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RESEARCH PAPER

The nature of floral signals in *Arabidopsis*. I. Photosynthesis and a far-red photoresponse independently regulate flowering by increasing expression of *FLOWERING LOCUS T* (*FT*)

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Abstract

Arabidopsis **flowers in long day (LD) in response to signals transported from the photoinduced leaf to the shoot apex. These LD signals may include protein of the gene *FLOWERING LOCUS T* (*FT*) while in short day (SD) with its slower flowering, signalling may involve sucrose and gibberellin. Here, it is shown that after 5 weeks growth in SD, a single LD up-regulated leaf blade expression of *FT* and *CONSTANS* (*CO*) within 4–8 h, and flowers were visible within 2–3 weeks. Plants kept in SDs were still vegetative 7 weeks later. This LD response was blocked in *ft-1* and a *co* mutant. Exposure to different LD light intensities and spectral qualities showed that two LD photoresponses are important for up-regulation of *FT* and for flowering. Phytochrome is effective at a low intensity from far-red (FR)-rich incandescent lamps. Independently, photosynthesis is active in an LD at a high intensity from red (R)-rich fluorescent lamps. The photosynthetic role of a single high light LD is demonstrated here by the blocking of the flowering and *FT* increase on removal of atmospheric CO_2 or by decreasing the LD light intensity by 10-fold. These conditions also reduced leaf blade sucrose content and photosynthetic gene expression. An SD light integral matching that in a single LD was not effective for flowering, although there was reasonable *FT*-independent flowering after 12 SD at high light. While a single photosynthetic LD strongly amplified *FT* expression, the ability to respond to the LD required an additional but unidentified photoresponse. The implications of these findings for studies with mutants and for flowering in natural conditions are discussed.**

Key words: *Arabidopsis*, *CONSTANS*, far-red light, flowering, *FT*, long day, photosynthesis, sucrose.

Introduction

Flowering of *Arabidopsis thaliana* (L.) Heynh. is regulated environmentally by daylength and cold (Boss *et al*., 2004; Searle and Coupland, 2004; Imaizumi and Kay, 2006: Turck *et al*., 2008). Its light response in long days (LD) involves phytochrome (*PHY*) and the blue photoreceptors (Goto *et al*., 1991; Reed *et al*., 1994; Bagnall and King, 2001; Endo *et al*., 2007, and references therein). These photoreceptors interact with endogenous oscillators to activate expression in leaf blade vascular tissue of two 'floral' genes, *CONSTANS* (*CO*) and *FLOWERING LOCUS T* (*FT*) (Halliday *et al*., 2003; Imaizumi *et al*., 2003; Takada and Goto, 2003; An *et al*., 2004; Valverde *et al*., 2004; Endo *et al*., 2007).

Genetically, the link between *FT* and flowering is shown by the delayed flowering in *ft* mutants and early flowering in overexpression lines (Koorneef *et al*., 1991; Kardailsky *et al*., 1999; Kobayashi *et al*., 1999; Yoo *et al*., 2005). Furthermore, recent evidence implies LD

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Abbreviations: *CO*, *CONSTANS*; FR, far red light; *FT*, *FLOWERING LOCUS T*; GA, gibberellin; LD, long day conditions; R, red light; SD, short day conditions.

floral signalling by FT protein which may act as a signal transmitted from the leaf to the shoot apex in *Arabidopsis*, *Cucurbita* spp, and rice (Corbesier *et al*., 2007; Jaeger and Wigge, 2007; Lin *et al*., 2007; Mathieu *et al*., 2007; Tamaki *et al*., 2007). At the shoot apex, FT then interacts in a putative transcriptional complex with protein of the *FD* gene (Abe *et al*., 2005; Wigge *et al*., 2005).

Far-red (FR) light acting via phytochrome up-regulates *FT* expression in *Arabidopsis* (Cerdän and Chory, 2003; Halliday *et al*., 2003; Valverde *et al*., 2004) and, in parallel, FR promotes flowering (Reed *et al*., 1994; Bagnall and King, 2001, and references therein). Red (R) light acting via phytochrome blocks the *FT* increase and flowering is delayed. Paradoxically, however, high light intensities from R-rich fluorescent lamps are traditionally used to show LD up-regulation of *FT* (Suarez-Lopez *et al*., 2001; Imaizumi *et al*., 2003; Takada and Goto, 2003; Valverde *et al*., 2004; Abe *et al*., 2005; Wigge *et al*., 2005; Yoo *et al*., 2005).

Here, for *Arabidopsis* this paradox is explained by showing independent LD photoregulation of both *FT* and flowering by photosynthesis in high intensity R-rich light and by phytochrome in low intensity FR-rich light. Importantly, to assess cause and effect in the link between photosynthesis, *FT*, and flowering, conditions were used which give rapid flowering after exposure to a single LD. In a limited way, the question of sucrose regulation of the extremely late short day (SD) flowering of *Arabidopsis* (Ericksson *et al*., 2006) is also addressed.

Materials and methods

Plant material, growing conditions, and light treatments

Plants of *A. thaliana* (L.) Heynh. ecotype Columbia and various mutant lines in Columbia were grown in 8 h SDs at 22 °C under an irradiance of 100 μmol m^{-2} s^{-1} from fluorescent lamps. There was a very limited flowering by 3 weeks (up to 3% of 1000 plants in various experiments). These precociously flowering plants were removed and the remainder were still vegetative at 3 months.

For flower induction, the plants were always 5 weeks old when exposed to one or up to five LDs. The LD light extension was for a duration of 16 h from incandescent lamps at 10 μmol m^{-2} s^{-1} or from fluorescent lamps at either 10 μmol m^{-2} s^{-1} or 100 μmol m^{-2} s^{-1}. In one experiment (Fig. 7), light intensity in 8 h SDs was increased ~3-fold (to 270 μmol m^{-2} s^{-1} or 360 μmol m^{-2} s^{-1}) for a total of 12 d; each SD was terminated by a 10 min exposure to incandescent lamps.

The spectral output of the fluorescent lamps used here is enriched in yellow/orange and R wavebands, and the incandescent bulbs provide predominantly FR light (Supplementary Fig. S1 available at *JXB* online). The R:FR ratio of 0.8 in the FR-rich light provides spectral conditions close to direct sunlight (R:FR 1.1–1.25) especially as there is a substantial further enrichment for FR at twilight (Smith, 1982). Exposure to an LD from R-rich fluorescent lamps (R:FR 4.5) is often used but does not match natural conditions as closely as the FR-rich LD treatment. Compared with these fluorescent and incandescent lamps, there are matching responses of growth and flowering of *Arabidopsis* to light from narrow-band R and FR sources (Bagnall and King, 2001; Hisamatsu *et al*., 2005). Therefore, subsequently, the LDs here from fluorescent lamps is referred to as a R-rich LDs (LD-*R*) at a high light intensity (100 μmol m^{-2} s^{-1}) and as a low light R-rich LDs (LD-*lR*) at a 10-fold lower intensity An LD from incandescent lamps at low light is referred to as an FR-rich LD (LD-*FR*).

Flowering began 10–20 d after an LD (flower buds were visible ~2–3 d after bolt appearance) and was recorded both as percentage flowering and as days from the start of the LD until petal appearance. The findings of all experiments presented here have been confirmed in repeat studies.

For genetic studies of *CO*/*FT* in Columbia, the *co* mutant was from the SAIL T-DNA collection and carries a T-DNA insertion at bp 342 after the ATG (Laubinger *et al*., 2006). It causes late flowering in LD as with other *co* mutants (Putterill *et al*., 1995), produces very low levels of *CO* (Dr I Searle, Max Planck Institute, Koeln, Germany, personal communication), and lacks detectable *FT* expression in LD (T Hisamatsu, data not shown). The *ft-1* mutant was a third backcross line in Columbia crossed in from Landsberg *erecta*. Eighth backcross material of this *ft-1* line became available at the end of this study and it responded in a similar way to the third backcross material.

To block photosynthesis, 16 plants per treatment were enclosed in 7. 0 l clear plastic boxes which were flushed with CO_2-free or normal air at 2 l min^{-1}. Scrubbing of CO_2 was through a 1.0 l plastic cylinder filled with indicating soda lime. All CO_2 was quickly removed (<4 min), as measured with a gas analysis system. At the flow rates used, the soda lime column remained effective for up to 48 h. To balance the humidity of the air streams, both the CO_2-free and the normal air were aspirated in sealed 2.0 l water columns before entering the boxes.

Statistical analysis

Some statistical analysis involved analysis of variance (ANOVA) and calculation of $LSD_{0.05}$. Otherwise errors are shown as means ±SE. In many instances, the error was smaller than the symbol and is not visible in the figures. Unless indicated otherwise, 16 plants per treatment were assessed for calculating percentagr flowering and flowering time. All experiments reported here have been repeated at least once.

Quantitative real-time PCR analysis of gene expression

For studies of gene expression, the youngest fully expanded leaves were harvested from ⩾16 plants. Where harvests were during the dark period, a green safe light was used. Total RNA was extracted using an RNeasy Plant Mini Kit (Qiagen, Clifton Hills, Victoria, Australia) and treated with RNase-free DNase (Qiagen) according to the manufacturer's instructions An aliquot of 1 or 2 μg of total RNA was reverse-transcribed using Super Script II (Invitrogen, Mt Waverley, Victoria, Australia) according to the manufacturer's instructions. The cDNA was diluted 5- or 25-fold, and 4 μl was used in a 10 μl Q-PCR with SYBR Green JumpStart Taq ReadyMix (Sigma Aldrich, Castle Hill, NSW, Australia) performed on a Rotor-Gene 2000 Real-Time Cycler (Corbett Research, Sydney, Australia). The Q-PCR assays were repeated three times and, for any claimed treatment effects, the result was confirmed in at least one further independent experiment. All samples were normalized using the 'Comparative Quantification' analysis method (Rotogene-5 software, Corbett Research), and RNA expression is compared directly after normalization against an *ACTIN2* loading standard.

Primer pairs previously characterized were: *CO*, *FT*, and *SOC1* (Halliday *et al*., 2003) and *ACTIN2* (Hisamatsu *et al*., 2005). The internal standard, *ACTIN2* (At3g18780), was constant in the samples assayed. Further details of these assays are given in

Hisamatsu *et al*. (2005). The means presented are averages from three technical replicates, and all experiments have been repeated.

Measurement of leaf and shoot apex sucrose content

As shown in Supplementary Fig. S2 at *JXB* online, the 'shoot apex' used for the sucrose assays refers to a tissue piece no bigger than 250 μm diameter after dissection and which weighed <1 μg dry weight. It included the true shoot apex, some basal pith tissue, and up to two leaf primordia as large as the apex itself (Supplementary Fig. S2 at *JXB* online). This 'shoot apex' is smaller by several orders of magnitude than the 3 mm tissues pieces harvested as shoot tips but incorrectly described in the literature as 'the shoot apex'. Dissecting this minute shoot apex was not difficult and the GCMS-SIM assays of sucrose showed high reproducibility due to the inclusion of a [^{13}C]sucrose internal standard. Sensitivity of the microbalance was a limitation so four to five apices were combined in each assay and sufficient apices were collected for 5 replicate assays. The methods for sucrose extraction and quantification by GCMS-SIM are given in detail in King and Ben-Tal (2001).

Results

A sensitive LD flowering response

Arabidopsis (ecotype Columbia) flowered rapidly on exposure to a single 16 h LD light extension either at high light intensity (100 μmol m^{-2} s^{-1}) from R-rich (LD-*R*) fluorescent lamps or at a low intensity (10 μmol m^{-2} s^{-1}) from FR-rich (LD-*FR*) incandescent lamps (Fig. 1A). These plants were 5 weeks old when exposed to this single LD, and flower buds were visible 2 weeks later. Untreated control plants in SD were still vegetative 7 weeks later (i.e. 3 months after germination). Obligate and precise LD flowering in response to a single LD confirms earlier reports for *Arabidopsis* after its exposure to an R-rich LD (Corbesier *et al*., 1996) or to an FR-rich LD (Gocal *et al*., 2001).

In Fig. 1B the conditions for a single cycle floral induction by an R- (LD-*R*) or FR-rich LD (LD-*FR*) are shown schematically along with the SD control. Plants exposed to a low intensity (10 μmol m^{-2} s^{-1}) R-rich LD (LD-*lR*) were essentially vegetative (Fig. 1A), and this provided an additional 'control' treatment.

Based on published light response curves for photosynthesis of *Arabidopsis* (Walters *et al*., 1999), only the higher intensity R-rich LD would contribute photosynthetically. Thus, the restricted flowering in the R-rich low light LD-*lR* treatment (Fig. 1A) could suggest photosynthetic regulation of flowering in a high light intensity LD. The spectral contrast between flowering at a low light intensity after exposure to a single FR-rich LD but not after an R-rich LD, indicates an additional LD light response and one which, for these incandescent lamps, would involve *PHYB*, as shown previously (Bagnall and King, 2001).

Referencing time of the day to the time from the daily light-on signal (hour 0) is consistent with other studies.

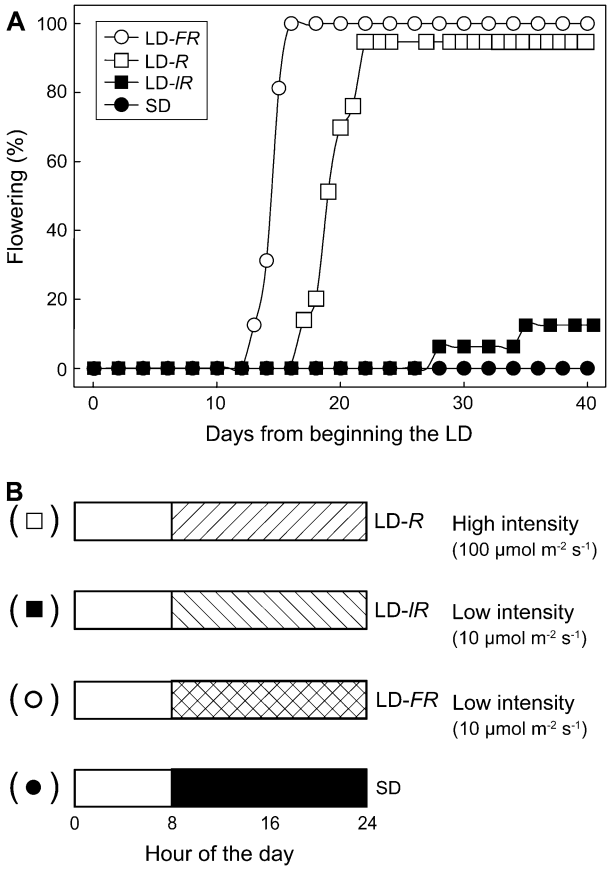

Fig. 1. A rapid LD flowering response of *Arabidopsis*, ecotype Columbia. Effect of a single LD on flowering of plants of Columbia (% plants flowering; A). The four daylength treatments imposed here and in further studies are shown schematically in (B). Prior to the single LD exposure, all plants were grown in 8 h SD for 5 weeks. After the LD, they were returned to SDs for daily recording of flowering. At 40 d (75 d from germination) there was little or no flowering of plants in SDs or after a single low intensity R-rich LD. There were 16 replicate plants for flowering assays. The shaded areas show the 'overnight' 16 h light or dark exposure.

However, the response to a single LD cycle is sometimes referred back to the end of the 8 h SD as this adds focus to the rapidity of the response to the LD.

Following the single LD there was a rapid response at the shoot tip; its expression of *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*) began to increase by hour 20 (Supplementary Fig. S3 at *JXB* online) and the size of the apex had increased dramatically by hour 24–48 (Gocal *et al*., 2001). Based on this evidence of early change at the shoot tip/apex, the subsequent analysis of the timing of leaf blade gene expression has focused on changes during the first LD.

An FT mutant blocks LD flowering

Flowering of *Arabidopsis* is inhibited in *ft* mutants (Koorneef *et al*., 1991; Kardailsky *et al*., 1999; Kobayashi *et al*., 1999; Yoo *et al*., 2005) and, here, *ft-1* in Columbia

completely inhibited the flowering of plants exposed to high intensity R-rich LD (Fig. 2). The point mutation in *ft-1* is near to the C-terminal group (Kardailsky *et al.*, 1999) and there is detectable mRNA production (Yoo *et al.*, 2005); nevertheless, its protein product is apparently sufficiently defective for *ft-1* to block LD flowering.

The lack of effect of *ft-1* on the late onset of flowering in SDs not only shows the specificity of *FT* for LDs but, more importantly, that *FT* accounts for all the flowering response to high intensity R-rich LD (LD-*R*). In contrast, flowering in FR-rich LD (LD-*FR*) was inhibited in *ft-1* (Fig. 2B) but it was not blocked completely, and the same result was found in a repeat experiment with *ft-1* backcrossed eight times into Columbia (not shown). Apparently, floral signalling in an R-rich LD involves *FT* alone but in an FR-rich LD there may be an additional signalling component, and this possibility is examined in the companion paper (Hisamatsu and King, 2008).

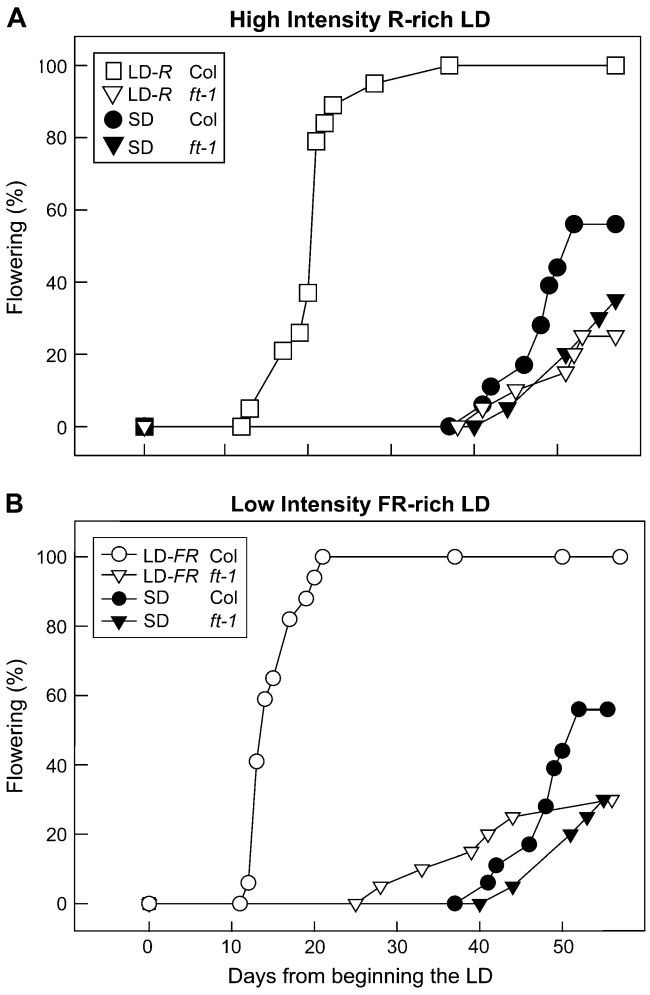

Fig. 2. The *ft-1* mutant blocks LD flowering. Plants of Columbia or *ft-1* were held in SD (filled symbols) or exposed to five LDs (open symbols) from high intensity R-rich fluorescent lamps (A) or from low intensity FR-rich lamps (B). These daylength treatments are described in Fig. 1.

CO regulates *FT* (Valverde *et al.*, 2004; see review in Turck *et al.*, 2008) and it was found that a *co* mutant in Columbia delayed flowering in LD-*R* and LD-*FR* (Supplementary Fig. S4 at *JXB* online) and blocked *FT* expression (not shown). However, this *co* mutant was not as effective as *ft-1*.

LDs and FT expression

Within 4–8 h of commencing a single florally inductive LD (hour 12–16), *FT* expression in the leaf blade increased rapidly and dramatically (Fig. 3A, B). Normalization to the highest value emphasizes the timing of the LD increase in *FT* in any one LD light exposure. When all four daylength conditions were included in a repeat experiment (Fig. 3C), their normalization to the FR-rich LD allows comparison across light treatments. An early and substantial increase in *FT* expression was only evident in the two florally effective LDs (LD-*R* and LD-*FR*). There was little or no increase in SDs or in a low intensity R-rich LD (LD-*lR*). Thus, as for flowering (Fig. 1), there is separate spectral and light intensity specificity for LD upregulation of *FT* expression. There is no obvious explanation for the delayed response of *FT* to an FR-rich LD relative to an R-rich LD.

In parallel with increase in *FT* expression, a florally effective R- or FR-rich LD increased *CO* expression (Fig. 4). In a low intensity R light LD (LD-*lR*) there was some increase in *CO* expression, but this was apparently not sufficient to affect *FT* expression which was weak (Fig. 3C) and matched by poor or nil flowering (Fig. 1A). A repeat experiment with more frequent sampling confirmed the FR-rich LD increase in *CO* expression (Supplementary Fig. S5 at *JXB* online).

None of the LD treatments increased *SOC1* expression in the leaf blade relative to plants in SD (Fig. 4), which confirms the findings of Wigge *et al.* (2005). In contrast, LDs increased expression of *SUC2*, a photosynthetically regulated gene, but only in a high light, R-rich LD (Fig. 4). This finding emphasizes the potential for a photosynthetic input in this high light LD.

Taken together, these studies (Figs 1–3) show that activation of *FT* in LDs accounts for flowering. In addition, they highlight inputs by two photoresponses, a low intensity (non-photosynthetic) response in a low light FR-rich LD and a photosynthetic input in a high intensity R-rich LD.

Photosynthetic regulation of FT and flowering

Photosynthetic regulation of *FT* in a high intensity R-rich LD is implied by the evidence that flowering and *FT* expression were both restricted by a 10-fold reduction in the LD light intensity (Figs 1, 3). To confirm this role for photosynthesis, plants were exposed to CO_2-free air to block photosynthesis during the 16 h high light LD

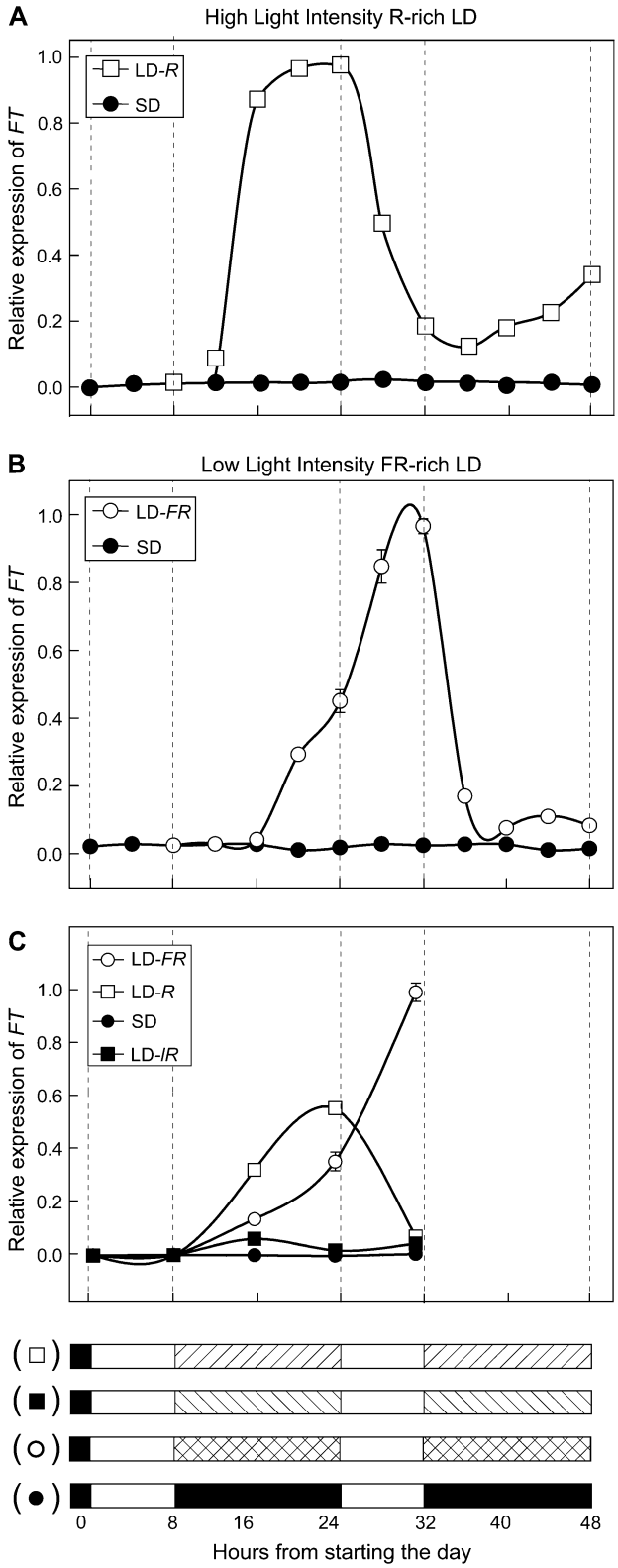

Fig. 3. An LD rapidly up-regulates *FT* expression. *FT* expression was assayed in the leaf blade over the first day of LD exposure and during a repeat of this LD. Compared with the SD control, both a high light R-rich LD (A) and an FR-rich LD (B) dramatically increased *FT* expression. In a repeat experiment, shown in (C), *FT* expression was assayed for all four daylength treatments described in Fig. 1. The dashed lines and schematic underneath the figures show the daily timing of light and of dark. Gene expression is normalized to the maximum value taken as 1.0. All values are means ±SE (n=3). The error bars are generally not evident because they were smaller than the symbols.

exposure (LD-*R*). Flowering was delayed by the removal of atmospheric CO_2 for the single 16 h 'overnight' period of high light intensity (Fig. 5A) and, in parallel, the normal *FT* increase was restricted (Fig. 5B). The same treatment had no effect on plants exposed to this LD at a 10-fold lower intensity (LD-*lR*); their flowering was late and *FT* expression was low (Fig. 5). Two further studies confirmed the strong delay of flowering when CO_2-free conditions were imposed for the entire period of a two LD high light period or for the 16 h 'overnight' periods of two high light LDs (not shown).

A role for photosynthesis in a high light LD is also supported by the measurements of sucrose content of the leaf and shoot apex. At a high light intensity, the LD caused an early doubling of leaf blade sucrose (<8 h after starting the LD, Fig. 6A), but there was little or no increase for plants in 16 h of darkness (SD) or exposed to a low intensity LD (LD-*lR* or LD-*FR*). At the shoot apex, sucrose increased in parallel with its increase in the LD leaf (Fig. 6B, C). Furthermore, exposure to CO_2-free air during a 16 h high light LD extension blocked sucrose increase in the leaf blade and shoot apex (Table 1).

LD increases in expression in the leaf blade of photosynthetically regulated genes (Fig. 4; Supplementary Fig. S6 at *JXB* online) also support this claim of a photosynthetic role for a high light LD. For example, up-regulation of *SUC2,* a gene which regulates sucrose transport, was only evident in a high light LD (Fig. 4). Comparable high light LD increases are shown in Supplementary Fig. S6 at *JXB* online, not only for *SUC2*, but also for genes involved in sucrose accumulation (*SUS1*), its perception (*AKIN1* a SNF1-like kinase), carbon interconversions (e.g. *ADPG* and *INVERTASE*), and other aspects of photosynthetic carbon fixation (*SUC3*).

In addition to the finding that *ft-1* completely blocked the high light LD response (Fig. 2), there was no flowering when only sucrose was allowed to increase. First, when *co* was used to block the *FT* increase, a high light LD was ineffective for flowering but the LD still increased the sucrose content of the leaf and shoot apex (Table 1). Secondly, in SD with its low *FT* expression (Fig. 3), Columbia did not flower when the light intensity in a single SD was increased to 360 μmol m^{-2} s^{-1} (not shown). Assuming a linear increase in photosynthesis with light intensity (Walters *et al.*, 1999), the daily photosynthetic gain in an 8 h SD at a light intensity of 360 μmol m^{-2} s^{-1} exceeds that for a 24 h LD exposure at 100 μmol m^{-2} s^{-1}.

Taken together, these findings imply that photosynthesis in the LD leaf regulates flowering by up-regulating *FT*

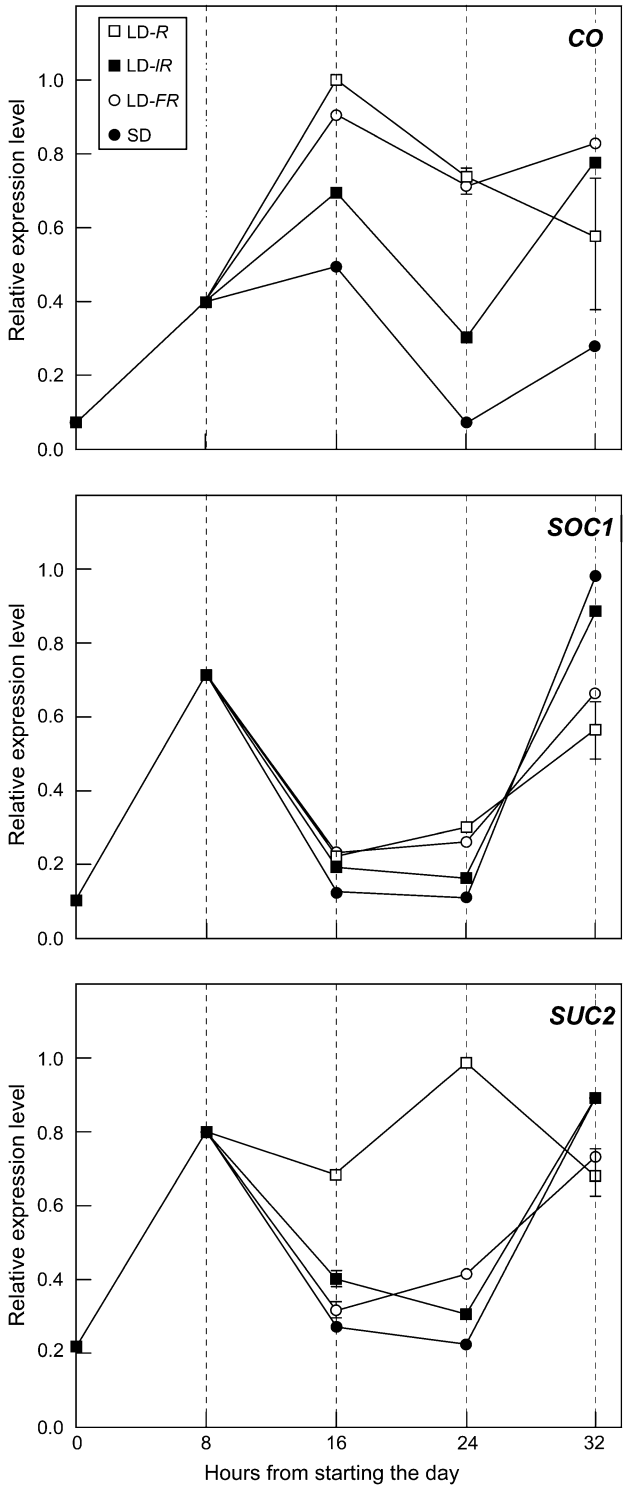

Fig. 4. Effects of LD on leaf blade gene expression. Expression in the leaf blade is shown for three genes *CO*, *SOC1*, and *SUC2*. *FT* expression in this experiment is shown in Fig. 3C. Other conditions were as for Figs 1 and 3.

expression. Secondly, because increasing photosynthesis for one SD was not sufficient to trigger flowering, LD specification to allow photosynthetic up-regulation of *FT* must be signalled via an additional photoresponse..

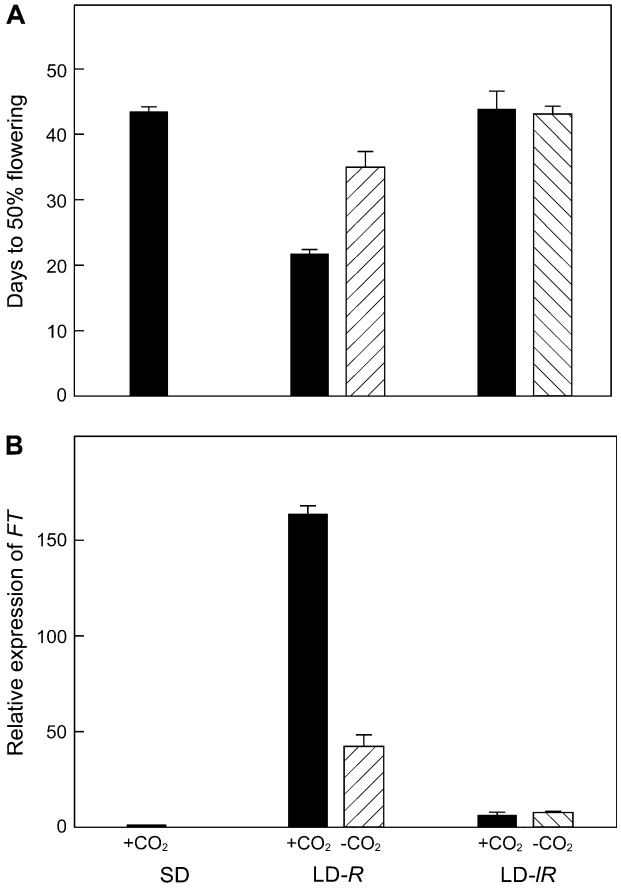

Fig. 5. Blocking photosynthesis during an LD can inhibit flowering and *FT* expression. Flowering (A) and *FT* expression (B) are compared following an exposure to normal (solid bar) or CO_2-free air (hatched bar) during a 16 h high light LD (LD-*R*, 100 μmol m^{-2} s^{-1}) or to an LD at a 10-fold lower intensity from the same fluorescent lamps (LD-*lR*, 10 μmol m^{-2} s^{-1}).

Can photosynthetic sucrose act directly in SD floral induction?

When grown in SD, *Arabidopsis* eventually flowers (>7 weeks later Fig. 2) and there is an associated increase in shoot tip sucrose (Ericksson *et al*., 2006). Here, 5-week-old plants of Columbia flowered rapidly in 8 h SDs (2–3 weeks) when the light intensity was increased for 12 d to 270 μmol m^{-2} s^{-1} (Fig. 7). These findings were confirmed in a repeat experiment involving a 12 d exposure in SD at 360 μmol m^{-2} s^{-1} (not shown). A single high light SD exposure did not induce flowering (as noted above) and SD plants held at 100 μmol m^{-2} s^{-1} were still vegetative when the experiment was terminated at 42 d (Fig. 7). Furthermore, SD flowering was independent of *FT* because high light stimulated flowering equally well for *ft-1* and Columbia (Fig. 7).

Compared with the *FT*-dependent flowering response to a single high light LD, the *FT*-independent SD response required more cycles of high light (12 d) and the response

was slower (e.g. 20.0 ± 1.4 d to flower in Fig. 1 versus 29.0 ± 1.4 d in Fig. 7). Such differences strengthen the claim that high light in LD acts via *FT* as a dominant LD floral signal.

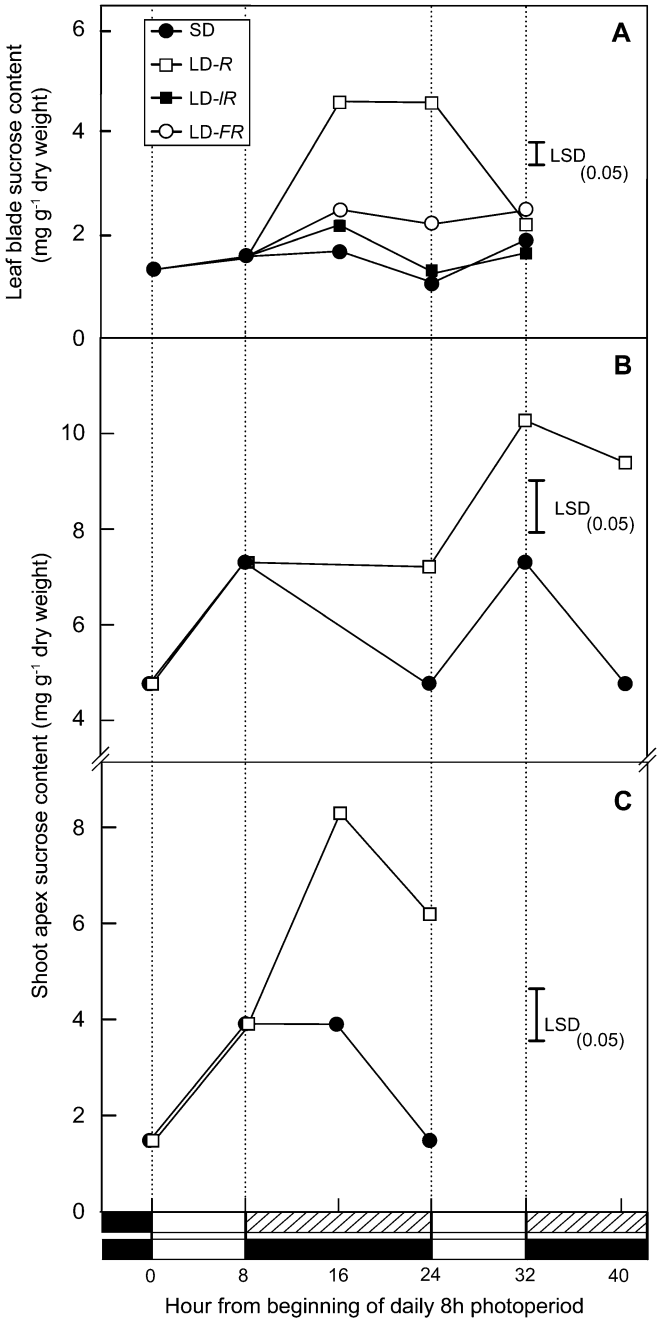

Fig. 6. Sucrose content of the leaf and shoot apex increases within hours in a high intensity R-rich LD from fluorescent lamps. Daily change in sucrose content of the leaf (A); or shoot apex (B, C) for plants in SD or exposed to an LD at a high intensity (LD-*R*; 100 μmol m^{-2} s^{-1}), at a low intensity (LD-*lR*; 10 μmol m^{-2} s^{-1}), or to an FR-rich LD (LD-*FR*; 10 μmol m^{-2} s^{-1}). The hatched bars indicate the timing of exposure to the various LD light extensions, the white bars indicate the daily 8 h light period of the SD, and the black bars indicate darkness. Each value is the mean of five replicates. Errors are shown as the LSD (*P?*=0.05) for all the data in each experiment. Other conditions are as for Fig. 1.

Discussion

Information on LD photoresponse(s) in the leaf blade is essential for any understanding of floral signals transported from the leaf to the shoot apex. Here, with *Arabidopsis*, its rapid and obligate flowering after a single LD has allowed identification of two LD photoresponses which act by up-regulating *FT* expression. Phytochrome is effective in an FR-rich LD at a low light intensity from incandescent lamps. In contrast, in a high light intensity LD from R-rich fluorescent lamps, photosynthesis is important and this novel observation has implications for studies with mutants and for understanding the role of *FT* in natural conditions.

There are at least two photoresponses controlling FT and flowering in LDs

Published studies of LD up-regulation of *FT* expression have introduced roles for both R and FR light but,

Table 1. *Effect of daylength and photosynthetic input on leaf and shoot apex sucrose content (mg g^{-1} dry weight)*

An 8 h SD light period was followed by a 16 h overnight dark period (SD) or a 16 h LD exposure to light from fluorescent lamps (LD-*R*) at a light intensity of 100 μmol m^{-2} s^{-1} in either normal or CO_2-free air. The values are means and SE of five replicate assays for apices and three replicates for the leaf for the Columbia ecotype of *Arabidopsis* and a *co* mutant of Columbia.

	SD	LD-*R*	
		Normal air	CO_2-free air
Columbia leaf	1.7±0.1	4.1±0.1	1.6±0.2
co mutant leaf	1.9±0.1	5.2±0.4	
co mutant shoot apex	3.6±0.5	5.7±0.7	0.8±0.2

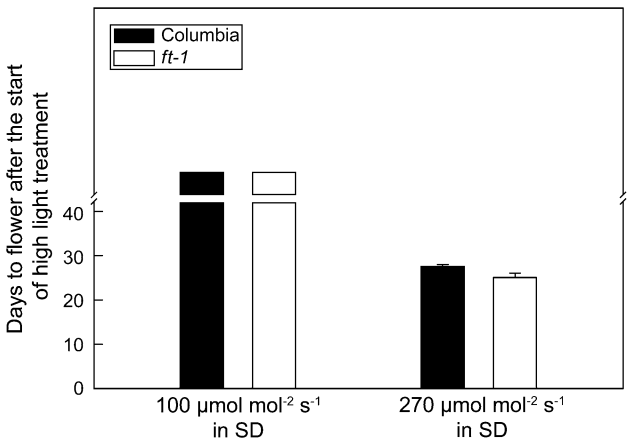

Fig. 7. An increase in light intensity in SD causes flowering equally well for Columbia and *ft-1*. Columbia (solid bar) and *ft-1* (open bar) were held for 12 d in an 8 h SD terminated daily by a 10 min exposure to low intensity FR-rich light from incandescent lamps. Half the plants were exposed to the normal SD light intensity of 100 μmol m^{-2} s^{-1} and half to an intensity of 270 μmol m^{-2} s^{-1}. None of the plants held at 100 μmol m^{-2} s^{-1} had flowered when the experiment was terminated after 42 d (77 d after germination). Values are means ±SE (*n*=18).

sometimes, without resolving which photoreceptors were active. However, when light action involves phytochrome, it is only FR light which enhances *FT* expression (Cerdän and Chory, 2003; Halliday *et al*., 2003; Valverde *et al*., 2004) and flowering (Goto *et al*., 1991; Reed *et al*., 1994; Bagnall and King, 2001, and references therein). Here, use of an additional non-florally inductive, low intensity R-rich LD separates the FR-rich phytochrome response from an intensity-dependent, R-rich photosynthetic input. The evidence provided of contributions by two photoresponses resolves the paradox that both R and FR light promote *FT* expression and flowering.

All the response to R-light LD is blocked in the *ft-1* mutant but, interestingly, flowering is not blocked completely by *ft-1* in a low intensity FR-rich LD (Fig. 2B). This result implies additional, albeit weak, signalling in an FR-rich LD. Gene redundancy involving the *FT*-related gene, *TSF*, seems unlikely. The *tsf* mutant delays flowering (Yamaguchi *et al*., 2005), but this would not explain the difference in response to *ft-1* between an FR- and an R-rich LD (Fig. 2). An alternative involves the known phytochrome up-regulation of gibberellin (GA) biosynthesis in shoots exposed to an FR-rich LD (Hisamatsu *et al*., 2005), and this explanation is examined in the companion paper (Hisamatsu and King, 2008).

Photosynthetic regulation of FT and flowering in LDs

In a leaf exposed to a high light, R-rich LD (LD-*R*), photosynthesis is important for *FT*-dependent floral signalling because: (i) this LD rapidly increases *FT* expression in the leaf blade (in 4–8 h; hours 12–16); (ii) *ft-1* completely blocks this LD-*R* flowering; (iii) this LD increases leaf sucrose and expression of genes regulating sucrose synthesis; (iv) exposure to this LD in CO_2-free air restricts increases in both *FT* and sucrose and delays flowering; and (v) a 10-fold reduction in light intensity during this LD restricts *FT* expression, prevents any sucrose increase, and delays flowering.

Support for a florigenic effect of photosynthetically derived sucrose acting in the leaf via *FT* is provided by the failure of applied sucrose to reverse late flowering of *ft-1* despite its effectiveness with five other late flowering mutants (Roldán *et al*., 1999). Nevertheless, how sucrose might act to up-regulate *FT* is unclear in the study of Roldán *et al*. (1999). They grew their plants in total darkness, so it is likely that sucrose was acting as an energy source. Similarly, the action of sucrose as an energy source for *FT* expression fits with applied sucrose reversing late flowering in lines with restricted carbon metabolism (Yu *et al*., 2000; see review in Bernier and Perilleux, 2005).

It is not clear why an increase in photosynthesis regulates *FT* expression in one LD but not in one SD at a much higher light intensity. Speculatively, in LDs, there must be an additional photoresponse potentiating the *FT* response which is then amplified by photosynthetic input. Blue light acting via the cryptochromes in a fluorescent light LD might determine daylength specificity, although it would not be part of the response to light intensity because there is little or no intensity dependence for blue light activation of *CO* expression and, it is assumed, of *FT* (Imaizumi *et al*., 2003). A more complex alternative which allows for 'gating' of the *FT* response by an endogenous rhythm introduces R light regulation of the rhythm phase, as has been reported for a number of plant responses including rhythmic CO_2 fixation in *Lemna perpusilla* Torr.(Hillman, 1971) and for a flowering rhythm in the SD plant *Chenopodium rubrum* L. (King and Cumming, 1972).

The possibility of parallel inputs to *FT* by photosynthesis, by FR-rich light, and by a potential third blue light input introduces unexpected complexity, particularly to studies with mutants. For example, delayed flowering of a Columbia *phyA* mutant could be the result of reduced photosynthetic input to *FT* since this mutant has half the wild-type leaf area and, in addition, a reduced photosynthetic pigment content (Walters *et al*., 1999; Bagnall and King, 2001). Consistent with a photosynthetic effect in *phyA*, higher light intensities reverse its late flowering. Furthermore, in the same studies, *phyA* did not delay flowering in non-photosynthetic, low light, FR-rich LD conditions (Bagnall and King, 2001). A more complex model may also be required to explain the effects of *GIGANTEA* (*GI*) on flowering. It acts as an upstream regulator of *CO* and *FT* via a link to a circadian rhythm (Mizoguchi *et al*., 2005) but also regulates sucrose interconversion to starch (Eimert *et al*., 1995), and its protein interacts with *SPINDLY* (*SPY*) to modulate GA actions on flowering (Tseng *et al*., 2004).

FT-independent flowering in high light SDs

Arabidopsis flowers early when a cell wall invertase is overexpressed near the shoot apex to enhance sucrose unloading there (Heyer *et al*., 2004). Also, sucrose increases at the shoot apex when *Arabidopsis* flowers in a photosynthetic LD (Fig. 6, and see Corbesier *et al*., 1998) and, in an SD, there is a dramatic increase in shoot tip sucrose content when Columbia eventually flowers (Ericksson *et al*., 2006). Direct regulation of flowering by transported sucrose is also favoured by the evidence presented of *FT*-independent flowering after 12 d exposure to a high light intensity in SDs (Fig. 7). Potentially, such promotion of flowering by sucrose would involve activation of *LEAFY* at the shoot apex, a possibility raised by Blázquez *et al*. (1998) from their studies of sucrose/GA-regulated increases in *LEAFY::GUS* expression.

On the other hand, *FT*-independent flowering in SDs is far weaker than *FT*-dependent flowering in a single LD. SD flowering required many more cycles of high light (12 cycles at 270 μmol m^{-2} s^{-1}) and with a far greater light integral (Fig. 1, versus Fig. 7), as also reported

previously for two other LD plants, *Sinapis alba* L. (Bodson *et al*., 1977) and *Fuchsia hybrida* (King and Ben-Tal, 2001). A simple photosynthetic regulation is unlikely, especially because when the *co* mutant was used to block *FT* increase and flowering (Supplementary Fig. S4 at *JXB* online), shoot apex sucrose still increased (Table 1). Perhaps, if maintained for 12 d this same sucrose increase would be sufficient for flowering, but there does not appear to be any evidence to support or deny such a speculation.

FT regulation in natural conditions

There have been no studies of flowering of *Arabidopsis* which combine the effects of seasonal light intensity and daylength. However, in the LD species *L. temulentum* there is strong additivity between photosynthetic input and the LD response (King and Evans, 1991). Thus, in late spring and summer, 'photosynthetic' amplification could become important for LD, *FT*-regulated flowering but photosynthesis may not be as dominant as the direct *FT* response to daylength, stop. This claim is consistent with the quantitative relationship to expression of an *FT* homologue associated with latitudinal adaptation for autumn bud set in *Populus tricocarpa* (Böhlenius *et al*., 2006).

In SD species, seasonal photosynthetic differences should be less relevant as *FT* expression and flowering increase in response to exposure to a prolonged daily dark period, as in rice (Izawa *et al*., 2002) and *Pharbitis nil* Chois (Hayama *et al*., 2007). Interestingly, an SD dark period leads to loss of spectrophotometrically detectable PHY Pfr in *P. nil* after 1–2 h (King *et al*., 1978). Thus, a reduction in the level of Pfr, either in darkness in SD species or with an FR exposure in LD species, leads to *FT* up-regulation. There is complexity yet to be explained for *P. nil* where *FT* expression is not related simply to that of *CO* (Hayama *et al*., 2007). It is also unclear why an SD plant does not up-regulate FT in LD, and vice versa for an LD response type.

Supplementary material

Supplementary Figures S1–S6 may be found at *JXB* online.

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