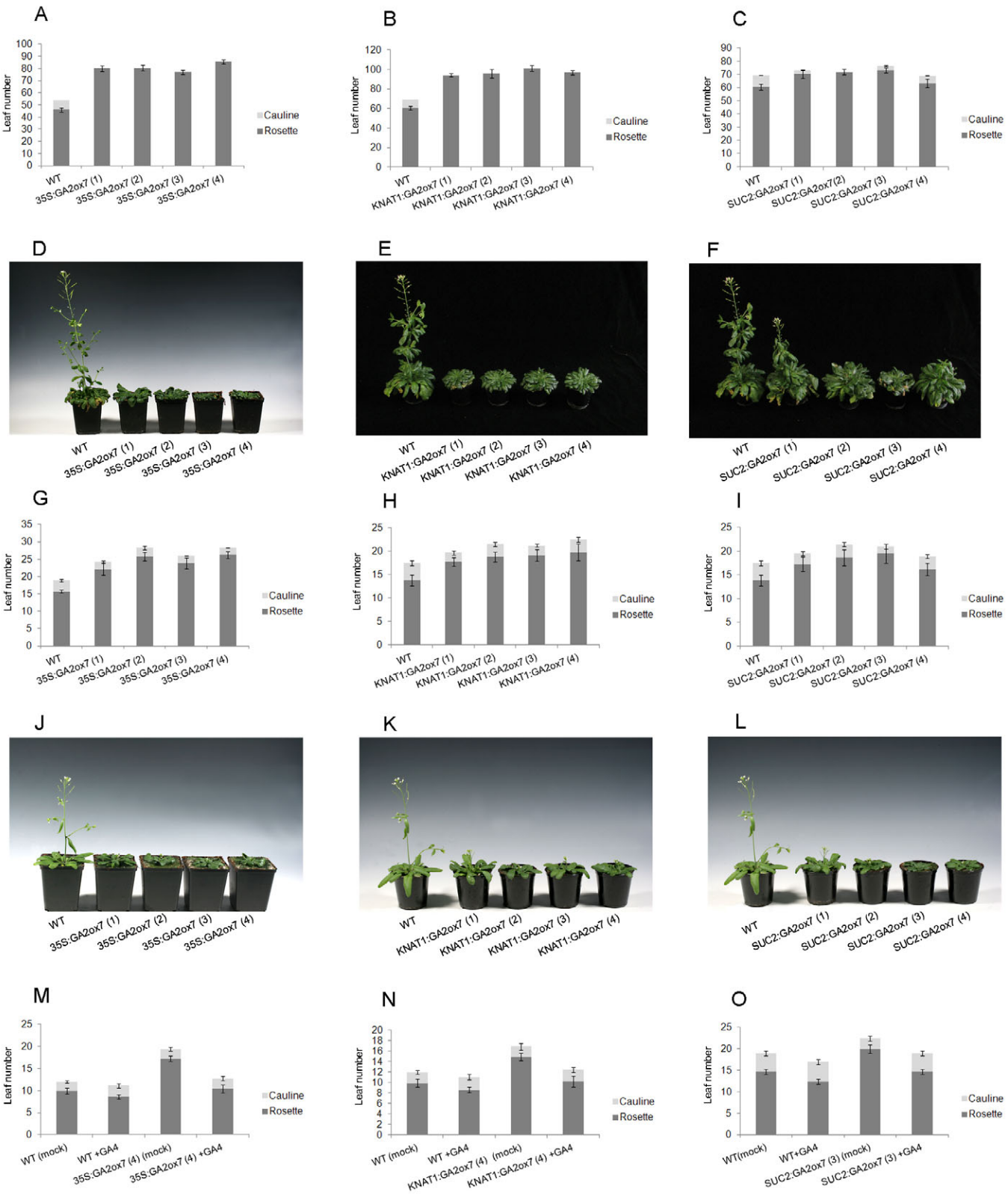

Fig. 2. Flowering time of the transgenic lines under LDs and SDs. (**A-C**) Flowering time of plants overexpressing *GA2ox7* in all tissues from the *CaMV 35S* promoter (A), in the SAM from the *KNAT1* promoter (B) and in the vasculature from the *SUC2* promoter (C) grown in SDs. Data are mean±s.d. of at least 10 plants. (**D-F**) Phenotypes of transgenic lines grown under SDs are shown below flowering time graphs. (**G-I**) Flowering time of *35S:GA2ox7* (G), *KNAT1:GA2ox7* (H) and *SUC2:GA2ox7* (I) plants under LDs. Data are mean±s.d. (**J-L**) Phenotypes of transgenic lines grown under LDs are shown below flowering-time graphs. (**M-O**) GA4 (10 μM) treatment of seedlings of *35S:GA2ox7* (M), of apices of *KNAT1:GA2ox7* (N) and of leaves of *SUC2:GA2ox7* (O). GA treatment was performed throughout the growth of the plant twice a week. Data are mean±s.d.

The effect of exogenous GA4 treatment on the late-flowering phenotype of the transgenic plants was also tested. GA4 application accelerated flowering of the transgenic lines under LDs, and at the end of the treatment the transgenic lines flowered with a similar number of leaves to the wild-type mock-treated plants (Fig. 2M,N,O).

To test whether the delay in flowering under LDs caused by *KNAT1:GA2ox7* was enhanced by *SUC2:GA2ox7*, the two latest flowering transgenic lines were crossed and flowering time was scored in the F1 generation (supplementary material Fig. S1G,H). The double overexpressor *KNAT1:GA2ox7 SUC2:GA2ox7* flowered later than either progenitor and at a similar stage to *35S:GA2ox7*. Therefore, the effect of overexpressing *GA2ox7* in the leaves and meristem is additive on flowering time under LDs.

Taken together, the flowering-time experiments indicate that under LDs GA acts both in the vasculature and at the SAM to promote flowering. However, the requirement for GA at the meristem is reduced in LDs compared with SDs, whereas in the vascular tissue the effect of GA on flowering appears stronger under LDs than SDs.

FT and *TSF* mRNA levels are regulated by GA in the phloem under long days

Many of the genes comprising the photoperiodic flowering pathway are expressed in the phloem companion cells, where the *SUC2* promoter is active. Therefore, whether *SUC2:GA2ox7* delays flowering by reducing the transcript levels of the photoperiodic pathway genes *FT*, *TSF*, *CO* and *GI* was tested (Fig. 3A-D). Several of these genes are regulated by the circadian clock so their RNA levels were measured every 3 hours through a 24-hour cycle under LDs. In wild-type plants, *FT* mRNA level showed the expected diurnal pattern with a strong increase at 12 hours after dawn and a peak at 16 hours. *SUC2:GA2ox7* showed a similar diurnal pattern in *FT* mRNA, but its rise in expression was slightly delayed and its abundance was significantly reduced between 12 and 16 hours after dawn. The *SUC2:GA2ox7* transformants with the highest *GA2ox7* transcript levels (Fig. 1B) showed the strongest reduction in *FT* (supplementary material Fig. S1I). A similar but less pronounced effect was observed for the mRNA of the *FT* paralogue *TSF* (Fig. 3B). By contrast, the mRNAs of *CO* and *GI*,

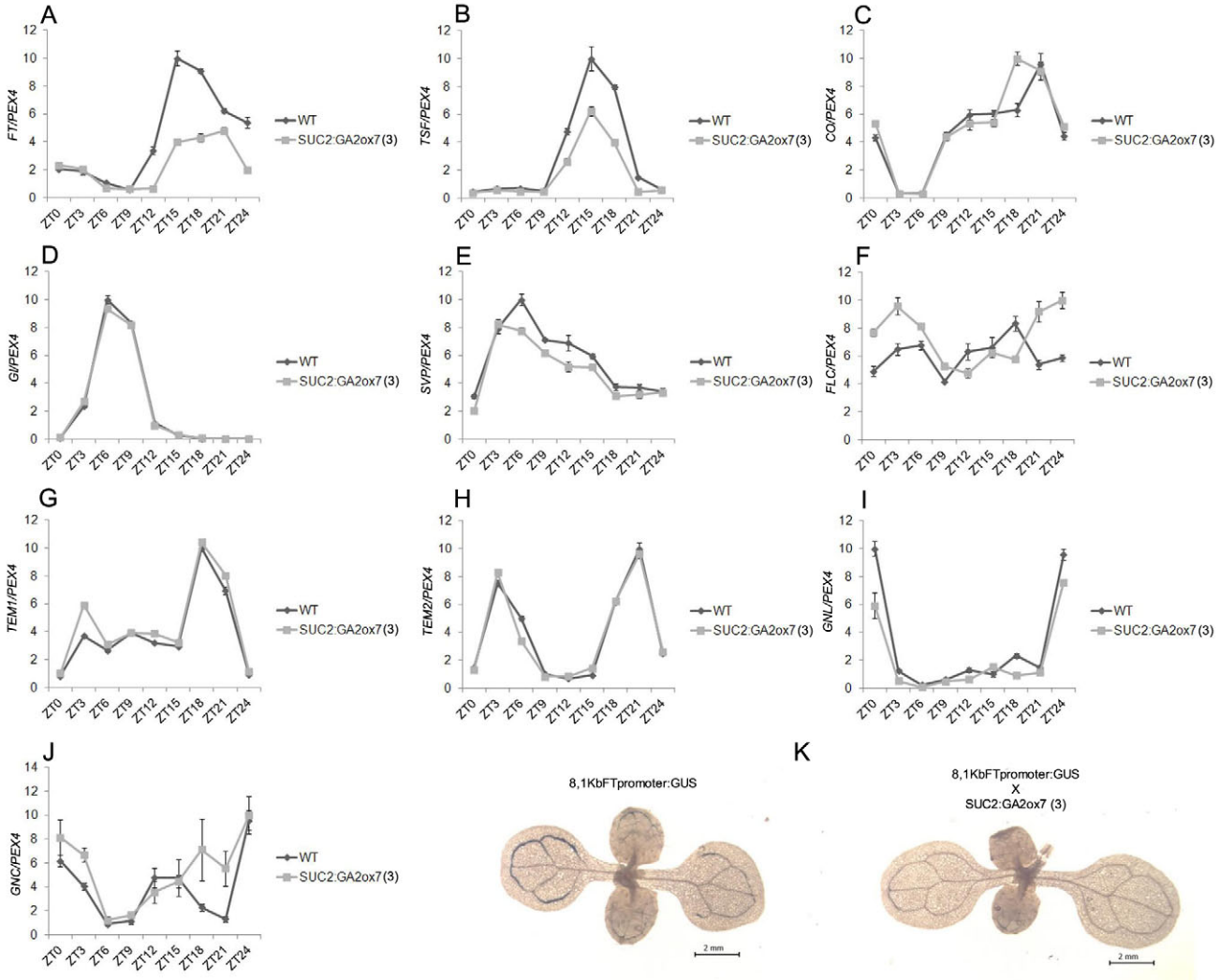

Fig. 3. *SUC2:GA2ox7* reduces the expression of photoperiodic genes *FT* and *TSF*. (**A-J**) Temporal expression patterns of *FT* (A), *TSF* (B), *CO* (C), *GI* (D), *SVP* (E), *FLC* (F), *TEM1* (G) and *TEM2* (H), and of GA downstream acting genes *GNL* (I) and *GNC* (J) in *SUC2:GA2ox7* plants compared with Col wild type. mRNA levels were measured by q-RT-PCR in leaves of 12-day-old seedling harvested throughout a LD. All q-PCR analyses were performed with at least three independent RNA samples. Time is expressed as hours from dawn (ZT, zeitgeber). Data are mean±s.d. (**K**) Histochemical localization of GUS activity in 10-day-old seedlings of 8.1 kb *FT* promoter:GUS and 8.1 kb *FT* promoter:GUS × *SUC2:GA2ox7* grown in LDs. Scale bars: 2 mm.

which act earlier in the photoperiodic pathway than *FT* and *TSF*, were not significantly reduced in *SUC2:GA2ox7* compared with wild type (Fig. 3C,D).

Several repressors of *FT* transcription have been described, including *SVP* (Lee et al., 2007; Li et al., 2008), *FLC* (Searle et al., 2006), *TEM1* and *TEM2* (Castillejo and Pelaz, 2008). Increased expression of the mRNAs of these repressors in *SUC2:GA2ox7* plants could explain the reduced level of *FT* and *TSF* transcripts, and therefore these mRNAs were quantified in the transgenic plants (Fig. 3E-H). No significant difference between *SUC2:GA2ox7* and Col wild type was observed for *SVP*, *TEM1* and *TEM2* transcript levels, indicating that increased levels of these mRNAs cannot explain the reduced expression of *FT* and *TSF*. *FLC* mRNA levels were slightly increased at the beginning of the light period in the *SUC2:GA2ox7* plants, suggesting that the increase in abundance of this mRNA may be the cause of the reduced levels of *FT* and *TSF* mRNAs (Fig. 3F). To test this further, *flc* mutant and wild-type plants were treated with paclobutrazol (PAC), an inhibitor of GA biosynthesis, and *FT* transcript levels were quantified. Interestingly, *FT* transcript was reduced to similar levels in wild-type and *flc* PAC-treated plants (supplementary material Fig. S1J). This result supports the idea that lowering GA content reduces *FT* expression and suggests that the effect of GA levels in regulating *FT* is likely to be independent of *FLC*.

Finally, *GNC* and *GNL* were recently described to act as repressors of flowering downstream of GA (Richter et al., 2010). *GNL* mRNA levels did not differ in *SUC2:GA2ox7* compared with Col (Fig. 3I), showing the same diurnal peak of abundance in both genotypes. *GNC* transcript levels slightly increased 18 hours after dawn in *SUC2:GA2ox7* plants compared with Col (Fig. 3J), but this difference is probably not sufficient to explain the reduced levels of *FT* transcript, which are observed earlier in the diurnal cycle (12 hours after dawn) (Fig. 3A).

An 8.1 kb fragment has previously been described to contain the *FT* promoter and recreates the spatial pattern of expression of *FT* (Adrian et al., 2010; Takada and Goto, 2003). The *SUC2:GA2ox7* transgenic line and Col were crossed to an *8.1KbFTpro:GUS* plant and GUS expression was analyzed in the F1 plants (Fig. 3K). As expected, *8.1KbFTpro:GUS*/– seedlings showed GUS signal in the vasculature of the cotyledons and leaves. By contrast, in *8.1KbFTpro:GUS*/– *SUC2:GA2ox7*/– seedlings, which were similarly stained, no GUS signal was detected. Thus, in wild-type plants GA acts to increase *FT* mRNA through the defined 8.1 kb *FT* promoter.

Ectopic expression of *FT* suppresses the late flowering caused by *SUC2:GA2ox7*

To assess whether the reduced level of *FT* and *TSF* mRNA was the cause of delayed flowering of *SUC2:GA2ox7* plants, a transgene expressing *FT* from a heterologous promoter was introduced into *SUC2:GA2ox7* plants. Ectopic expression of *FT* can overcome the effect of loss-of-function of *FT* and *TSF* (Jang et al., 2009; Michaels et al., 2005; Yamaguchi et al., 2005). The *GAS1:FT* construct overexpresses *FT* mRNA only in minor veins and to a lesser extent than other phloem-specific promoters (Corbesier et al., 2007; Haritatos et al., 2000). The *SUC2:GA2ox7* × *GAS1:FT* plants flowered much earlier than those carrying only *SUC2:GA2ox7* and after producing a similar number of leaves to *GAS1:FT* plants (Fig. 4A,B), supporting the idea that the late flowering of *SUC2:GA2ox7* is caused by reduced *FT* mRNA levels.

In addition, the effects of impairing GA signalling in the companion cells on *FT* expression and flowering time were tested by expressing from the *SUC2* promoter the dominant mutant form of GAI that represses GA signalling (Peng et al., 1997). *SUC2:gai-D* plants were late flowering and showed reduced *FT* mRNA levels, similar to the effects observed in the *SUC2:GA2ox7* plants (supplementary material Fig. S2A,B).

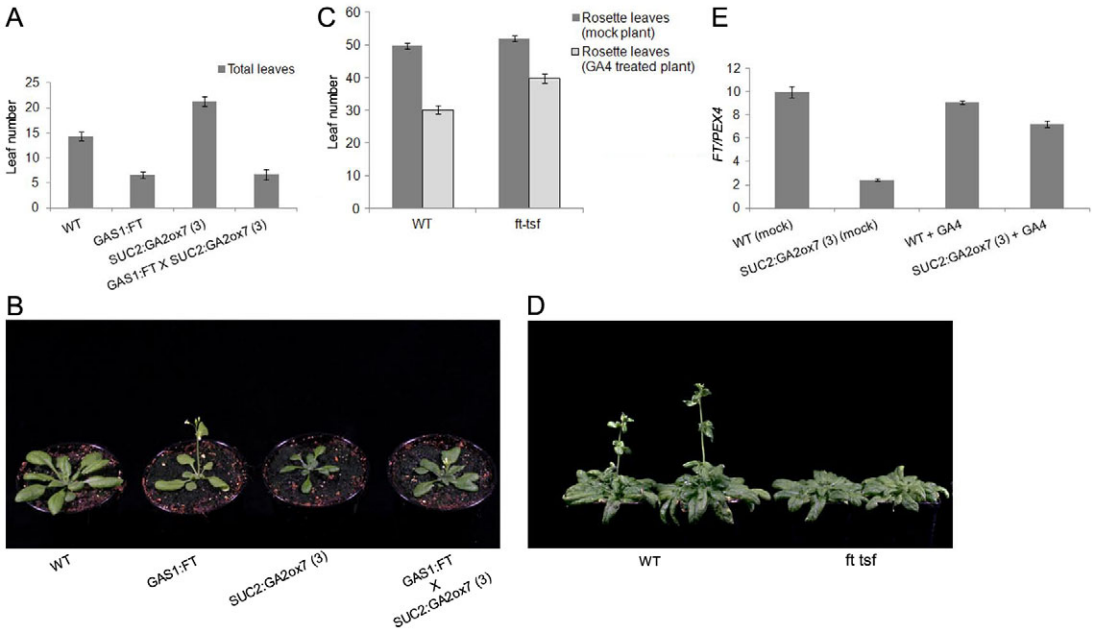

Fig. 4. The *ft tsf* double mutant shows less sensitivity to leaf applications of GA in the acceleration of flowering. (**A**,**B**) Effect of ectopic expression of *FT* in *SUC2:GA2ox7* plants grown in LDs. Col wild-type, *SUC2:GA2ox7* and *GAS1:FT* plants were used as controls. (**C**,**D**) Effect of GA4 on flowering time of *ft tsf* and Col wild-type plants under SDs. GA4 (10 μM) was applied to leaves twice weekly. (**E**) Effect of GA4 on *FT* expression in *SUC2:GA2ox7* and Col wild-type plants in LDs. GA treatment was carried out in leaves of 10-day-old plants and tissues were collected 24 hours after. Data are mean±s.d.

The above experiments suggested that GA and GA signalling act in the vascular tissue to increase *FT* and *TSF* mRNA levels and thereby promote flowering. Therefore, whether *FT* and *TSF* are required in the leaf for GA treatments of leaves to promote flowering was tested. Leaves of *ft-10 tsf-1* double mutants and wild-type plants grown under SD were treated with GA4 (Fig. 4C,D). Wild-type plants showed significant acceleration of flowering upon GA treatment, producing 20 leaves fewer than the mock-treated plants. By contrast, GA application to leaves of *ft-10 tsf-1* mutants caused flowering to occur after production of only 10 leaves fewer than the mock-treated plants. Therefore, *ft-10 tsf-1* double mutants still respond to GA leaf treatments, but their response is strongly attenuated compared with wild-type plants. This result is consistent with GA leaf treatments acting partly through *FT* and *TSF* to promote flowering. In addition, leaves of *SUC2:GA2ox7* and Col wild type were also treated with GA and after 24 hours the *FT* transcript level was quantified (Fig. 4E). Wild-type plants did not show any significant change in *FT* expression after GA application, which is probably due to the saturating level of GA at this stage. By contrast, *SUC2:GA2ox7* showed an approximately threefold increase of *FT* transcript in the GA-treated compared with the mock-treated plants. Therefore, depletion of GA in the leaves of *SUC2:GA2ox7* caused *FT* downregulation, which could be restored by applying active GA.

Induction of *SPL*s but not *SOC1* transcription is delayed in the meristem of *KNAT1:GA2ox7* plants under LDs

The level of *FT* mRNA was similar in *KNAT1:GA2ox7* and Col plants under LDs (supplementary material Fig. S2C), confirming that the delay in flowering of this plant occurred by a different mechanism than for *SUC2:GA2ox7* plants.

During the transition to flowering, expression of many genes is induced at the shoot apex, and this can be synchronized by transferring plants from SDs to LDs. To determine how these gene expression patterns are affected by *KNAT1:GA2ox7*, the transgenic plants and Col were grown for 3 weeks in SDs and then transferred to LDs. Apices were harvested for in situ hybridization before transfer and then after 3, 5 and 9 days in LDs.

In Col shoot meristems, *SOC1* mRNA was not detected after 3 weeks in SDs, but increased in the meristem after 3, 5 and 9 LDs (Fig. 5A). Similarly, in the *KNAT1:GA2ox7* plants, *SOC1* mRNA was detected in the meristem after exposure to 3, 5 and 9 LDs. However, unlike Col plants, flower development was not initiated throughout this period. Consistent with this result, an increase in *SOC1* transcript in apices of Col and *KNAT1:GA2ox7* plants was detected after transfer to LDs (Fig. 5B). Thus, the meristem of *KNAT1:GA2ox7* plants responds normally to the LD signal in terms of *SOC1* mRNA induction, demonstrating that GA is required to promote later steps in floral induction.

The *SPL* genes are expressed in the shoot apical meristem downstream of *SOC1* (Jung et al., 2011; Torti et al., 2012) and play important roles in the activation of floral meristem identity genes *FUL* and *AP1* (Wang et al., 2009; Yamaguchi et al., 2009). Therefore, the expression patterns of *SPL* mRNAs were also studied. In Col plants transferred to LDs, the mRNAs of *SPL4* and *SPL5* were strongly detected in the rib meristem region after exposure to 3-5 LDs (Fig. 6B,C). Similarly, *SPL9* mRNA was detected on the flanks of the meristems of Col plants exposed to 3-5 LDs (Fig. 6D). By contrast, in *KNAT1:GA2ox7*, expression of *SPL4* and *SPL9* mRNAs was strongly reduced so that their mRNAs only appeared weakly after exposure to 5 LDs. *SPL5* mRNA level was even more strongly affected and was undetectable in the shoot meristem 5 LDs after transfer. *SPL3* mRNA was detected throughout the meristem and in leaf primordia in Col plants and increased in abundance during LD induction (Fig. 6A). Conversely, in *KNAT1:GA2ox7*, *SPL3* expression was strongly delayed and transcript was only weakly detectable after 5 LDs in leaf primordia.

These experiments indicate that although *KNAT1:GA2ox7* does not prevent the early induction of *SOC1* expression in the shoot meristem in response to LDs, it does prevent the subsequent activation of later acting genes such as *SPL3*, *SPL4*, *SPL5* and *SPL9*.

The effect of *KNAT1:GA2ox7* on *SPL* gene expression could be exerted at the level of FD, which binds directly to *SPL3*, *SPL4* and *SPL5* to promote their expression (Jung et al., 2011). Therefore, *fd* mutants were treated with active GA and the levels of *SPL3* and *SPL4* mRNAs were quantified in apices (supplementary material Fig. S2D,E). *SPL3* and *SPL4* mRNA levels increased in *fd* mutants treated with GA compared with the mock-treated plants, indicating that GA can activate these *SPL* genes independently of *FD*. However, the level of *SPL* expression is lower than in GA-treated wild-type plants, so a role for *FD* in this process cannot be excluded (supplementary material Fig. S2D,E).

Expression of SPL genes is negatively regulated by miR156 at the post-transcriptional level (Gandikota et al., 2007; Schwab et al., 2005). Therefore, whether downregulation of *SPL* genes in *KNAT1:GA2ox*7 was caused by increased levels of miR156 was tested in apices of wild type and *KNAT1:GA2ox7* (supplementary material Fig. S2F). Apices were harvested after growing plants in LDs for 6, 9, 11, 13 and 17 days. In Col wild type, the levels of

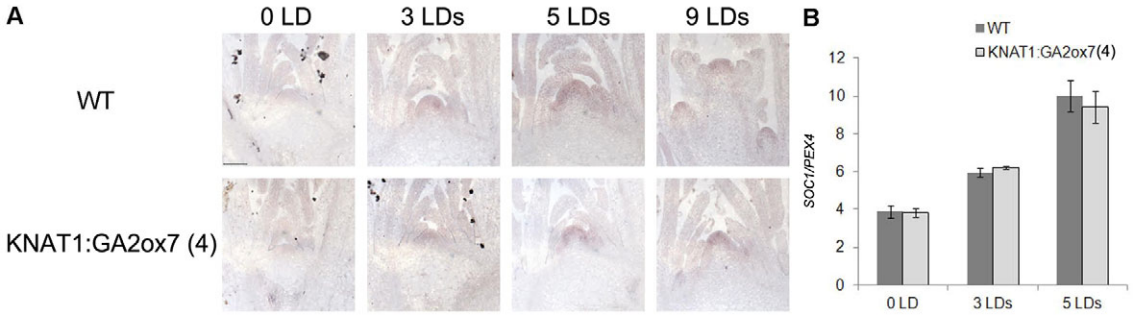

Fig. 5. Temporal and spatial expression pattern of *SOC1* in the transgenic lines. (**A**) Time courses of in situ hybridization on Col wild-type and *KNAT1:GA2ox7* plants. Plants were grown for 3 weeks in SDs (0 LD) and then transferred to LDs (3 LD, 5 LD, 9 LD). (**B**) *SOC1* expression levels in apices of *KNAT1:GA2ox7* and Col wild-type. Plants were grown for 3 weeks in SDs (0 LD) and then transferred to LDs (3 LD, 5 LD). Data are mean±s.d. Scale bar: 75 μm.

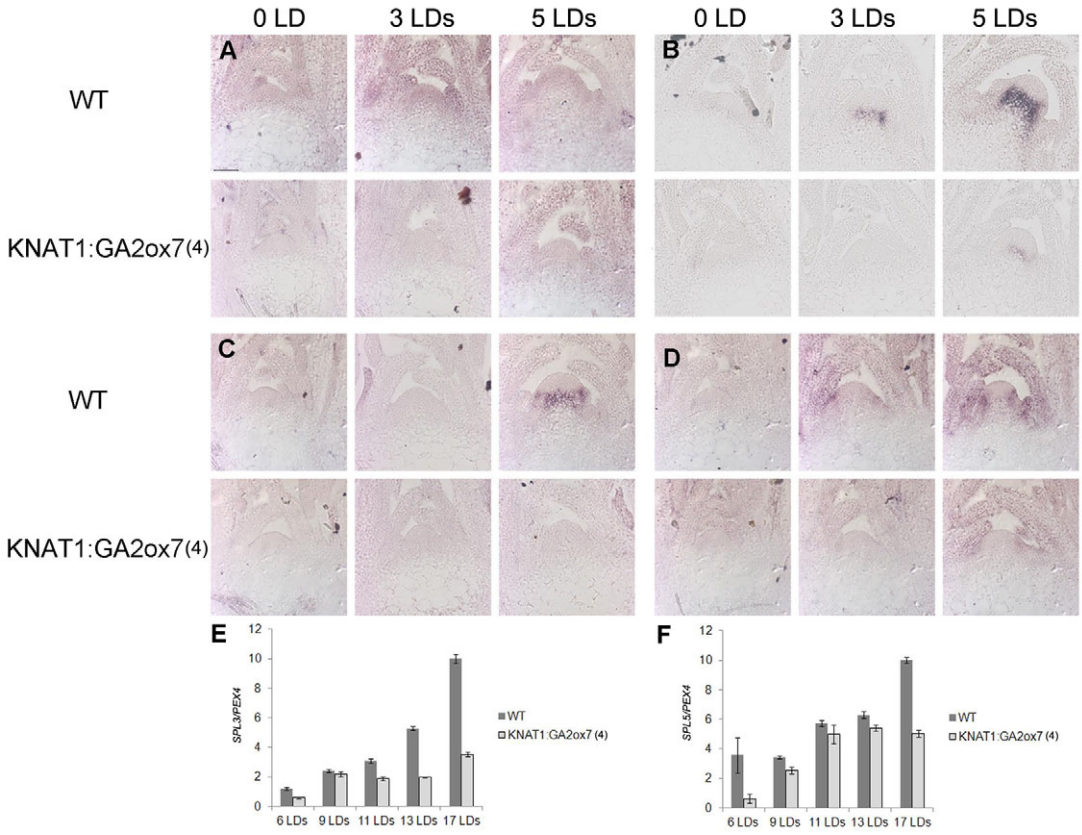

Fig. 6. Temporal and spatial expression patterns of *SPL* genes in the transgenic lines. Time courses of in situ hybridization on Col wild-type and *KNAT1:GA2ox7* plants grown for 3 weeks in SDs (0 LD) and then transferred to LDs (3 LD, 5 LD). (**A-D**) Specific probes were used to detect mRNAs of *SPL3* (A), *SPL4* (B), *SPL5* (C) and *SPL9* (D). (**E**,**F**) Temporal expression patterns of *SPL3* (E) and *SPL5* (F) in apices of Col wild-type and *KNAT1:GA2ox7* plants grown in continuous LDs. Sample were harvested at 6 LD, 9 LD, 11 LD, 13 LD and 17 LD. Data are mean±s.d. Scale bar: 50 μm.

miR156 progressively decreased along the time course, as previously described (Wang et al., 2009; Wu and Poethig, 2006), reaching the lowest level at 17 LDs (supplementary material Fig. S2F). Similarly, in *KNAT1:GA2ox7*, the expression pattern of miR156 followed the same kinetics as wild type and no significant differences in abundance of miR156 were detected. By contrast, the transcript levels of *SPL3* increased in apices of wild-type plants but not in *KNAT1:GA2ox7* (Fig. 6E). *SPL5* mRNA slightly increased along the time course in *KNAT1:GA2ox7* plants but the transcript levels were significantly reduced compared with wild type (Fig. 6F).

Taken together, the in situ hybridization and the qRT-PCR data suggest that in the shoot apical meristem GA increases *SPL* mRNA levels by acting after *SOC1* mRNA accumulation and not by decreasing miR156 levels.

DISCUSSION

The plant growth regulator GA has previously been shown to promote the transition to flowering of *Arabidopsis* mainly under non-inductive SDs. Here, we demonstrated that GA has defined tissue-specific roles during floral induction in response to inductive LDs.

Effects of tissue specific expression of *GA2ox7* on leaf size and height

Gibberellins regulate many phases of development, including height, leaf size and chlorophyll content of *Arabidopsis*. The strongest effect on plant height was observed in KNAT1:*GA2ox7* plants, suggesting that the major impact of GA in shoot elongation occurs in the meristem. This effect might be caused by ectopic expression of *GA2ox7* in cells in which it is not normally expressed or due to increased activity of *GA2ox7* in cells in which it is expressed in wild-type plants. The expression patterns of the class III GA2ox-encoding genes *GA2ox7* and *GA2ox8* are unknown, but expression of classes I and II GA2 oxidases have been detected in the shoot apical meristem of *Arabidopsis*, rice and maize (Bolduc and Hake, 2009; Jasinski et al., 2005; Sakamoto et al., 2001).

The severe short internode phenotype of *KNAT1:GA2ox7* plants is similar to that of loss-of-function GA biosynthetic mutants, consistent with the overexpression of *GA2ox7* depleting GA from the meristem. Bioactive GA is also present within the apex of flowering plants when internodes strongly extend. GA promotes cell division and expansion, suggesting that both contribute to internode elongation in the rib meristem region (Achard et al., 2009; Cowling and Harberd, 1999; Daykin et al., 1997). Although depletion of GA in the meristem showed the greatest effect on stem length and these plants were unable to appreciably extend stem internodes, a significant effect was also observed in the *SUC2:GA2ox7* plants, where GA is depleted in the phloem companion cells.

SUC2:GA2ox7 plants also showed a dark green phenotype associated with increased chlorophyll levels. GA regulates chlorophyll biosynthesis through the transcriptional repressors DELLAs and the downstream acting proteins GNL and GNC (Richter et al., 2010). Indeed, GA causes downregulation of *GNL*

and *GNC* mRNAs leading to reduced levels of protochlorophyllide oxidoreductases (PORs), thus modulating chlorophyll biosynthesis. In agreement with these findings, we showed that overexpression of *GA2ox7* causing depletion of GA in the companion cells led to increased chlorophyll levels in the leaves. However, no difference in abundance of *GNL* and *GNC* transcripts could be detected in total leaf mRNA. Perhaps if *GNC* and *GNL* are expressed throughout the leaf, reduction in expression in companion cells is undetectable in total leaf RNA; alternatively, other genes might be implicated in the regulation of GA-mediated chlorophyll biosynthesis. No effect could be observed on chlorophyll content by lowering GA in the SAM, suggesting that GA levels in the meristem do not affect chlorophyll biosynthesis.

The length of the leaf radius was consistently reduced when GA was depleted in companion cells and in the SAM. This phenotype was similar to that reported for *ga20ox1 ga20ox2* double mutants, which show reduced levels of GA4 and GA1 (Rieu et al., 2008a). Our data suggest that GA levels in the companion cells and shoot meristem contribute to this phenotype.

Effect on floral transition of misexpression of *GA2ox7* in phloem companion cells

The effects of the *SUC2:GA2ox7* and *KNAT1:GA2ox7* fusions on flowering time were separable at the physiological and molecular levels. Expression in phloem companion cells from the *SUC2* promoter caused a relatively stronger delay of flowering under LDs than SDs, although the increase in absolute number of leaves was similar under both conditions. By contrast, the *KNAT1* fusion caused the strongest effect under SDs, where it prevented flowering. The delay in flowering of *SUC2:GA2ox7* under LDs correlated with reduced levels of *FT* and *TSF* mRNAs, which were not observed in *KNAT1:GA2ox7* plants. A similar correlation between GA levels and *FT* mRNA abundance was previously observed in the *ga1-3* mutant exposed to long days enriched in far-red light (Hisamatsu and King, 2008). However, in those plants, GA levels are strongly reduced in all tissues, and GA depletion in other cell types might affect *FT* mRNA levels in the companion cells, as was observed for PHYB (Endo et al., 2005). However, our experiments, together with those of Hisamatsu and King (Hisamatsu and King, 2008) strongly suggest that GA is required in the companion cells for normal levels of *FT* and *TSF* mRNAs under LDs. We also provide genetic evidence that the reduced levels of *FT* and *TSF* mRNAs are causally related to the late flowering of the *SUC2:GA2ox7* plants. Introduction of a transgene expressing *FT* from a heterologous phloem-specific promoter, *GAS1*, suppressed the late flowering of *SUC2:GAox2* plants. Furthermore, GA applications to leaves increased *FT* and *TSF* mRNA levels in *SUC2:GA2ox7* plants, as previously shown for *ga1-3* plants (Hisamatsu and King, 2008), and restored early flowering. The full effect of GA applications to the leaves on flowering time required an increase in *FT* and *TSF* mRNA levels, supported by the observation that *ft-10 tsf-1* double mutants were less sensitive to GA leaf applications, although they did still respond to the treatment. Previously, Hisamatsu and King (Hisamatsu and King, 2008) discussed an *FT* independent role of GA applications, and this is probably explained by a spatially separated function for GA in the shoot meristem, as mentioned in the following section. The mechanism by which GA increases *FT* and *TSF* mRNA levels is presumably via DELLA protein accumulation. Indeed, we demonstrated that expression of gai-D, a dominant mutant form of the GAI DELLA protein (Peng et al., 1997), in companion cells reduced *FT* and *TSF* mRNA levels. Therefore, when DELLA proteins accumulate in the companion cells, they likely inhibit proteins required for transcriptional activation of *FT*. No effect on mRNAs of previously identified regulators of *FT* was observed, demonstrating that depletion of GA does not affect the transcription of known repressors or activators of *FT*, although we cannot exclude that these proteins are regulated at the post-translational level.

Effect on floral transition of misexpression of *GA2ox7* in the shoot meristem

The role of GA at the apex in the promotion of flowering has mainly been studied under SDs. Under these conditions, GA levels increase at the apex prior to the floral transition, and this correlates with increased expression of the floral meristem identity gene *LFY* (Eriksson et al., 2006). GA also promotes expression of genes that act earlier in floral induction, particularly increasing transcription of *SOC1* (Achard et al., 2004; Moon et al., 2003). Applications of exogenous GA to wild-type plants caused increased abundance of *SOC1* mRNA, whereas in *ga1-3* and *gai* mutants, *SOC1* mRNA level was reduced. However, all published analyses of *SOC1* expression in response to GA were carried out by RT-PCR, and as *SOC1* is also expressed in leaves (Michaels et al., 2005), the increase in expression detected in apical samples may not be in the shoot meristem. In addition, the effect of GA on *SOC1* mRNA was mainly analyzed at single time points, making it difficult to assess its effect on the dynamics of *SOC1* expression during floral induction. By performing in situ hybridization to follow *SOC1* mRNA in the meristem over several days after inducing flowering by exposure to LDs, our work identifies a role for GA in the meristem after induction of *SOC1*.

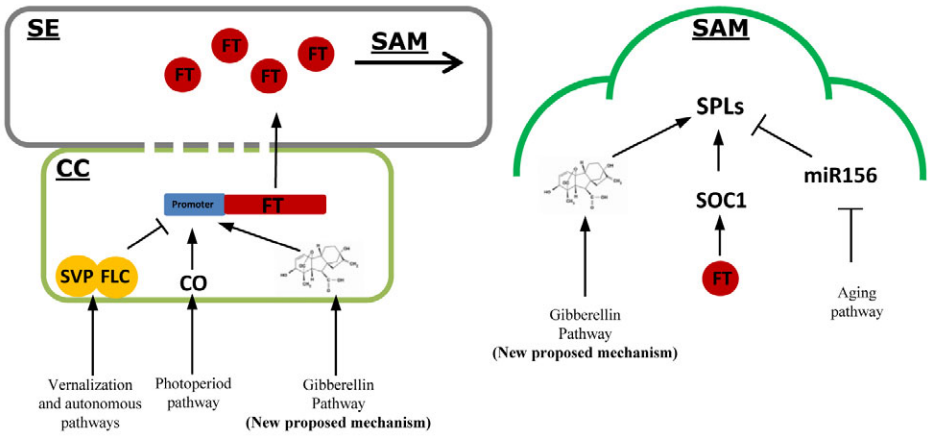

Fig. 7. Spatially separated roles of GA in controlling the floral transition under long days. GA signalling regulates the floral transition in LDs by increasing *FT* mRNA levels in the leaf vasculature, and of the levels of *SPL* gene mRNAs at the shoot apical meristem. Other pathways also regulate *FT*. FT protein moves to the SAM, where it activates expression of the floral integrator *SOC1*. At the SAM, GA promotes expression of *SPL3, SPL4, SPL5* and *SPL9*, and this occurs without transcriptional changes in *SOC1*. CC, companion cell; SE, sieve element; SAM, shoot apical meristem.

Transfer of wild-type plants from SDs to LDs causes a rapid induction of *SOC1* mRNA in the meristem within 1-3 days (Borner et al., 2000; Samach et al., 2000). The *SPL* genes are induced slightly later, with *SPL4*, *SPL5* and *SPL9* mRNAs rising in the meristem 3-5 days after transfer (Torti et al., 2012; Wang et al., 2009). The dynamics of *SOC1* mRNA induction was not changed in *KNAT1:GA2ox7* plants, indicating that reducing GA in the meristem does not affect *SOC1* induction in the meristem, in contrast to what was observed under SDs (Achard et al., 2004; Moon et al., 2003). However, expression of *SPL3*, *SPL4*, *SPL5* and *SPL9* all occurred markedly later, indicating that GA has a role in floral induction under LDs between activation of *SOC1* transcription and the activation of *SPL* gene expression (Fig. 7). By contrast, no effects on *SPL9* mRNA or miR156 were detected by RT-PCR in 2-week-old plants treated with GA or in *ga1-3* mutants, compared with wild-type (Wang et al., 2009), but this single time point would not have been sufficient to detect the effect of GA on the dynamics of *SPL* activation. GA-dependent activation of *SPL* gene expression may contribute to the induction of floral meristem identity genes by GA, because SPLs have been shown to bind directly to floral meristem identity genes such as *LFY* (Wang et al., 2009; Yamaguchi et al., 2009). As transcription of *SPL* genes is induced in the SAM both by the photoperiodic (Torti et al., 2012; Wang et al., 2009) and GA pathways, they might both activate *LFY* transcription via SPL proteins. However, the GA and photoperiod pathways are likely also to have additional independent branches leading to *LFY* activation, because they were previously shown to activate *LFY* transcription through independent promoter motifs (Blazquez and Weigel, 2000). The mechanism by which GA regulates *SPL* expression presumably involves post-translational regulation of transcription factors required to increase *SPL* expression. These GA regulated factors might act together with SOC1, which has recently been shown to bind directly to *SPL* genes. Taken together, our data provide a basis for identifying the molecular mechanisms by which under inductive photoperiods GA signalling facilitates the activation of *FT* transcription in leaves and transcription of the *SPL* genes in the meristem.

Acknowledgements
We thank Peter Huijser (Cologne) for advice on detection of *SPL* mRNAs and miR156, and Jarod Rollins and Fernando Andres for comments on the manuscript. Sara Bergonzi kindly provided primers for miRNA156 quantification.

Funding
This work was supported by a Marie Curie studentship to A.P. through the EC training programme SYSFLO and by a core grant of the Max Planck Society to G.C.

Competing interests statement
The authors declare no competing financial interests.

Supplementary material
Supplementary material available online at
http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.077164/-/DC1

References

Abe, M., Kobayashi, Y., Yamamoto, S., Daimon, Y., Yamaguchi, A., Ikeda, Y., Ichinoki, H., Notaguchi, M., Goto, K. and Araki, T. (2005). FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. *Science* **309**, 1052-1056.
Achard, P., Herr, A., Baulcombe, D. C. and Harberd, N. P. (2004). Modulation of floral development by a gibberellin-regulated microRNA. *Development* **131**, 3357-3365.
Achard, P., Gusti, A., Cheminant, S., Alioua, M., Dhondt, S., Coppens, F., Beemster, G. T. S. and Genschik, P. (2009). Gibberellin signaling controls cell proliferation rate in Arabidopsis. *Curr. Biol.* **19**, 1188-1193.
Adrian, J., Farrona, S., Reimer, J. J., Albani, M. C., Coupland, G. and Turck, F. (2010). cis-regulatory elements and chromatin state coordinately control temporal and spatial expression of FLOWERING LOCUS T in Arabidopsis. *Plant Cell* **22**, 1425-1440.
An, H. L., Roussot, C., Suarez-Lopez, P., Corbesler, L., Vincent, C., Pineiro, M., Hepworth, S., Mouradov, A., Justin, S., Turnbull, C. et al. (2004). CONSTANS acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of Arabidopsis. *Development* **131**, 3615-3626.
Blazquez, M. A. and Weigel, D. (2000). Integration of floral inductive signals in Arabidopsis. *Nature* **404**, 889-892.
Blazquez, M. A., Soowal, L. N., Lee, I. and Weigel, D. (1997). LEAFY expression and flower initiation in Arabidopsis. *Development* **124**, 3835-3844.
Blazquez, M. A., Green, R., Nilsson, O., Sussman, M. R. and Weigel, D. (1998). Gibberellins promote flowering of Arabidopsis by activating the LEAFY promoter. *Plant Cell* **10**, 791-800.
Bolduc, N. and Hake, S. (2009). The maize transcription factor KNOTTED1 directly regulates the gibberellin catabolism gene ga2ox1. *Plant Cell* **21**, 1647-1658.
Borner, R., Kampmann, G., Chandler, J., Gleissner, R., Wisman, E., Apel, K. and Melzer, S. (2000). A MADS domain gene involved in the transition to flowering in Arabidopsis. *Plant J.* **24**, 591-599.
Bowman, J. L., Alvarez, J., Weigel, D., Meyerowitz, E. M. and Smyth, D. R. (1993). Control of flower development in Arabidopsis thaliana by Apetala1 and interacting genes. *Development* **119**, 721-743.
Cardon, G., Hohmann, S., Klein, J., Nettesheim, K., Saedler, H. and Huijser, P. (1999). Molecular characterisation of the Arabidopsis SBP-box genes. *Gene* **237**, 91-104.
Cardon, G. H., Hohmann, S., Nettesheim, K., Saedler, H. and Huijser, P. (1997). Functional analysis of the Arabidopsis thaliana SBP-box gene SPL3: a novel gene involved in the floral transition. *Plant J.* **12**, 367-377.
Castillejo, C. and Pelaz, S. (2008). The balance between CONSTANS and TEMPRANILLO activities determines FT expression to trigger flowering. *Curr. Biol.* **18**, 1338-1343.
Corbesier, L., Vincent, C., Jang, S. H., Fornara, F., Fan, Q. Z., Searle, I., Giakountis, A., Farrona, S., Gissot, L., Turnbull, C. et al. (2007). FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. *Science* **316**, 1030-1033.
Cowling, R. J. and Harberd, N. P. (1999). Gibberellins control Arabidopsis hypocotyl growth via regulation of cellular elongation. *J. Exp. Bot.* **50**, 1351-1357.
Daykin, A., Scott, I. M., Francis, D. and Causton, D. R. (1997). Effects of gibberellin on the cellular dynamics of dwarf pea internode development. *Planta* **203**, 526-535.
de Lucas, M., Daviere, J. M., Rodriguez-Falcon, M., Pontin, M., Iglesias-Pedraz, J. M., Lorrain, S., Fankhauser, C., Blazquez, M. A., Titarenko, E. and Prat, S. (2008). A molecular framework for light and gibberellin control of cell elongation. *Nature* **451**, 480-484.
Dill, A., Thomas, S. G., Hu, J. H., Steber, C. M. and Sun, T. P. (2004). The Arabidopsis F-box protein SLEEPY1 targets gibberellin signaling repressors for gibberellin-induced degradation. *Plant Cell* **16**, 1392-1405.
Endo, M., Nakamura, S., Araki, T., Mochizuki, N. and Nagatani, A. (2005). Phytochrome B in the mesophyll delays flowering by suppressing FLOWERING LOCUS T expression in Arabidopsis vascular bundles. *Plant Cell* **17**, 1941-1952.
Eriksson, S., Bohlenius, H., Moritz, T. and Nilsson, O. (2006). GA(4) is the active gibberellin in the regulation of LEAFY transcription and Arabidopsis floral initiation. *Plant Cell* **18**, 2172-2181.
Feng, S. H., Martinez, C., Gusmaroli, G., Wang, Y., Zhou, J. L., Wang, F., Chen, L. Y., Yu, L., Iglesias-Pedraz, J. M., Kircher, S. et al. (2008). Coordinated regulation of Arabidopsis thaliana development by light and gibberellins. *Nature* **451**, 475-479.
Fornara, F., de Montaigu, A. and Coupland, G. (2010). SnapShot: control of flowering in Arabidopsis. *Cell* **141**, 550.
Fu, X. D., Richards, D. E., Fleck, B., Xie, D. X., Burton, N. and Harberd, N. P. (2004). The Arabidopsis mutant sleepy1(gar2-1) protein promotes plant growth by increasing the affinity of the SCFSLY1 E3 ubiquitin ligase for DELLA protein substrates. *Plant Cell* **16**, 1406-1418.
Gandikota, M., Birkenbihl, R. P., Hohmann, S., Cardon, G. H., Saedler, H. and Huijser, P. (2007). The miRNA156/157 recognition element in the 3′ UTR of the Arabidopsis SBP box gene SPL3 prevents early flowering by translational inhibition in seedlings. *Plant J.* **49**, 683-693.
Gocal, G. F. W., Sheldon, C. C., Gubler, F., Moritz, T., Bagnall, D. J., MacMillan, C. P., Li, S. F., Parish, R. W., Dennis, E. S., Weigel, D. et al. (2001). GAMYB-like genes, flowering, and gibberellin signaling in Arabidopsis. *Plant Physiol.* **127**, 1682-1693.
Harberd, N. P., Belfield, E. and Yasumura, Y. (2009). The angiosperm gibberellin-GID1-DELLA growth regulatory mechanism: how an "inhibitor of an inhibitor" enables flexible response to fluctuating environments. *Plant Cell* **21**, 1328-1339.

Haritatos, E., Ayre, B. G. and Turgeon, R. (2000). Identification of phloem involved in assimilate loading in leaves by the activity of the galactinol synthase promoter. *Plant Physiol.* **123**, 929-937.
Hisamatsu, T. and King, R. W. (2008). The nature of floral signals in Arabidopsis. II. Roles for FLOWERING LOCUS T (FT) and gibberellin. *J. Exp. Bot.* **59**, 3821-3829.
Jaeger, K. E. and Wigge, P. A. (2007). FT protein acts as a long-range signal in Arabidopsis. *Curr. Biol.* **17**, 1050-1054.
Jang, S., Torti, S. and Coupland, G. (2009). Genetic and spatial interactions between FT, TSF and SVP during the early stages of floral induction in Arabidopsis. *Plant J.* **60**, 614-625.
Jasinski, S., Piazza, P., Craft, J., Hay, A., Woolley, L., Rieu, I., Phillips, A., Hedden, P. and Tsiantis, M. (2005). KNOX action in Arabidopsis is mediated by coordinate regulation of cytokinin and gibberellin activities. *Curr. Biol.* **15**, 1560-1565.
Jung, J.-H., Ju, Y., Seo, P. J., Lee, J.-H. and Park, C.-M. (2011). The SOC1-SPL module integrates photoperiod and gibberellic acid signals to control flowering time in Arabidopsis. *Plant J.* **69**, 577-588.
Kardailsky, I., Shukla, V. K., Ahn, J. H., Dagenais, N., Christensen, S. K., Nguyen, J. T., Chory, J., Harrison, M. J. and Weigel, D. (1999). Activation tagging of the floral inducer FT. *Science* **286**, 1962-1965.
Kobayashi, Y. and Weigel, D. (2007). Move on up, it's time for change-mobile signals controlling photoperiod-dependent flowering. *Genes Dev.* **21**, 2371-2384.
Kobayashi, Y., Kaya, H., Goto, K., Iwabuchi, M. and Araki, T. (1999). A pair of related genes with antagonistic roles in mediating flowering signals. *Science* **286**, 1960-1962.
Lee, J. H., Yoo, S. J., Park, S. H., Hwang, I., Lee, J. S. and Ahn, J. H. (2007). Role of SVP in the control of flowering time by ambient temperature in Arabidopsis. *Genes Dev.* **21**, 397-402.
Li, D., Liu, C., Shen, L., Wu, Y., Chen, H., Robertson, M., Helliwell, C. A., Ito, T., Meyerowitz, E. and Yu, H. (2008). A repressor complex governs the integration of flowering signals in Arabidopsis. *Dev. Cell* **15**, 110-120.
Markwell, J., Osterman, J. C. and Mitchell, J. L. (1995). Calibration of the Minolta SPAD-502 leaf chlorophyll meter. *Photosynth. Res.* **46**, 467-472.
Mathieu, J., Warthmann, N., Kuttner, F. and Schmid, M. (2007). Export of FT protein from phloem companion cells is sufficient for floral induction in Arabidopsis. *Curr. Biol.* **17**, 1055-1060.
McGinnis, K. M., Thomas, S. G., Soule, J. D., Strader, L. C., Zale, J. M., Sun, T. P. and Steber, C. M. (2003). The Arabidopsis SLEEPY1 gene encodes a putative F-box subunit of an SCF E3 ubiquitin ligase. *Plant Cell* **15**, 1120-1130.
Michaels, S. D., Himelblau, E., Kim, S. Y., Schomburg, F. M. and Amasino, R. M. (2005). Integration of flowering signals in winter-annual Arabidopsis. *Plant Physiol.* **137**, 149-156.
Moon, J., Suh, S. S., Lee, H., Choi, K. R., Hong, C. B., Paek, N. C., Kim, S. G. and Lee, I. (2003). The SOC1 MADS-box gene integrates vernalization and gibberellin signals for flowering in Arabidopsis. *Plant J.* **35**, 613-623.
Nakajima, M., Shimada, A., Takashi, Y., Kim, Y. C., Park, S. H., Ueguchi-Tanaka, M., Suzuki, H., Katoh, E., Iuchi, S., Kobayashi, M. et al. (2006). Identification and characterization of Arabidopsis gibberellin receptors. *Plant J.* **46**, 880-889.
Peng, J. R., Carol, P., Richards, D. E., King, K. E., Cowling, R. J., Murphy, G. P. and Harberd, N. P. (1997). The Arabidopsis GAI gene defines a signaling pathway that negatively regulates gibberellin responses. *Genes Dev.* **11**, 3194-3205.
Phillips, A. L., Ward, D. A., Uknes, S., Appleford, N. E. J., Lange, T., Huttly, A. K., Gaskin, P., Graebe, J. E. and Hedden, P. (1995). Isolation and expression of 3 gibberellin 20-oxidase cDNA clones from Arabidopsis. *Plant Physiol.* **108**, 1049-1057.
Pnueli, L., Gutfinger, T., Hareven, D., Ben-Naim, O., Ron, N., Adir, N. and Lifschitz, E. (2001). Tomato SP-interacting proteins define a conserved signaling system that regulates shoot architecture and flowering. *Plant Cell* **13**, 2687-2702.
Ranjan, A., Fiene, G., Fackendahl, P. and Hoecker, U. (2011). The Arabidopsis repressor of light signaling SPA1 acts in the phloem to regulate seedling de-etiolation, leaf expansion and flowering time. *Development* **138**, 1851-1862.
Reeves, P. H. and Coupland, G. (2001). Analysis of flowering time control in Arabidopsis by comparison of double and triple mutants. *Plant Physiol.* **126**, 1085-1091.
Richter, R., Behringer, C., Muller, I. K. and Schwechheimer, C. (2010). The GATA-type transcription factors GNC and GNL/CGA1 repress gibberellin signaling downstream from DELLA proteins and PHYTOCHROME-INTERACTING FACTORS. *Genes Dev.* **24**, 2093-2104.
Rieu, I., Eriksson, S., Powers, S. J., Gong, F., Griffiths, J., Woolley, L., Benlloch, R., Nilsson, O., Thomas, S. G., Hedden, P. et al. (2008a). Genetic analysis reveals that C(19)-GA 2-oxidation is a major gibberellin inactivation pathway in Arabidopsis. *Plant Cell* **20**, 2420-2436.
Rieu, I., Ruiz-Rivero, O., Fernandez-Garcia, N., Griffiths, J., Powers, S. J., Gong, F., Linhartova, T., Eriksson, S., Nilsson, O., Thomas, S. G. et al. (2008b). The gibberellin biosynthetic genes AtGA20ox1 and AtGA20ox2 act, partially redundantly, to promote growth and development throughout the Arabidopsis life cycle. *Plant J.* **53**, 488-504.
Sakamoto, T., Kobayashi, M., Itoh, H., Tagiri, A., Kayano, T., Tanaka, H., Iwahori, S. and Matsuoka, M. (2001). Expression of a gibberellin 2-oxidase gene around the shoot apex is related to phase transition in rice. *Plant Physiol.* **125**, 1508-1516.
Samach, A., Onouchi, H., Gold, S. E., Ditta, G. S., Schwarz-Sommer, Z., Yanofsky, M. F. and Coupland, G. (2000). Distinct roles of CONSTANS target genes in reproductive development of Arabidopsis. *Science* **288**, 1613-1616.
Schmid, M., Uhlenhaut, N. H., Godard, F., Demar, M., Bressan, R., Weigel, D. and Lohmann, J. U. (2003). Dissection of floral induction pathways using global expression analysis. *Development* **130**, 6001-6012.
Schomburg, F. M., Bizzell, C. M., Lee, D. J., Zeevaart, J. A. D. and Amasino, R. M. (2003). Overexpression of a novel class of gibberellin 2-oxidases decreases gibberellin levels and creates dwarf plants. *Plant Cell* **15**, 151-163.
Schwab, R., Palatnik, J. F., Riester, M., Schommer, C., Schmid, M. and Weigel, D. (2005). Specific effects of MicroRNAs on the plant transcriptome. *Dev. Cell* **8**, 517-527.
Schwarz, S., Grande, A. V., Bujdoso, N., Saedler, H. and Huijser, P. (2008). The microRNA regulated SBP-box genes SPL9 and SPL15 control shoot maturation in Arabidopsis. *Plant Mol. Biol.* **67**, 183-195.
Searle, I., He, Y. H., Turck, F., Vincent, C., Fornara, F., Krober, S., Amasino, R. A. and Coupland, G. (2006). The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in Arabidopsis. *Genes Dev.* **20**, 898-912.
Takada, S. and Goto, K. (2003). TERMINAL FLOWER2, an Arabidopsis homolog of HETEROCHROMATIN PROTEIN1, counteracts the activation of FLOWERING LOCUS T by CONSTANS in the vascular tissues of leaves to regulate flowering time. *Plant Cell* **15**, 2856-2865.
Torti, S., Fornara, F., Vincent, C., Andrés, F., Nordström, K., Göbel, U., Knoll, D., Schoof, H. and Coupland, G. (2012). Analysis of the Arabidopsis shoot meristem transcriptome during floral transition identifies distinct regulatory patterns and a leucine-rich repeat protein that promotes flowering. *Plant Cell* **24**, 444-462.
Turck, F., Fornara, F. and Coupland, G. (2008). Regulation and identity of florigen: FLOWERING LOCUS T moves center stage. *Annu. Rev. Plant Biol.* **59**, 573-594.
Wang, J. W., Czech, B. and Weigel, D. (2009). miR156-regulated SPL transcription factors define an endogenous flowering pathway in Arabidopsis thaliana. *Cell* **138**, 738-749.
Wigge, P. A., Kim, M. C., Jaeger, K. E., Busch, W., Schmid, M., Lohmann, J. U. and Weigel, D. (2005). Integration of spatial and temporal information during floral induction in Arabidopsis. *Science* **309**, 1056-1059.
Willige, B. C., Ghosh, S., Nill, C., Zourelidou, M., Dohmann, E. M. N., Maier, A. and Schwechheimer, C. (2007). The DELLA domain of GA INSENSITIVE mediates the interaction with the GA INSENSITIVE DWARF1A gibberellin receptor of Arabidopsis. *Plant Cell* **19**, 1209-1220.
Wilson, R. N., Heckman, J. W. and Somerville, C. R. (1992). Gibberellin is required for flowering in Arabidopsis thaliana under short days. *Plant Physiol.* **100**, 403-408.
Woodger, F. J., Millar, A., Murray, F., Jacobsen, J. V. and Gubler, F. (2003). The role of GAMYB transcription factors in GA-regulated gene expression. *J. Plant Growth Regul.* **22**, 176-184.
Wu, G. and Poethig, R. S. (2006). Temporal regulation of shoot development in Arabidopsis thaliana by miR156 and its target SPL3. *Development* **133**, 3539-3547.
Wu, G., Park, M. Y., Conway, S. R., Wang, J. W., Weigel, D. and Poethig, R. S. (2009). The sequential action of miR156 and miR172 regulates developmental timing in Arabidopsis. *Cell* **138**, 750-759.
Xu, Y. L., Li, L., Wu, K. Q., Peeters, A. J. M., Gage, D. A. and Zeevaart, J. A. D. (1995). The GA5 locus of Arabidopsis-thaliana encodes a multifunctional gibberellin 20-Oxidase-molecular cloning and functional expression. *Proc. Natl. Acad. Sci. USA* **92**, 6640-6644.
Yamaguchi, A., Kobayashi, Y., Goto, K., Abe, M. and Araki, T. (2005). TWIN SISTER OF FT (TSF) acts as a floral pathway integrator redundantly with FT. *Plant Cell Physiol.* **46**, 1175-1189.
Yamaguchi, A., Wu, M. F., Yang, L., Wu, G., Poethig, R. S. and Wagner, D. (2009). The microRNA-regulated SBP-box transcription factor SPL3 is a direct upstream activator of LEAFY, FRUITFULL, and APETALA1. *Dev. Cell* **17**, 268-278.
Yamaguchi, S. (2008). Gibberellin metabolism and its regulation. *Annu. Rev. Plant Biol.* **59**, 225-251.
Yang, H. P., Schmuke, J. J., Flagg, L. M., Roberts, J. K., Allen, E. M., Ivashuta, S., Gilbertson, L. A., Armstrong, T. A. and Christian, A. T. (2009). A novel real-time polymerase chain reaction method for high throughput quantification of small regulatory RNAs. *Plant Biotechnol. J.* **7**, 621-630.