GIGANTEA Enables Drought Escape Response via Abscisic Acid-Dependent Activation of the Florigens and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1*[1[C][W]]

Matteo Riboni, Massimo Galbiati, Chiara Tonelli, and Lucio Conti*

Department of Life Sciences, University of Milan, 20133 Milan, Italy (M.R., M.G., C.T., L.C.); and Fondazione Filarete, 20139 Milan, Italy (M.G., C.T., L.C.)

ORCID ID: 0000-0002-7837-4227 (L.C.).

Downloaded from https://academic.oup.com/plphys/article/162/3/1706/6110857 by U.S. Department of Justice user on 16 August 2022

Modulation of the transition to flowering plays an important role in the adaptation to drought. The drought-escape (DE) response allows plants to adaptively shorten their life cycle to make seeds before severe stress leads to death. However, the molecular basis of the DE response is unknown. A screen of different Arabidopsis (*Arabidopsis thaliana*) flowering time mutants under DE-triggering conditions revealed the central role of the flower-promoting gene *GIGANTEA* (*GI*) and the florigen genes *FLOWERING LOCUS T* (*FT*) and *TWIN SISTER OF FT* (*TSF*) in the DE response. Further screens showed that the phytohormone abscisic acid is required for the DE response, positively regulating flowering under long-day conditions. Drought stress promotes the transcriptional up-regulation of the florigens in an abscisic acid- and photoperiod-dependent manner, so that early flowering only occurs under long days. Along with the florigens, the floral integrator *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* is also up-regulated in a similar fashion and contributes to the activation of *TSF*. The DE response was recovered under short days in the absence of the floral repressor *SHORT VEGETATIVE PHASE* or in *GI*-overexpressing plants. Our data reveal a key role for GI in connecting photoperiodic cues and environmental stress independently from the central *FT*/*TSF* activator *CONSTANS*. This mechanism explains how environmental cues may act upon the florigen genes in a photoperiodically controlled manner, thus enabling plastic flowering responses.

The timing of the floral transition has significant consequences for the reproductive success of plants and consequently their adaptability to various environmental conditions. Plasticity in flowering time in response to changes in water availability has been documented in several plant species (Xu et al., 2005; Lafitte et al., 2006; Sherrard and Maherali, 2006; Franks et al., 2007; Franks, 2011; Ivey and Carr, 2012). As water scarcity results in a reduction of growing seasons, the drought-escape (DE) response defines the ability of plants to complete their life cycle before stress conditions lead to lethality (McKay et al., 2003; Verslues and Juenger, 2011). Thus, in natural environments, the onset of the DE response represents a key adaptive trait in triggering an acceleration of the floral transition and reproductive success (Franks, 2011). Despite its ecological significance, a DE response has not yet been ascribed to a mechanism of flowering gene regulation. Therefore, a key question is, what mechanism transduces a drought-derived signal into affecting the floral transition?

The floral transition is controlled by internal and external factors and occurs when the shoot apical meristem (SAM) receives appropriate signals and switches from producing vegetative leaves to producing flowers, fruits, and seeds (Bernier et al., 1993). The study of the model plant Arabidopsis (*Arabidopsis thaliana*) resulted in the definition of four major pathways involved in flowering time control: the photoperiodic, the vernalization, the autonomous, and the GA pathways (Amasino, 2010; Andrés and Coupland, 2012).

Flowering in annual Arabidopsis ecotypes is strongly promoted by long-day (LD) photoperiod conditions, typical of spring/early summertime. The photoperiodic pathway is characterized by three key components, whose regulation and activity is required for correct daylength measurement: *GIGANTEA* (*GI*), *CONSTANS* (*CO*), and *FLOWERING LOCUS T* (*FT*; Putterill et al., 1995; Fowler et al., 1999; Kardailsky et al., 1999; Kobayashi et al., 1999; Park et al., 1999). Mutations in any of these genes delay flowering under long days (LDs), with little effect under short-day (SD) conditions. Daylength duration is perceived in the leaves, where a systemic signal (known as the florigen) originates (Evans, 1971). During LDs, light promotes the interaction between GI and a family of light-sensing F-box ubiquitin ligases, which results in the degradation

[1] This work was supported by the BIOGESTECA 15083/RCC project, funded by Regione Lombardia, by the AGRISOST project, funded by Fondazione Umberto Veronesi per il Progresso delle Scienze, Milan, Italy, and by the University of Milan (Ph.D. studentship to M.R. and research fellowship to L.C.).

* Corresponding author; e-mail lucio.conti@unimi.it.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Lucio Conti (lucio.conti@unimi.it).

[C] Some figures in this article are displayed in color online but in black and white in the print edition.

[W] The online version of this article contains Web-only data.

www.plantphysiol.org/cgi/doi/10.1104/pp.113.217729

 © 2013 American Society of Plant Biologists. All Rights Reserved.

of a set of transcriptional repressors at the *CO* promoter (Imaizumi et al., 2005; Sawa et al., 2007; Fornara et al., 2009). LDs also promote the stabilization of the CO protein and the consequent activation of the florigen genes *FT* and *TWIN SISTER OF FT* (*TSF*) in the phloem companion cells (An et al., 2004; Valverde et al., 2004; Yamaguchi et al., 2005; Jang et al., 2009). However, the FT protein moves to the SAM, where it interacts with the bZIP transcription factor FLOWERING LOCUS D (FD) to orchestrate the floral transition (Abe et al., 2005; Wigge et al., 2005; Corbesier et al., 2007; Jaeger and Wigge, 2007; Mathieu et al., 2007). *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*) encodes a MADS box transcription factor and represents an early target of the FT/FD complex in the SAM (Lee et al., 2000; Lee and Lee, 2010).

Mutations in the autonomous pathway cause a delay in flowering irrespective of the photoperiod. The autonomous pathway promotes flowering by down-regulating the floral repressor *FLOWERING LOCUS C* (*FLC*; Michaels and Amasino, 1999, 2001). The late-flowering phenotype of autonomous pathway mutants can be reverted by vernalization, which targets *FLC* chromatin by imposing a silenced epigenetic state (Kim et al., 2009). GAs play a key role in flowering, particularly under short days (SDs), since GA-deficient mutants do not flower under those conditions (Wilson et al., 1992).

In nature, plants are exposed to a variety of external cues with remarkable, yet contrasting, effects on flowering. For instance, warm temperatures (28°C) substantially accelerate flowering compared with cool temperatures (16°C) in Arabidopsis (Blázquez et al., 2003; Balasubramanian et al., 2006). Abiotic stresses such as UV-C exposure accelerate flowering (Martínez et al., 2004). Conversely, intermittent cold treatment and salt stress inhibit flowering (Achard et al., 2006; Seo et al., 2009). Recent data show the importance of nutrient availability and the opposing role of nitrate and phosphate on flowering (Kant et al., 2011). Thus, plants are able to discriminate the type of external "stress" and to integrate this information into the flowering network. A key goal in flowering studies, therefore, is to define the mechanistic basis underlying such integration and its physiological significance.

FT is a central node of floral integration, since its expression depends on multiple inputs (Pin and Nilsson, 2012). *FT* is mainly controlled in a photoperiodic manner. However, other external stimuli have been shown to directly converge at the *FT* promoter, including blue light and warm temperature (Liu et al., 2008b; Kumar et al., 2012). Besides being positively controlled, *FT* expression is further fine-tuned via modulation of the activity of several repressor complexes, including FLC/SHORT VEGETATIVE PHASE (SVP), TEMPRANILLO1, and SCHLAFMÜTZE/APETALA2-like (Castillejo and Pelaz, 2008; Li et al., 2008; Mathieu et al., 2009).

Warm temperature is arguably the best-characterized paradigm for stress-dependent *FT* up-regulation. However, warm ambient temperature triggers *FT* up-regulation both under SD and LD conditions (Kumar et al., 2012). Here, we propose a model for the interaction between photoperiod and drought stress, whereby photoperiod-activated GI enables the abscisic acid (ABA) and drought-mediated activation of *FT*/*TSF* and *SOC1*. Consequently, plants can maximize their fitness by coordinating stress responses according to seasonal cues.

RESULTS

Early Drought Stress Triggers the DE Response in Arabidopsis

To assess the presence of a DE response strategy in Arabidopsis and to define the genetic basis underlying this adaptive trait, we set up conditions to impose a persistent drought stress starting from early stages of development. Three-day-old seedlings were either watered daily to maintain a relative water soil content at 80% to 90% or not watered to allow soil moisture to decrease to 30% (Supplemental Fig. S1A). A bona-fide water stress condition was reached within 6 d after sowing, as confirmed by the increase in the ABA-dependent markers *ABSCISIC ACID INSENSITIVE2* (*ABI2*) and *RESPONSIVE TO ABA18* (*RAB18*; Lång and Palva, 1992; Nemhauser et al., 2006; Supplemental Fig. S1, B and C). These water deficiency conditions, maintained throughout the duration of the experiment, were nevertheless compatible with plant growth and survival and resulted in a robust early-flowering response. Compared with normal watering, drought-treated Columbia (Col-0) and Landsberg *erecta* (L*er*) wild-type plants produced fewer vegetative leaves as well as an early bolting time, indicative of the DE response (Fig. 1, A–E). The early-flowering phenotype was reflected in the early up-regulation of the floral markers *LEAFY* and *APETALA1* (Blázquez et al., 1997; Hempel et al., 1997) in plants undergoing drought stress compared with normal watering controls (Supplemental Fig. S1, D and E).

The DE Response Requires GI, FT/TSF, and SOC1

To determine whether the DE response observed in wild-type accessions was mediated by any of the known flowering time genes, we imposed DE-triggering conditions under LDs upon different late flowering time mutants that are representatives of all known floral pathways (Fig. 1, A–D).

Mutants in the autonomous pathway (*lumnidependens* [*ld*], *fve*, *fy*, and *fca*, which flower late irrespective of the photoperiod) and the GA pathway (*ga1*, impaired in GA production) produced a DE response relatively similar to the wild type, as they were consistently early flowering under DE-triggering conditions (Fig. 1, A–D). A complete absence of the DE response was observed in *gi* mutants both in the Col-0 (*gi-100*) and L*er* (*gi-4*) backgrounds (Fig. 1, A–D and F). We confirmed the requirement for *GI* in triggering the DE response by analyzing independent alleles of *gi* (*gi-1*, *gi-2*, *gi-5*, and *gi-6*), ruling out an allele- or

Downloaded from https://academic.oup.com/plphys/article/162/3/1706/6110857 by U.S. Department of Justice user on 16 August 2022

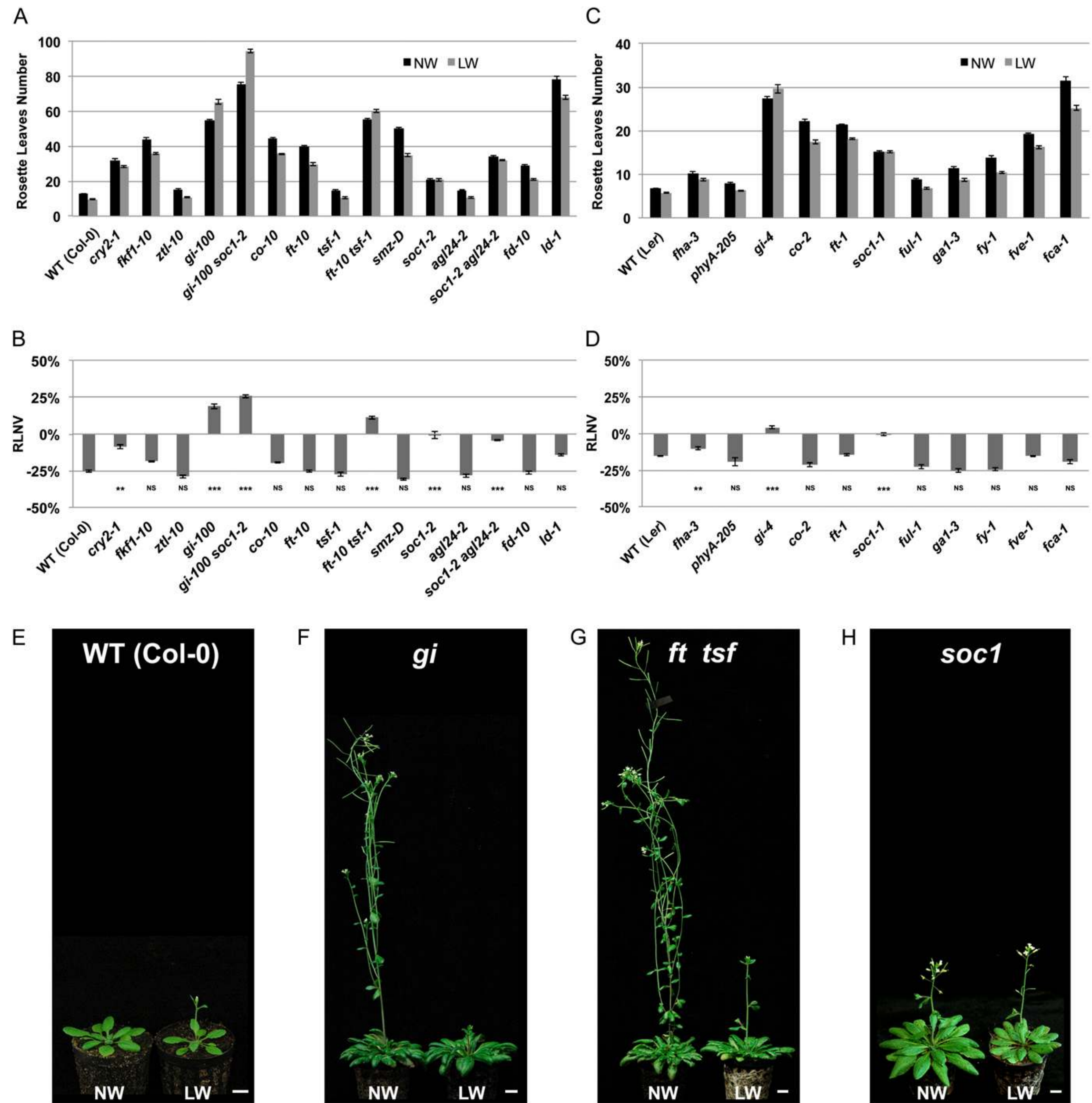

Figure 1. The DE response requires components of the photoperiodic pathway. A and C, Rosette leaf mean numbers in wild-type (WT) Col-0 (A) and L*er* (C) and flowering time mutants grown under LDs. Plants were subjected to normal-watering (NW; black bars) or low-watering (LW; gray bars) regimes. Error bars represent SE ($n = 15$). B and D, Quantification of the DE response for each genotype detailed in A and C, respectively, expressed as relative leaf number variation (RLNV). Numbers indicate percentage variations in number of leaves in plants grown under the low-watering relative to the normal-watering condition. Error bars represent SE. Student's *t* test values are as follows: $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, $^{NS}P > 0.05$, not significant. E to H, Images of representative plants of the indicated genotypes grown under LDs and subjected to normal-watering or low-watering regimes. Wild-type Col-0 plants are 3 weeks old (E), *gi-2* plants are 12 weeks old (F), *ft-10 tsf-1* plants are 16 weeks old (G), and *soc1-2* plants are 8 weeks old (H). Bars = 1 cm. [See online article for color version of this figure.]

ecotype-specific effect (Supplemental Fig. S2, A and B). Furthermore, *gi* plants also displayed a significant delay in flowering time under a restricted watering regime, but this was more pronounced in the Col-0 background compared with L*er* (Fig. 1, A–D and F; Supplemental Fig. S2, A and B).

Downloaded from https://academic.oup.com/plphys/article/162/3/1706/6110857 by U.S. Department of Justice user on 16 August 2022

Despite the known functional dependence of GI on light-sensing protein interactors such as FLAVIN-BINDING, KELCH REPEAT, F-BOX1 (FKF1) and ZEITLUPE (ZTL), responsible for GI-mediated *CO* activation and clock function, respectively (Imaizumi et al., 2005; Kim et al., 2007), no evident defects in the DE response were found in single *fkf1* and *ztl* mutants (Fig. 1, A and B).

Interestingly, we found that mutants in the blue light photoreceptor *CRYPTOCHROME2* (*cry2-1* in Col-0 and *fha-3* in L*er*) were significantly impaired in their DE responses (Fig. 1, A–D). As CRY2 affects the photoperiodic pathway at different levels, including the promotion of GI protein stability (Yu et al., 2008; Zuo et al., 2011), this finding may support the central role of GI in mediating the DE response.

In accordance with GI being ultimately responsible for the photoperiodic activation of the florigen genes *FT* and *TSF*, *ft tsf* double mutants (but not their respective single mutants) lacked the DE response, largely mimicking the *gi* mutants (Fig. 1, A–D and G). Although these data point to a florigen-dependent mechanism for DE activation, this response does not appear to require the activity of *CO*, a transcriptional regulator of *FT* and *TSF* that acts downstream of *GI* in mediating the photoperiodic response. Also, no DE response defects were observed in *phytochrome A* mutants, which affect CO protein levels (Valverde et al., 2004) and thus are largely downstream of *GI* (Fig. 1, C and D).

GI-dependent but *CO*-independent pathways of *FT* activation have been described (Jung et al., 2007; Sawa and Kay, 2011). One such pathway involves the GI-dependent activation of *microRNA172*, resulting in the posttranscriptional gene silencing of the *AP2-like* genes (a class of *FT* transcriptional repressors; Yant et al., 2010). If this was the case, we would expect a reduction in the DE response in plants carrying an activation-tagged allele of the *AP2-like* gene *SCHLAFMÜTZE* (*smz-D*; Mathieu et al., 2009). However, *smz-D* plants exhibited an unaltered DE response, suggesting another mode of GI action (Fig. 1, A and B).

Despite the central role of the florigen proteins in mediating the DE response, no defects were found in *fd*, whose wild-type gene product represents a key FT interactor in the SAM (Fig. 1, A and B; Abe et al., 2005; Wigge et al., 2005). This could be due to *FLOWERING LOCUS D PARALOG*, mediating florigen signaling in the SAM redundantly with FD (Jaeger et al., 2013).

A strongly reduced DE response was present in *soc1* plants (*soc1-1* in L*er* and *soc1-2* in Col-0) but not in *fruitfull* (*ful*), both related MADS box-type transcription factors and downstream targets of FT in the SAM (Fig. 1, A–D and H; Gu et al., 1998; Samach et al., 2000). Previously, it was shown that mutations in *AGAMOUS-LIKE24* (*AGL24*), a SOC1 interactor and regulator, aggravated the *soc1* mutant flowering phenotype, suggesting partial redundancy between these two genes (Lee et al., 2008; Liu et al., 2008a). However, no DE response defects were apparent in *agl24* single mutants, and *soc1 agl24* were indistinguishable from *soc1* mutants with respect to their DE responses (Fig. 1, A and B). Also, while *gi soc1* double mutants were later flowering than *gi*, they were similar in their lack of DE, suggesting that *GI* and *SOC1* were largely operating in the same pathway in the context of the DE response (Fig. 1, A and B). Taken together, our data reveal a cooption of *GI*, but not *CO*, to activate DE response in a florigen- and *SOC1*-dependent manner.

Downloaded from https://academic.oup.com/plphys/article/162/3/1706/6110857 by U.S. Department of Justice user on 16 August 2022

The Onset of the DE Response Is Photoperiod Dependent

We analyzed the DE phenotype of plants grown under SDs to test its photoperiod dependency. In contrast to LDs, wild-type plants (L*er* or Col-0) did not generate the DE response under SDs (Fig. 2, , B, E, and G). Interestingly, SD-grown Col-0 wild-type plants (but not L*er*) produced a significant delay in the floral transition under drought conditions compared with normal watering, reminiscent of that previously observed in *gi* or *ft tsf* mutants under LDs. Thus, the DE response appears to be dependent upon GI mediating LD photoperiodic cues, a finding that prompted us to test whether artificial ectopic expression of *GI* would be sufficient in restoring the DE response under SDs. *35S:GI* (L*er*) and *35S:HEMAGGLUTININ-GI* (Col-0) recovered the DE response, supporting the photoperiod dependency model for DE activation (Fig. 2, A, B, and H).

Under LDs, *35S:GI* and *35S:SOC1* plants did not display a DE response. This could be due to their early floral transition, occurring before the perception of any significant drought stress stimulus (Fig. 2D). *35S:SOC1* plants did not recover the DE response under SDs, exhibiting early flowering irrespective of the irrigation conditions (Fig. 2, A–C). Double hemizygous *35S:GI/−* *35S:SOC1/−* plants under SDs were earlier than their respective parental lines (Fig. 2, A and B) but did not produce the DE response, further indicating that *SOC1* action is downstream of *GI* in the context of DE response activation. High levels of *SOC1* may thus saturate the floral induction process independently of LDs, resulting in a lack of DE response. On the other hand, the partial reactivation of the photoperiodic response resulting from *GI* overexpression is sufficient to reinstall the DE response, even in the absence of favorable photoperiodic cues.

DE Response Recovery under SDs in *svp* Mutants

Drought stress can only promote flowering under LDs via a florigen-dependent mechanism. Therefore, we hypothesized that by relieving the repressive state at the promoter of the florigen genes, we could restore the DE response under SDs.

Several *FT* repressors have been characterized, namely the gene products *FLC*, *FLOWERING LOCUS M* (*FLM*), *AP2-like* (e.g. *SMZ*, *SCHNARCHZAPFEN* [*SNZ*], *TARGET OF EAT1* [*TOE1*], and *TOE2*), and *SVP* (Yant et al., 2009). Under LDs, the effect of *flc* and *flm* mutations did not appear to alter the DE response (Fig. 2, C and D). In contrast,

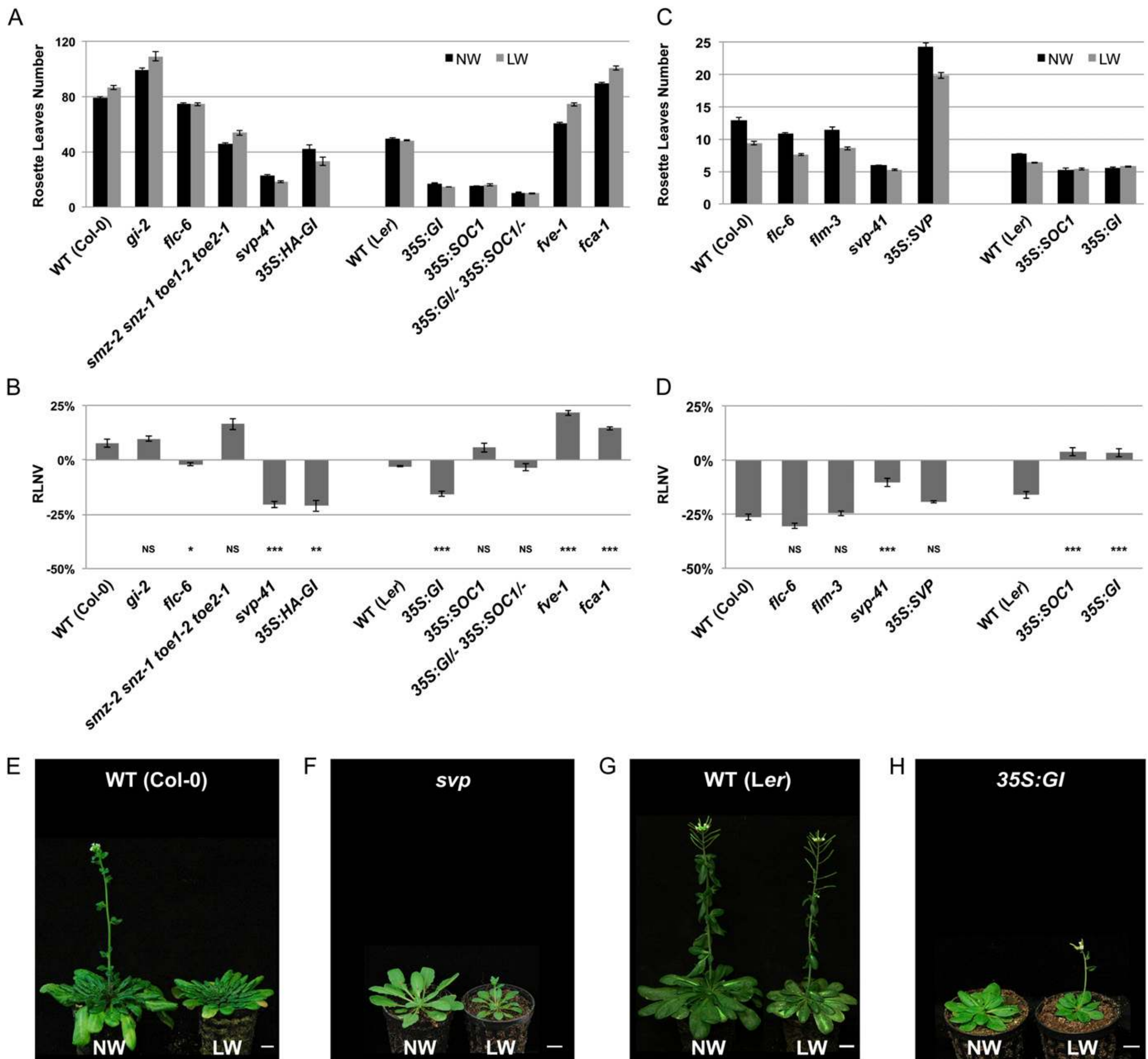

Figure 2. The onset of the DE response is photoperiod dependent. A, Rosette leaf mean number of wild-type (WT) plants and flowering time mutants grown under SDs. Plants were subjected to normal-watering (NW; black bars) or low-watering (gray bars) regimes. Error bars represent SE ($n = 15$). B, Quantification of the DE response for each genotype detailed in A expressed as relative leaf number variation (RLNV). Error bars represent SE. Student's *t* test values are as follows: $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$, $^{NS}P > 0.05$, not significant. C, Rosette leaf mean number of wild-type plants and flowering time mutants grown under LDs. Plants were subjected to normal-watering (black bars) or low-watering (gray bars) regimes. Error bars represent SE ($n = 15$). D, Quantification of the DE response for each genotype detailed in C expressed as relative leaf number variation. Error bars represent SE. Student's *t* test values are as follows: $***P \leq 0.001$, $^{NS}P > 0.05$, not significant. E to H, Images of representative plants of the indicated genotypes grown under SDs and subjected to normal-watering or low-watering regimes. Wild-type Col-0 plants are 16 weeks old (E), *svp-41* plants are 8 weeks old (F), wild-type L*er* plants are 12 weeks old (G), and *35S:GI* plants are 7 weeks old (H). Bars = 1 cm. [See online article for color version of this figure.]

no significant DE response occurred in *svp* mutants, which exhibited an extremely early-flowering phenotype, independent of the irrigation regime (Fig. 2, C and D).

Under SDs, no DE was observed in *flm* or *smz snz toe1 toe2* mutants (Fig. 2, A and B; Supplemental Fig. S3, A and B). As *SMZ* requires *FLM* to exert its repressive function on *FT* (Mathieu et al., 2009), these data indicate that the *SMZ*/*FLM* transcriptional repressor complex is not responsible for the lack of DE response under SDs. Rather, our results indicate an

Downloaded from https://academic.oup.com/plphys/article/162/3/1706/6110857 by U.S. Department of Justice user on 16 August 2022

important role for the FLC/SVP complex in preventing the DE response under SDs. As expected, *flc* mutants were slightly earlier flowering under SDs compared with the wild type (Fig. 2, A and B; Supplemental Fig. S3, A and B). However, unlike the wild type, *flc* plants did not exhibit a floral delay when grown under drought conditions. Interestingly, *svp* plants were able to recover a strong DE response under SDs (Fig. 2, A, B, and F).

Although lacking the DE response, L*er* wild-type plants did not exhibit a flowering delay under drought conditions when grown under SDs (Fig. 2, A, B, and G). The fact that the L*er* ecotype carries a weaker allele of *FLC* compared with Col-0 (Lee et al., 1994), coupled with the lack of a floral delay in *flc* mutants (Col-0 background) under SDs, could account for this observation. In support of this hypothesis, *fca* and *fve* mutants (L*er*; characterized by increased levels of *FLC*; Sheldon et al., 2000), produced a significant floral delay under drought conditions compared with normal watering (Fig. 2, A and B). Noticeably, compared with *fca*, *fve* plants exhibited a more pronounced floral delay, which correlates with the high levels of *SVP* being present in this particular genotype (Li et al., 2008).

Drought-induced changes in *FLC*/*SVP* transcript levels could account for such a floral delay. *FLC* transcript levels (but not *SVP*) were slightly but reproducibly increased under drought conditions in both LDs and SDs (Supplemental Fig. S4, A and B). However, such an increment in *FLC* transcript levels is unlikely to play a significant role under LDs, as *fve*, *fy*, *ld*, and *fca* plants did not exhibit obvious DE defects (Fig. 1, A–D). Also, plants ectopically expressing *SVP* (*35S:SVP*) under LDs did not exhibit DE defects (Fig. 2, C and D).

Taken together, these data indicate that SVP, likely in association with its interactor FLC, contributes to preventing the DE response upon drought conditions under SDs. Conversely, LD conditions overcome the FLC/SVP repression largely posttranscriptionally to enable the DE response.

The Phytohormone ABA Promotes the DE Response under LDs and Affects Flowering in a Photoperiod-Dependent Manner

The phytohormone ABA plays a pivotal role in orchestrating several drought responses, but its role in flowering time is poorly understood (Fujita et al., 2011). Mutants impaired in ABA biosynthesis, *aba deficient1* and *aba deficient2* (*aba1-6* and *aba2-4*), flowered later than the wild type even under normal watering conditions, indicating a positive role for ABA in controlling the floral transition (Fig. 3, A and C). Despite being significantly later flowering than the wild type, *aba2-4* plants were consistently earlier than *aba1-6* (Student's t test, $P = 0.02$), which could reflect the relative severity of this particular allele.

Under drought stress conditions, *aba1* mutant plants exhibited a reduced DE response compared with the wild type (Fig. 3, A and B). However, because of the residual DE response in *aba1* mutants, other non-ABA-dependent pathways are likely to contribute to the early-flowering phenotype caused by drought. Alternatively, residual ABA production in these mutants (ethyl methanesulfonate generated, nucleotide substitution alleles, and unlikely to be null) was sufficient to generate the DE response. To distinguish between these possibilities, we analyzed an *ABA1* transfer DNA (T-DNA) insertion line (Morris et al., 2006), which could represent a more severe allele. These *aba1* mutants showed a late-flowering phenotype, similar to the *aba1-6* allele, under normal watering conditions (Supplemental Fig. S5). However, unlike *aba1-6* plants, they could not survive under drought stress conditions, thus precluding an evaluation of their DE response.

To further confirm such a positive role of ABA in flowering, we analyzed the phenotype of higher order mutants in the ABA negative regulator PROTEIN PHOSPHATASE2C (PP2C) gene family, known to result in hypersensitized ABA signaling (Rubio et al., 2009). Compared with the wild type, *hypersensitive to aba1* (*hab1-1*), *aba insensitive1* (*abi1-2*), *abi2-2*, and *hab1-1 abi1-2 pp2ca-1* mutants were significantly earlier flowering, even under normal watering conditions (Fig. 3A). Under drought stress conditions, their DE response was relatively similar to the wild type, likely as a result of the combined contribution of increased ABA accumulation and increased sensitivity (Fig. 3B). In agreement with the floral promotive role of ABA under LDs, the early flowering of *hab1-1 abi1-2 pp2ca-1* plants was accompanied by strongly increased *FT* (but not *TSF*) transcript accumulation (Fig. 3D).

We hypothesized that the constitutive activation of ABA signaling might overcome the lack of DE under SDs. However, *hab1-1 abi1-2 pp2ca-1* plants were significantly later flowering compared with the wild type (producing more than 20 vegetative leaves) under normal watering regimes (Fig. 3E). *FLC* levels (but not *SVP*) were elevated in SD-grown *hab1-1 abi1-2 pp2ca-1* compared with the wild type, which could contribute to the phenotype observed (Fig. 3G). In contrast, ABA biosynthesis-defective mutants (*aba1-6*) did not exhibit altered flowering time compared with the wild type (Fig. 3E). Under drought conditions, both ABA constitutive signaling and biosynthesis mutants generated a flowering delay, similar to the wild type (Fig. 3F).

Our results indicate that ABA acts as a positive regulator of flowering under LD conditions but suppresses flowering under noninductive SDs.

ABA Up-Regulates *FT*/*TSF* and *SOC1* Expression in a Photoperiod-Dependent Manner

We sought to precisely monitor the expression of flowering genes in DE-defective genotypes. Normally irrigated or drought-stressed plants were grown under SDs and then shifted to LDs to allow the DE response. Upon LD shift in wild-type plants, *FT* and *TSF* transcripts

Downloaded from https://academic.oup.com/plphys/article/162/3/1706/6110857 by U.S. Department of Justice user on 16 August 2022

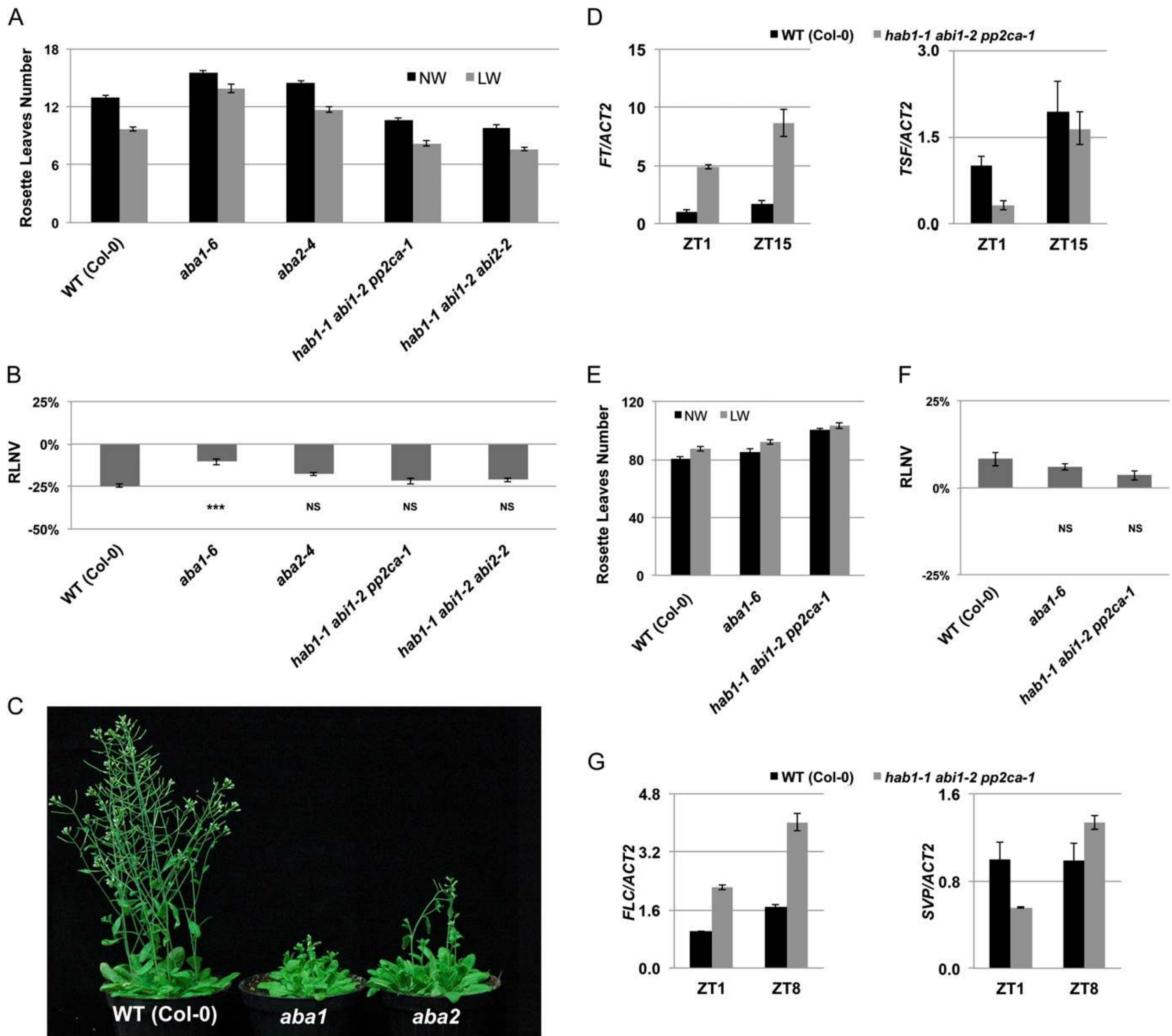

Figure 3. ABA is required for the DE response by positively regulating flowering. A, Rosette leaf mean number of the wild type (WT) and ABA biosynthesis or signaling mutants grown under LDs. Plants were subjected to normal-watering (NW; black bars) or low-watering (LW; gray bars) regimes. Error bars represent SE ($n = 15$). B, Quantification of the DE response for each genotype detailed in A expressed as relative leaf number variation (RLNV). Error bars represent SE. Student's *t* test values are as follows: $^{***}P \leq 0.001$, $^{NS}P > 0.05$, not significant. C, Images of ABA biosynthesis-deficient plants (*aba1-6* and *aba2-4*) compared with the Col-0 wild type. Four-week-old plants grown under LDs are shown. D, Real-time quantitative PCR of *FT* and *TSF* transcripts in 11-d-old *hab1-1 abi1-2 pp2ca-1* or wild-type (Col-0) seedlings. Plants were harvested at Zeitgeber times 1 and 15 (ZT1 and ZT15) in a 16-h-light/8-h-dark photoperiodic regime. Values represent fold change variations of *FT* and *TSF* transcript levels relative to Zeitgeber time 1 (arbitrarily set at 1 in Col-0). *ACTIN2* expression (*ACT2*) was used for normalization; error bars represent SD of two technical replicates. A representative experiment of two biological replicates is shown. E, Rosette leaf mean number of the wild type and ABA biosynthesis or signaling mutants grown under SDs. Plants were subjected to normal-watering (black bars) or low-watering (gray bars) regimes. Error bars represent SE ($n = 15$). F, Quantification of the DE response for each genotype detailed in E expressed as relative leaf number variation. Error bars represent SE; Student's *t* test values are as follows: $^{NS}P > 0.05$, not significant. G, Real-time quantitative PCR of *FLC* and *SVP* transcripts in 3-week-old *hab1-1 abi1-2 pp2ca-1* or wild-type (Col-0) seedlings. Plants were harvested at Zeitgeber times 1 and 8 (ZT1 and ZT8) in an 8-h-light/16-h-dark photoperiodic regime. Values represent fold change variations of *FLC* and *SVP* transcript levels relative to Zeitgeber time 1 (arbitrarily set at 1 in Col-0). *ACT2* expression was used for normalization; error bars represent SD of two technical replicates. A representative experiment of two biological replicates is shown. [See online article for color version of this figure.]

Downloaded from https://academic.oup.com/plphys/article/162/3/1706/6110857 by U.S. Department of Justice user on 16 August 2022

levels strongly increased at dusk, coinciding with the first and second photoextension periods (Fig. 4A). Under drought conditions, *FT* and *TSF* up-regulation was dramatically increased compared with normally watered controls, especially during the second LD (Fig. 4A). Consistent with the DE response occurring in coincidence with LDs, no obvious *FT* or *TSF* transcript increases were detectable under SDs, irrespective of watering regime (Fig. 4, compare A with F). This was further confirmed by the lack of *FT*/*TSF* up-regulation in *gi* mutants despite the transfer to LDs (Fig. 4B). It is unlikely that the higher florigen transcript accumulation under drought stress derived from increased GI levels, as little variation in *GI* gene expression was apparent at any time point during the experiment, independent of the irrigation regimes (Supplemental Fig. S4C). Rather, the boost in *FT* and *TSF* expression was strongly ABA dependent, as it was nearly abolished in *aba1-6* plants (Fig. 4C). Moreover, we found that *aba1-6* had generally reduced photoperiod-dependent up-regulation of *FT* and *TSF* transcript levels compared with the wild type under normal watering conditions, especially upon the first photoextension period. Thus, ABA promotes flowering by contributing to florigen transcript accumulation and by potentiating florigen levels under drought conditions.

Upon a shift to LD conditions, *SOC1* transcripts were also up-regulated in a drought-dependent manner in

Downloaded from https://academic.oup.com/plphys/article/162/3/1706/6110857 by U.S. Department of Justice user on 16 August 2022

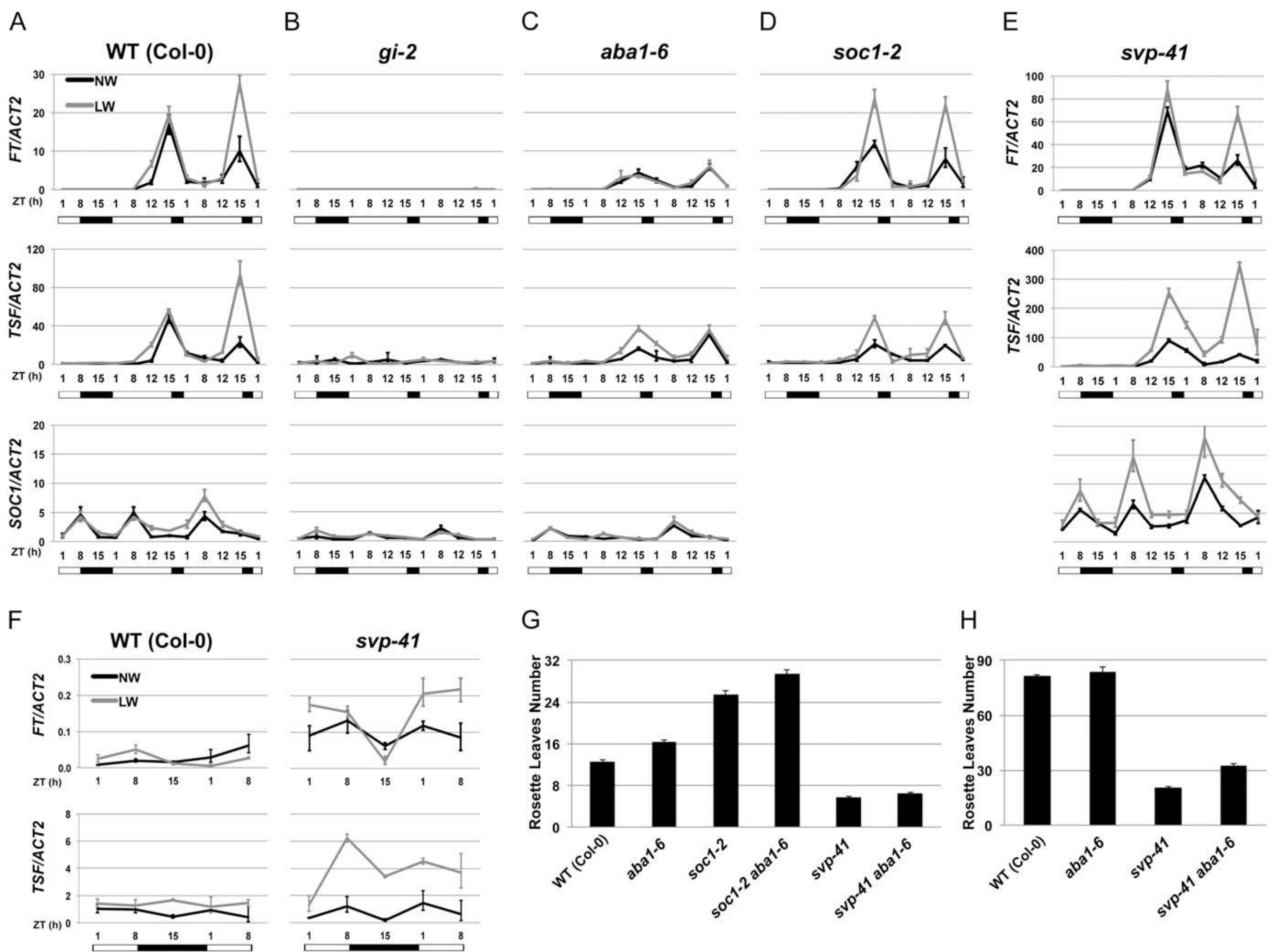

Figure 4. ABA- and photoperiod-dependent up-regulation of *FT*, *TSF*, and *SOC1* transcripts. A to E, Real-time quantitative PCR of *FT*, *TSF*, and *SOC1* transcripts in 3-week-old wild-type (WT) Col-0 (A), *gi-2* (B), *aba1-6* (C), *soc1-2* (D), and *svp-41* (E) seedlings. Plants were subjected to normal-watering (NW; black lines) or low-watering (LW; gray lines) regimes and harvested at the indicated time points coinciding with the light phase (white bar) or in the dark (black bar) during an SD-to-LD shift. At each time point, values represent fold change variations of *FT*, *TSF*, and *SOC1* transcript levels relative to Col-0 under normal watering. *ACT2* expression was used for normalization; error bars represent SD of two technical replicates. A representative experiment of two biological replicates is shown. F, Closeup of the *FT* and *TSF* pattern of expression during the SD part of the experiment illustrated in A and E. G, Rosette leaf mean number of wild-type Col-0 and the indicated single and double mutants grown under LDs. Error bars represent SE (n = 10–12). H, Same as G but grown under SDs. Error bars represent SE (n = 10–12).

wild-type plants (Fig. 4A). Such up-regulation was abolished in *gi* mutants, suggesting that it was mediated by the photoperiod (Fig. 4B). We then established that *SOC1* up-regulation under drought conditions required ABA and that ABA was also necessary for maintaining wild-type *SOC1* transcript levels even under normal watering conditions (Fig. 4C). Thus, similar to the florigen genes, *SOC1* is subjected to both ABA and photoperiod transcriptional control.

FT positively regulates *SOC1* expression (Michaels et al., 2005; Yoo et al., 2005) and is responsible for *SOC1* up-regulation in the SAM (Jang et al., 2009). Other floral integrators and *FT* targets are up-regulated in the SAM, namely *FUL* and *AGL24*, but these did not display a strong drought dependency in their expression (Supplemental Fig. S4, D and E). *SOC1* is also expressed in leaves before the floral transition and could play a role in *FT* activation (Lee et al., 2000; Samach et al., 2000; Searle et al., 2006). The observed drought-dependent *SOC1* up-regulation occurred very early after the LD shift; therefore, it is unlikely to reflect varying *SOC1* levels in the SAM (Fig. 4A). In *soc1* mutants grown under normal watering conditions, the expression levels of *TSF* (but not *FT*) were generally lower than in the wild type (Fig. 4D). Under drought conditions, *soc1* mutants exhibited strongly reduced *TSF* up-regulation but no obvious change in *FT* expression. Thus, besides acting downstream of the florigen in the SAM, *SOC1* also acts upstream of the *TSF* gene, possibly conveying an ABA-dependent signal. As observed previously, *FT* activation is independent of *SOC1* (Searle et al., 2006) but still strongly ABA dependent. In support of this model of ABA independently acting on *FT* and *SOC1*, *aba1 soc1* plants were later flowering than *soc1* single mutants, indicating that ABA deficiency can delay flowering through pathways other than *SOC1* (i.e. *FT*; Fig. 4G).

SVP has been shown to negatively regulate *FT* and *SOC1* expression (Li et al., 2008; Jang et al., 2009). Because *svp* mutants recovered the DE response under SDs, we anticipated a photoperiod-independent up-regulation of the florigens and/or *SOC1* upon drought conditions in the *svp* mutants. Compared with the wild type, the levels of *FT* were higher (up to 5-fold) in normally watered *svp* plants under the SD part of the experiment (Fig. 4, E and F). However, no strong *FT* up-regulation occurred at these time points upon drought conditions. Unlike *FT*, *TSF* levels did not greatly differ in *svp* mutants compared with the wild type under normal watering, but they were increased upon drought conditions (Fig. 4, E and F). However, this *TSF* up-regulation was relatively small if compared with the changes in *TSF* transcript levels occurring under LDs in wild-type plants (Fig. 4A). Under normal irrigation, *SOC1* transcript levels were strongly increased in *svp* plants under SDs, resembling those observed in the wild type under LDs (Fig. 4, A and E). Strikingly, under drought conditions, the levels of *SOC1* were further increased, implying that SVP normally prevents the drought-dependent activation of *SOC1* under SDs (Fig. 4E). As expected, upon the shift to LDs, *svp* plants exhibited a dramatic *SOC1* and florigen gene up-regulation compared with the wild type. Moreover such up-regulation was further boosted under drought conditions (Fig. 4E).

In summary, *svp* mutants recover the DE response under SDs, and this is reflected in *SOC1* drought-dependent up-regulation, but not *FT* and only marginally *TSF*. To substantiate the involvement of ABA in mediating this drought-dependent signal in *svp* plants, we generated *aba svp* double mutants. Under LDs, these plants were slightly but significantly later flowering than *svp* single mutants (Student's *t* test, $P = 0.02$; Fig. 4G). This could suggest that the contribution of ABA to flowering in the *svp* mutant background was additive and largely masked by the strong photoperiod-mediated activation of *FT*. However, under SDs, *aba svp* plants were much more late flowering than *svp* single mutants. This finding is consistent with the idea that, under SDs, the ABA-promotive role in flowering genes (e.g. *SOC1*) is normally impaired due to SVP repression (Fig. 4H).

DISCUSSION

Role of GI in the DE Response

In this work, we identified GI as a key component mediating the DE response in Arabidopsis. However, a key question emerges regarding what kind of signal GI transduces to activate the DE response. In the simplest scenario, GI mediates daylength, effectively enabling the superimposition of drought/ABA stimuli upon the *FT*/*TSF* promoters when daylength is favorable. The fact that DE is absent under SDs (phenocopying *gi* mutants under LDs) is in accord with this model. However, GI mediates different signaling pathways that could directly affect drought stress perception and/or responses, perhaps independently of its photoperiodic role. *gi* mutants were shown to be hypertolerant to oxidative stress, to be insensitive to salt-mediated floral delay, and to be primed for cold tolerance (Kurepa et al., 1998; Cao et al., 2005; Seo et al., 2009; Kim et al., 2013). In addition, *gi* mutants exhibit an enhanced starch accumulation, a relevant aspect to consider in the light of recent data highlighting the importance of starch metabolism and carbon signaling in flowering (Eimert et al., 1995; Wahl et al., 2013). However, the contribution of starch accumulation in ameliorating drought stress is currently poorly understood (Harb et al., 2010). Intriguingly, *FT* and EARLY FLOWERING3 (a target and an interactor of GI, respectively) have been recently involved in the control of guard cell activity (Kinoshita et al., 2011). Taken together, these observations may suggest a more complex model whereby GI mediates stress stimuli in concert and/or downstream of its photoperiodic role. Perhaps *gi* plants have a constitutive drought-tolerant phenotype (e.g. as a result of reduced *FT* expression in stomata), which alters their perception of drought stress. A future goal will be to investigate

Downloaded from https://academic.oup.com/plphys/article/162/3/1706/6110857 by U.S. Department of Justice user on 16 August 2022

these possible mechanisms of GI action and to establish their relationship (if any) with the photoperiod.

Although we could not identify the exact role of GI action within the DE response, our expression data indicate that photoperiod-stimulated GI activity is essential for the up-regulation of *FT*/*TSF* gene expression under drought stress (Figs. 4, A and B, and 5). Therefore, we anticipate that the underlying mechanism will be different from other modes of environmental up-regulation of *FT/TSF* (e.g. warm ambient temperature), which can occur independently of photoperiodic cues (Balasubramanian et al., 2006; Kumar et al., 2012). The precise biochemical function of GI protein is still largely unknown, as it was found in association with different protein complexes, thus arguing against a single mode of action. GI activates flowering mainly through the CO-FT module, although it can also promote flowering independently of these genes (Kim et al., 2005; Mizoguchi et al., 2005; Jung et al., 2007; Sawa and Kay, 2011). GI has been shown to physically interact with different floral repressors, including SVP, FLC, and TEM, and to directly bind to the *FT* promoter, providing a CO-independent mode of *FT* activation (Sawa and Kay, 2011). Thus, under LDs, GI may promote the DE response by regulating chromatin accessibility and/or interfere with repressor activity at the florigen promoters to allow their ABA-dependent up-regulation (Fig. 5). Whether this model can be also applied to *SOC1* activation is still unclear, as *SOC1* up-regulation under drought conditions may largely derive from increased florigen levels. The observation that *ft tsf* double mutants are unable to trigger a DE response argues in favor of a florigen-dependent mechanism of *SOC1* activation under drought conditions.

Our results highlight the importance of the SVP/FLC complex in preventing the DE response under SDs, but this was not reflected in the recovery of *FT* and *TSF* drought-dependent up-regulation (Fig. 2, A and B). This suggests the involvement of additional transcriptional repressors at the florigen promoter, hindering their ABA responsiveness (Fig. 5). Rather, the loss of SVP/FLC activity recovered the ABA-dependent *SOC1* up-regulation (Fig. 5). Accordingly, the early-flowering phenotype of *svp* mutants was strongly attenuated under SDs in the *svp aba1* double mutants, suggesting that SVP normally prevents ABA from positively activating *SOC1* (Fig. 4H). An increase in SVP/FLC complex activity (as in *fve* or *fca* mutants) strongly delayed flowering under SDs and drought conditions without affecting the DE response under LDs (Figs. 1, C and D, and 2, A and B). Similarly *35S:SVP* plants did not exhibit DE response defects under LDs. These observations indicate that under LDs, GI-enabled, ABA-dependent florigen gene up-regulation prevails over floral repression (Fig. 5).

SOC1 Potentiates the Drought-Dependent *TSF* Up-Regulation

soc1 plants displayed strongly attenuated drought-dependent *TSF* up-regulation (Fig. 4D). Thus, the DE nonresponsive phenotype of *soc1* might derive from the combined effects of impaired *TSF* up-regulation and defective signaling downstream of *FT*. Beyond flowering time control, *SOC1* is emerging as an important regulator of several developmental and stress responses. In conjunction with *FUL*, *SOC1* controls meristem determinacy and cambial activity (Melzer et al., 2008). Furthermore, *SOC1* orchestrates freezing tolerance responses by negatively regulating the *C-REPEAT/DRE-BINDING FACTOR* genes (Seo et al., 2009). A genome-wide survey of *SOC1* binding sites revealed a significant enrichment in genes involved in the abiotic stress response process (Tao et al., 2012). The reduced *TSF* levels in *soc1* mutants coupled with the fact that drought-mediated *SOC1* up-regulation was strictly ABA dependent suggests a role for *SOC1* in mediating part of the ABA-dependent transcriptional control over *TSF*. We speculate that *SOC1* may also play a general role in coordinating other ABA-dependent responses.

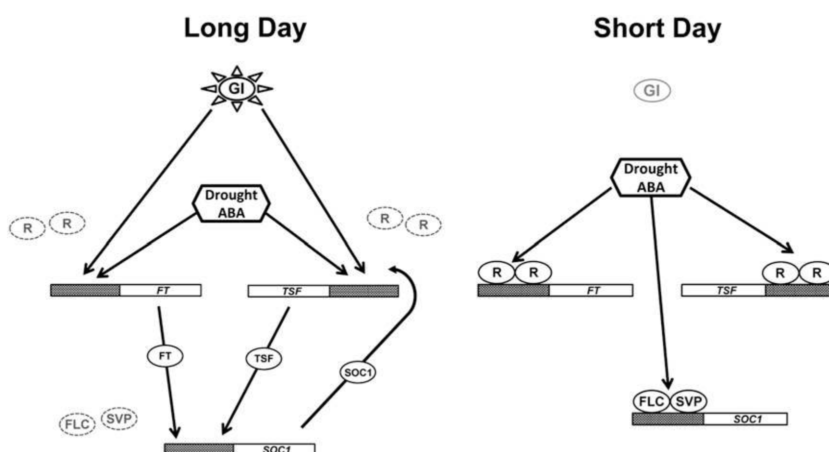

Figure 5. Photoperiod dependency of the DE response in Arabidopsis. Drought stress, largely via ABA signaling, promotes the DE response under LDs but not SDs. Photoperiod-activated GI may relieve the transcriptional repression at the *FT/TSF* promoters, thus facilitating their ABA-dependent up-regulation. Increased florigen levels trigger *SOC1* activation, which in turn contributes to *TSF* up-regulation. A floral delay occurs under SDs upon drought conditions. Drought and/or ABA may enhance the activity of different repressor complexes (e.g. SVP/FLC or other repressors [R]) through an unknown mechanism, thus interfering with the floral transition.

Downloaded from https://academic.oup.com/plphys/article/162/3/1706/6110857 by U.S. Department of Justice user on 16 August 2022

The Phytohormone ABA Participates in the Floral Transition, But Its Effect Is Photoperiod Dependent

ABA levels increase upon water scarcity to orchestrate different drought responses (Leung and Giraudat, 1998; Nambara and Marion-Poll, 2005). However, ABA is regarded as a general inhibitor of flowering, as exogenous ABA applications delay flowering (Blazquez et al., 1998; Domagalska et al., 2010). Also, *glucose insensitive1* (allelic to *aba2*) is early flowering compared with wild-type Wassilewskija (Cheng et al., 2002; Domagalska et al., 2010). However, plants overexpressing the ABA biosynthesis rate-limiting enzyme *NCED3* did not exhibit a significantly altered flowering phenotype (Domagalska et al., 2010). Recent findings suggest a positive role for ABA in stress-induced flowering by promoting the nuclear tethering of the OXIDATIVE STRESS2 (OXS2) zinc-finger transcription factor, an activator of *SOC1* (Blanvillain et al., 2011). The late-flowering phenotype we observed in independent ABA biosynthetic mutants (Col-0 background) coupled with their reduced DE response also indicates that endogenous ABA acts as a positive regulator of flowering under LDs. Supporting a positive role of ABA in flowering, constitutively activated ABA signaling mutants (e.g. *hab1-1 abi1-2 pp2ca-1*; Rubio et al., 2009) were early flowering under LDs. Also, the ectopic expression of the ABA-activated *Snrk2.6/OPEN STOMATA1* (a positive ABA signaling regulator) has been reported to produce an early-flowering phenotype (Zheng et al., 2010).

Alongside these positive ABA effects on flowering (which could be explained in terms of patterns of *SOC1* and florigen activation), our data reveal a negative role of drought and ABA under SDs. Compared with the wild type, *hab1-1 abi1-2 pp2ca-1* exhibited a late-flowering phenotype under this photoperiod condition (Fig. 3A). Also, in wild-type plants, drought caused a floral delay compared with the normal watering control, and this was strongly dependent upon FLC/SVP complex activity (Fig. 2, A and C). However, *FLC* (but not *SVP*) transcript levels were only slightly up-regulated in wild-type plants upon drought conditions and in *hab1-1 abi1-2 pp2ca-1* under SDs (Fig. 3G; Supplemental Fig. S4, A and B). These data point to a model where, in the absence of LDs, drought stress increases the repressor activity of the FLC/SVP complex largely at the posttranscriptional level (Fig. 5). It must be noted that drought-treated wild-type plants under SDs did not phenocopy *hab1-1 abi1-2 pp2ca-1* mutants undergoing normal watering in terms of the floral delay phenotype. These observations indicate that drought stress alone could not recapitulate the full effect of constitutive ABA signaling. Alternatively, the constitutive ABA activation of *hab1-1 abi1-2 pp2ca-1* mutants could result in additional effects that were independent of ABA.

Different hormonal signals participate to the floral transitions by affecting florigen levels. GAs accelerate flowering through the up-regulation of *FT* and *TSF* in the leaves (Galvão et al., 2012; Porri et al., 2012). Cytokinins specifically activate *TSF* transcription (D'Aloia et al., 2011). However, the mode of action of GAs and cytokinins with respect to *FT* and *TSF* up-regulation appears to be independent of the photoperiod conditions. Salicylic acid application also resulted in *FT* up-regulation and early flowering (Martínez et al., 2004). Interestingly, this early-flowering phenotype was dependent upon GI activity, but not CO, which is reminiscent of the DE response.

Expanding sets of gene expression data indicate a positive role for ABA and drought stress in the activation of florigen-like genes, including *TSF*, *BROTHER OF FT AND TFL1*, and *MOTHER OF FT AND TFL1* (Chung et al., 2010; Xi et al., 2010). In contrast, *ABI3* has been proposed to negatively regulate *TSF* (Suzuki et al., 2003). Our data indicate an important role for ABA in the transcriptional up-regulation of *FT* and *TSF*, but limited to the LD photoperiod (Fig. 4, A and C). Moreover, increased *FT* levels (but not *TSF*) were observed in the *hab1-1 abi1-2 pp2ca-1* ABA-hypersensitive mutants under LDs (Fig. 3D). Thus, *TSF* requires both drought- and ABA-specific components for its up-regulation. Indeed, besides the ABA-dependent activation of *TSF*, we found evidence for an ABA-independent mechanism of activation that could contribute to the residual DE response of *aba1* mutants (Figs. 3, A and B, and 4C). Conversely, the late-flowering phenotype of *hab1-1 abi1-2 pp2ca-1* mutants under SDs suggests also an inhibitory role for ABA in flowering. ABA is a mobile molecule, and its site of production and distribution are compatible with a role in the leaf vasculature (the site of florigen production) as well as the SAM (Endo et al., 2008; Seo and Koshiba, 2011). The opposing role of ABA in flowering may reflect a spatially distinct ABA signaling mechanism (the leaf and the SAM). Thus, a more precise understanding of the site of ABA action as well as the mechanism for the ABA-repressive role warrants further investigation.

CONCLUSION

Our data reveal an interaction between drought stress and photoperiod in the activation of the florigen genes, a process requiring photoperiod-activated GI protein and the phytohormone ABA. The ability to trigger a DE response allows plants to survive in ephemeral environments, characterized by sudden and unpredictable changes in water availability. As our data suggest the onset of the DE response to be tightly controlled by photoperiodic cues, drought episodes occurring in spring may be a cue for plants for yet harsher drought conditions to follow in the summertime, making a DE response advantageous. We propose that the broader significance for this photoperiod-drought stress interaction could be to allow water status signals to affect the floral transition, but limiting this to a particular temporal window (e.g. spring versus autumn).

Downloaded from https://academic.oup.com/plphys/article/162/3/1706/6110857 by U.S. Department of Justice user on 16 August 2022

MATERIALS AND METHODS

Plant Materials and Growing Conditions

In this study, we used wild-type Arabidopsis (*Arabidopsis thaliana*) plants in the Col-0 and L*er* backgrounds. Mutant or transgenic lines (obtained from the Nottingham Arabidopsis Stock Centre or other laboratories) are detailed in Supplemental Table S2. Seeds were stratified in the dark at 4°C for 2 d before sowing. Seeds were germinated and plants were grown in a controlled-environment cabinet at a temperature of 20°C to 23°C, 65% relative humidity, either under LD (16 h of light/8 h of dark) or SD (8 h of light/16 h of dark) photoperiods. Light was cool-white fluorescent tubes (Osram; Sylvania) at a fluency of 120 to 150 μmol m^{-2} s^{-1} (photosynthetically active radiation).

Plants were grown in Arabasket pots plus Araflat (BETATECH) filled with a blend (4:1, v/v) of loam sandy soil and peat (Vigorplant Italia). The soil water capacity was calculated as follows: Arabasket pots were filled with soil and air dried for 72 h in an oven at 45°C and then weighed (dry weight). Arabasket pots were subsequently soaked in water and weighed (wet weight). One hundred percent relative soil water content (RSWC) was calculated with the following formula: (wet weight − dry weight)/(wet weight − dry weight) × 100. The water evaporation rate in the growth chambers was then calculated by air drying the Arabasket pots and weighing them daily until the RSWC reached the target level of 30%. At least 15 plants were tested for each genotype in two parallel experiments: normal watering (80%–90% RSWC) and low watering (30% RSWC) conditions. The RSWC was kept constant by daily application of 4 mL of water to the normally watered plants and 2 mL every 2 d to the low-watering plants. Throughout all the experiments, random Arabasket pots were weighed to monitor the RSCW. In all experiments, plants received 2 mL of 1× solution of fertilizer every 3 weeks (nitrogen:phosphorus: potassium, 7.5:3:6 + iron; COMPO).

For the SD-to-LD shift experiments, stratified seeds (20–50) were sown in Arabasket pots, and plants were grown as described above. After 3 weeks, plants were harvested at the indicated time points of the subjective day and shifted to LDs. For each time point/treatment/genotype combination, plants were harvested in two biological replicates, each one consisting of approximately 50 seedlings pooled from three different Arabaskets. Two independent shift experiments were performed.

Isolation of Double Mutants and Genotyping

Mutant combinations were generated by crossing. The *agl24-2* and *svp-41* mutant alleles were genotyped as detailed previously (Michaels et al., 2003; Gregis et al., 2006). *gi-100* homozygous mutants were selected using the BASTA resistance carried by the T-DNA. The *aba1-6* mutants were selected by genomic PCR amplification with primers flanking the *aba1-6*-specific polymorphism followed by *Bsa*I restriction (Niyogi et al., 1998; Barrero et al., 2005). Genotyping primers for *soc1-2* and *aba1-6* and reverse transcription-PCR primers for *fd-10*, *fkf1-10*, and *ztl-10* are listed in Supplemental Table S3. *FD*, *FKF1*, and *ZTL* transcript abundance in the *fd-10*, *fkf1-10*, and *ztl-10* mutants was verified by reverse transcription-PCR (Supplemental Fig. S6).

Flowering Time Measurement and Quantification of the DE Response

Flowering time was measured by counting the number of vegetative leaves produced at bolting. Flowering time of mutant and transgenic plants used in this study is detailed in Supplemental Table S1. The DE response was calculated for each genotype as the percentage variation in the number of vegetative (rosette) leaves in plants grown under low-watering conditions (Leaves LW) relative to plants with a normal-watering regime (Leaves NW) by the following formula: (Leaves LW − Leaves NW)/Leaves NW (%). Each mutant genotype/treatment combination experiment described in this work was repeated two to four times.

RNA Extraction and Real-Time Quantitative PCR

Total RNA was extracted with TRIzol reagent (Invitrogen). A total of 1.5 μg of total RNA was used for complementary DNA synthesis with the SuperScript VILO cDNA Synthesis Kit (Invitrogen). Quantitative real-time PCR was performed with Fast SYBR Green Master Mix (Applied Biosystems), and amplification was real-time monitored on a 7900 HT Fast Real-Time PCR system (Applied Biosystems). Changes in gene expression were calculated relative to *ACT2* using the $\Delta\Delta$Ct method (Livak and Schmittgen, 2001). Quantitative real-time PCR primers are provided in Supplemental Table S3.

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers *GI* (AT1G22770), *TSF* (AT4G20370), *SOC1* (AT2G45660), *FT* (AT1G65480), *SVP* (AT2G22540), *CO* (AT5G15840), *FD* (AT4G35900), *FLC* (AT5G10140), *SMZ* (AT3G54990), *ABI2* (AT5G57050), *RAB18* (AT5G66400), *LFY* (AT5G61850), *AP1* (AT1G69120), *LD* (AT4G02560), *FVE* (AT2G19520), *FY* (AT5G13480), *FCA* (AT4G16280), *GA1* (AT4G02780), *FKF1* (AT1G68050), *ZTL* (AT5G57360), *CRY2* (AT1G04400), *PHYA* (AT1G09570), *FUL* (AT5G60910), *AGL24* (AT4G24540), *FLM* (AT1G77080), *SNZ* (AT2G39250), *TOE1* (AT2G28550), *TOE2* (AT5G60120), *ABA1* (AT5G67030), *ABA2* (AT1G52340), *ABI1* (AT4G26080), *ABI2* (AT5G57050), *PP2CA* (AT3G11410), and *ACT2* (AT3G18780).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. DE response induction in Arabidopsis.

Supplemental Figure S2. Absence of the DE response in independent *gi* alleles.

Supplemental Figure S3. DE response in floral repressor mutants under SDs.

Supplemental Figure S4. Floral gene regulation under drought stress upon SD-to-LD shifts.

Supplemental Figure S5. Mean rosette leaf numbers in *aba1* mutants.

Supplemental Figure S6. Characterization of T-DNA insertion alleles of *FD*, *FKF1*, and *ZTL*.

Supplemental Table S1. Flowering time of mutant and transgenic plants used in this study.

Supplemental Table S2. List of genotypes used in this study.

Supplemental Table S3. List of primers used in this study.

ACKNOWLEDGMENTS

We thank Drs. George Coupland, Peter Huijser (Max Planck Institute for Breeding Research), Pedro Rodriguez (Consejo Superior de Investigaciones Científicas), Markus Schmid (Max Planck Institute for Developmental Biology), Richard Amasino (University of Wisconsin), Martin Kater (University of Milan), and the Nottingham Arabidopsis Stock Centre for providing seed lines. We also thank Dr. Annabel Whibley (Centre National de la Recherche Scientifique) and Dr. Desmond Bradley (John Innes Centre) for insightful comments on the manuscript.

Received March 12, 2013; accepted May 27, 2013; published May 29, 2013.

LITERATURE CITED

Abe M, Kobayashi Y, Yamamoto S, Daimon Y, Yamaguchi A, Ikeda Y, Ichinoki H, Notaguchi M, Goto K, 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, Cheng H, De Grauwe L, Decat J, Schoutteten H, Moritz T, Van Der Straeten D, Peng J, Harberd NP (2006) Integration of plant responses to environmentally activated phytohormonal signals. Science **311:** 91–94

Amasino R (2010) Seasonal and developmental timing of flowering. Plant J **61:** 1001–1013

An H, Roussot C, Suárez-López P, Corbesier L, Vincent C, Piñeiro 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

Andrés F, Coupland G (2012) The genetic basis of flowering responses to seasonal cues. Nat Rev Genet **13:** 627–639

Balasubramanian S, Sureshkumar S, Lempe J, Weigel D (2006) Potent induction of Arabidopsis thaliana flowering by elevated growth temperature. PLoS Genet **2:** e106

Barrero JM, Piqueras P, González-Guzmán M, Serrano R, Rodríguez PL, Ponce MR, Micol JL (2005) A mutational analysis of the ABA1 gene of

Downloaded from https://academic.oup.com/plphys/article/162/3/1706/6110857 by U.S. Department of Justice user on 16 August 2022

Arabidopsis thaliana highlights the involvement of ABA in vegetative development. J Exp Bot **56:** 2071–2083

Bernier G, Havelange A, Houssa C, Petitjean A, Lejeune P (1993) Physiological signals that induce flowering. Plant Cell **5:** 1147–1155

Blanvillain R, Wei S, Wei P, Kim JH, Ow DW (2011) Stress tolerance to stress escape in plants: role of the OXS2 zinc-finger transcription factor family. EMBO J **30:** 3812–3822

Blázquez MA, Ahn JH, Weigel D (2003) A thermosensory pathway controlling flowering time in Arabidopsis thaliana. Nat Genet **33:** 168–171

Blazquez MA, Green R, Nilsson O, Sussman MR, Weigel D (1998) Gibberellins promote flowering of *Arabidopsis* by activating the *LEAFY* promoter. Plant Cell **10:** 791–800

Blázquez MA, Soowal LN, Lee I, Weigel D (1997) LEAFY expression and flower initiation in Arabidopsis. Development **124:** 3835–3844

Cao S, Ye M, Jiang S (2005) Involvement of GIGANTEA gene in the regulation of the cold stress response in Arabidopsis. Plant Cell Rep **24:** 683–690

Castillejo C, Pelaz S (2008) The balance between CONSTANS and TEMPRANILLO activities determines FT expression to trigger flowering. Curr Biol **18:** 1338–1343

Cheng W-H, Endo A, Zhou L, Penney J, Chen H-C, Arroyo A, Leon P, Nambara E, Asami T, Seo M, et al (2002) A unique short-chain dehydrogenase/reductase in *Arabidopsis* glucose signaling and abscisic acid biosynthesis and functions. Plant Cell **14:** 2723–2743

Chung KS, Yoo SY, Yoo SJ, Lee JS, Ahn JH (2010) BROTHER OF FT AND TFL1 (BFT), a member of the FT/TFL1 family, shows distinct pattern of expression during the vegetative growth of Arabidopsis. Plant Signal Behav **5:** 1102–1104

Corbesier L, Vincent C, Jang S, Fornara F, Fan Q, 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

D'Aloia M, Bonhomme D, Bouché F, Tamseddak K, Ormenese S, Torti S, Coupland G, Périlleux C (2011) Cytokinin promotes flowering of Arabidopsis via transcriptional activation of the FT paralogue TSF. Plant J **65:** 972–979

Domagalska MA, Sarnowska E, Nagy F, Davis SJ (2010) Genetic analyses of interactions among gibberellin, abscisic acid, and brassinosteroids in the control of flowering time in Arabidopsis thaliana. PLoS ONE **5:** e14012

Eimert K, Wang S-M, Lue WI, Chen J (1995) Monogenic recessive mutations causing both late floral initiation and excess starch accumulation in *Arabidopsis*. Plant Cell **7:** 1703–1712

Endo A, Sawada Y, Takahashi H, Okamoto M, Ikegami K, Koiwai H, Seo M, Toyomasu T, Mitsuhashi W, Shinozaki K, et al (2008) Drought induction of Arabidopsis 9-cis-epoxycarotenoid dioxygenase occurs in vascular parenchyma cells. Plant Physiol **147:** 1984–1993

Evans LT (1971) Flower induction and the florigen concept. Annu Rev Plant Physiol **22:** 365–394

Fornara F, Panigrahi KCS, Gissot L, Sauerbrunn N, Rühl M, Jarillo JA, Coupland G (2009) Arabidopsis DOF transcription factors act redundantly to reduce CONSTANS expression and are essential for a photoperiodic flowering response. Dev Cell **17:** 75–86

Fowler S, Lee K, Onouchi H, Samach A, Richardson K, Morris B, Coupland G, Putterill J (1999) GIGANTEA: a circadian clock-controlled gene that regulates photoperiodic flowering in Arabidopsis and encodes a protein with several possible membrane-spanning domains. EMBO J **18:** 4679–4688

Franks SJ (2011) Plasticity and evolution in drought avoidance and escape in the annual plant Brassica rapa. New Phytol **190:** 249–257

Franks SJ, Sim S, Weis AE (2007) Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. Proc Natl Acad Sci USA **104:** 1278–1282

Fujita Y, Fujita M, Shinozaki K, Yamaguchi-Shinozaki K (2011) ABA-mediated transcriptional regulation in response to osmotic stress in plants. J Plant Res **124:** 509–525

Galvão VC, Horrer D, Küttner F, Schmid M (2012) Spatial control of flowering by DELLA proteins in Arabidopsis thaliana. Development **139:** 4072–4082

Gregis V, Sessa A, Colombo L, Kater MM (2006) *AGL24, SHORT VEGETATIVE PHASE*, and *APETALA1* redundantly control *AGAMOUS* during early stages of flower development in *Arabidopsis*. Plant Cell **18:** 1373–1382

Gu Q, Ferrándiz C, Yanofsky MF, Martienssen R (1998) The FRUITFULL MADS-box gene mediates cell differentiation during Arabidopsis fruit development. Development **125:** 1509–1517

Harb A, Krishnan A, Ambavaram MMR, Pereira A (2010) Molecular and physiological analysis of drought stress in Arabidopsis reveals early responses leading to acclimation in plant growth. Plant Physiol **154:** 1254–1271

Hempel FD, Weigel D, Mandel MA, Ditta G, Zambryski PC, Feldman LJ, Yanofsky MF (1997) Floral determination and expression of floral regulatory genes in Arabidopsis. Development **124:** 3845–3853

Imaizumi T, Schultz TF, Harmon FG, Ho LA, Kay SA (2005) FKF1 F-box protein mediates cyclic degradation of a repressor of CONSTANS in Arabidopsis. Science **309:** 293–297

Ivey CT, Carr DE (2012) Tests for the joint evolution of mating system and drought escape in Mimulus. Ann Bot (Lond) **109:** 583–598

Jaeger KE, Pullen N, Lamzin S, Morris RJ, Wigge PA (2013) Interlocking feedback loops govern the dynamic behavior of the floral transition in *Arabidopsis*. Plant Cell **25:** 820–833 doi: 10.1105/tpc.113.109355

Jaeger KE, Wigge PA (2007) FT protein acts as a long-range signal in Arabidopsis. Curr Biol **17:** 1050–1054

Jang S, Torti S, 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

Jung J-H, Seo Y-H, Seo PJ, Reyes JL, Yun J, Chua N-H, Park C-M (2007) The *GIGANTEA*-regulated microRNA172 mediates photoperiodic flowering independent of *CONSTANS* in *Arabidopsis*. Plant Cell **19:** 2736–2748

Kant S, Peng M, Rothstein SJ (2011) Genetic regulation by NLA and microRNA827 for maintaining nitrate-dependent phosphate homeostasis in Arabidopsis. PLoS Genet **7:** e1002021

Kardailsky I, Shukla VK, Ahn JH, Dagenais N, Christensen SK, Nguyen JT, Chory J, Harrison MJ, Weigel D (1999) Activation tagging of the floral inducer FT. Science **286:** 1962–1965

Kim DH, Doyle MR, Sung S, Amasino RM (2009) Vernalization: winter and the timing of flowering in plants. Annu Rev Cell Dev Biol **25:** 277–299

Kim W-Y, Ali Z, Park H-J, Park SJ, Cha J-Y, Perez-Hormaeche J, Quintero FJ, Shin G, Kim MR, Qiang Z, et al (2013) Release of SOS2 kinase from sequestration with GIGANTEA determines salt tolerance in Arabidopsis. Nat Commun **4:** 1352–1364

Kim W-Y, Fujiwara S, Suh S-S, Kim J, Kim Y, Han L, David K, Putterill J, Nam HG, Somers DE (2007) ZEITLUPE is a circadian photoreceptor stabilized by GIGANTEA in blue light. Nature **449:** 356–360

Kim W-Y, Hicks KA, Somers DE (2005) Independent roles for *EARLY FLOWERING 3* and *ZEITLUPE* in the control of circadian timing, hypocotyl length, and flowering time. Plant Physiol **139:** 1557–1569

Kinoshita T, Ono N, Hayashi Y, Morimoto S, Nakamura S, Soda M, Kato Y, Ohnishi M, Nakano T, Inoue S-I, et al (2011) FLOWERING LOCUS T regulates stomatal opening. Curr Biol **21:** 1232–1238

Kobayashi Y, Kaya H, Goto K, Iwabuchi M, Araki T (1999) A pair of related genes with antagonistic roles in mediating flowering signals. Science **286:** 1960–1962

Kumar SV, Lucyshyn D, Jaeger KE, Alós E, Alvey E, Harberd NP, Wigge PA (2012) Transcription factor PIF4 controls the thermosensory activation of flowering. Nature **484:** 242–245

Kurepa J, Smalle J, Van Montagu M, Inzé D (1998) Oxidative stress tolerance and longevity in Arabidopsis: the late-flowering mutant gigantea is tolerant to paraquat. Plant J **14:** 759–764

Lafitte HR, Li ZK, Vijayakumar CHM, Gao YM, Shi Y, Xu JL, Fu BY, Yu SB, Ali AJ, Domingo J, et al (2006) Improvement of rice drought tolerance through backcross breeding: evaluation of donors and selection in drought nurseries. Field Crops Res **97:** 77–86

Lång V, Palva ET (1992) The expression of a rab-related gene, rab18, is induced by abscisic acid during the cold acclimation process of Arabidopsis thaliana (L.) Heynh. Plant Mol Biol **20:** 951–962

Lee H, Suh SS, Park E, Cho E, Ahn JH, Kim SG, Lee JS, Kwon YM, Lee I (2000) The AGAMOUS-LIKE 20 MADS domain protein integrates floral inductive pathways in Arabidopsis. Genes Dev **14:** 2366–2376

Lee I, Michaels SD, Masshardt AS, Amasino RM (1994) The late-flowering phenotype of FRIGIDA and mutations in LUMINIDEPENDENS is suppressed in the Landsberg erecta strain of Arabidopsis. Plant J **6:** 903–909

Lee J, Lee I (2010) Regulation and function of SOC1, a flowering pathway integrator. J Exp Bot **61:** 2247–2254

Lee J, Oh M, Park H, Lee I (2008) SOC1 translocated to the nucleus by interaction with AGL24 directly regulates leafy. Plant J **55:** 832–843

Downloaded from https://academic.oup.com/plphys/article/162/3/1706/6110857 by U.S. Department of Justice user on 16 August 2022

Leung J, Giraudat J (1998) Abscisic acid signal transduction. Annu Rev Plant Physiol Plant Mol Biol **49:** 199–222

Li D, Liu C, Shen L, Wu Y, Chen H, Robertson M, Helliwell CA, Ito T, Meyerowitz E, Yu H (2008) A repressor complex governs the integration of flowering signals in Arabidopsis. Dev Cell **15:** 110–120

Liu C, Chen H, Er HL, Soo HM, Kumar PP, Han J-H, Liou Y-C, Yu H (2008a) Direct interaction of AGL24 and SOC1 integrates flowering signals in Arabidopsis. Development **135:** 1481–1491

Liu H, Yu X, Li K, Klejnot J, Yang H, Lisiero D, Lin C (2008b) Photoexcited CRY2 interacts with CIB1 to regulate transcription and floral initiation in Arabidopsis. Science **322:** 1535–1539

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods **25:** 402–408

Martínez C, Pons E, Prats G, León J (2004) Salicylic acid regulates flowering time and links defence responses and reproductive development. Plant J **37:** 209–217

Mathieu J, Warthmann N, Küttner F, Schmid M (2007) Export of FT protein from phloem companion cells is sufficient for floral induction in Arabidopsis. Curr Biol **17:** 1055–1060

Mathieu J, Yant LJ, Mürdter F, Küttner F, Schmid M (2009) Repression of flowering by the miR172 target SMZ. PLoS Biol **7:** e1000148

McKay JK, Richards JH, Mitchell-Olds T (2003) Genetics of drought adaptation in Arabidopsis thaliana. I. Pleiotropy contributes to genetic correlations among ecological traits. Mol Ecol **12:** 1137–1151

Melzer S, Lens F, Gennen J, Vanneste S, Rohde A, Beeckman T (2008) Flowering-time genes modulate meristem determinacy and growth form in Arabidopsis thaliana. Nat Genet **40:** 1489–1492

Michaels SD, Amasino RM (1999) *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. Plant Cell **11:** 949–956

Michaels SD, Amasino RM (2001) Loss of *FLOWERING LOCUS C* activity eliminates the late-flowering phenotype of *FRIGIDA* and autonomous pathway mutations but not responsiveness to vernalization. Plant Cell **13:** 935–941

Michaels SD, Ditta G, Gustafson-Brown C, Pelaz S, Yanofsky M, Amasino RM (2003) AGL24 acts as a promoter of flowering in Arabidopsis and is positively regulated by vernalization. Plant J **33:** 867–874

Michaels SD, Himelblau E, Kim SY, Schomburg FM, Amasino RM (2005) Integration of flowering signals in winter-annual Arabidopsis. Plant Physiol **137:** 149–156

Mizoguchi T, Wright L, Fujiwara S, Cremer F, Lee K, Onouchi H, Mouradov A, Fowler S, Kamada H, Putterill J, et al (2005) Distinct roles of *GIGANTEA* in promoting flowering and regulating circadian rhythms in *Arabidopsis*. Plant Cell **17:** 2255–2270

Morris ER, Chevalier D, Walker JC (2006) *DAWDLE*, a forkhead-associated domain gene, regulates multiple aspects of plant development. Plant Physiol **141:** 932–941

Nambara E, Marion-Poll A (2005) Abscisic acid biosynthesis and catabolism. Annu Rev Plant Biol **56:** 165–185

Nemhauser JL, Hong F, Chory J (2006) Different plant hormones regulate similar processes through largely nonoverlapping transcriptional responses. Cell **126:** 467–475

Niyogi KK, Grossman AR, Björkman O (1998) *Arabidopsis* mutants define a central role for the xanthophyll cycle in the regulation of photosynthetic energy conversion. Plant Cell **10:** 1121–1134

Park DH, Somers DE, Kim YS, Choy YH, Lim HK, Soh M-S, Kim HJ, Kay SA, Nam HG (1999) Control of circadian rhythms and photoperiodic flowering by the Arabidopsis GIGANTEA gene. Science **285:** 1579–1582

Pin PA, Nilsson O (2012) The multifaceted roles of FLOWERING LOCUS T in plant development. Plant Cell Environ **35:** 1742–1755

Porri A, Torti S, Romera-Branchat M, Coupland G (2012) Spatially distinct regulatory roles for gibberellins in the promotion of flowering of Arabidopsis under long photoperiods. Development **139:** 2198–2209

Putterill J, Robson F, Lee K, Simon R, Coupland G (1995) The CONSTANS gene of Arabidopsis promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. Cell **80:** 847–857

Rubio S, Rodrigues A, Saez A, Dizon MB, Galle A, Kim T-H, Santiago J, Flexas J, Schroeder JI, Rodriguez PL (2009) Triple loss of function of protein phosphatases type 2C leads to partial constitutive response to endogenous abscisic acid. Plant Physiol **150:** 1345–1355

Samach A, Onouchi H, Gold SE, Ditta GS, Schwarz-Sommer Z, Yanofsky MF, Coupland G (2000) Distinct roles of CONSTANS target genes in reproductive development of Arabidopsis. Science **288:** 1613–1616

Sawa M, Kay SA (2011) GIGANTEA directly activates Flowering Locus T in Arabidopsis thaliana. Proc Natl Acad Sci USA **108:** 11698–11703

Sawa M, Nusinow DA, Kay SA, Imaizumi T (2007) FKF1 and GIGANTEA complex formation is required for day-length measurement in Arabidopsis. Science **318:** 261–265

Searle I, He Y, Turck F, Vincent C, Fornara F, Kröber S, Amasino RA, 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

Seo E, Lee H, Jeon J, Park H, Kim J, Noh Y-S, Lee I (2009) Crosstalk between cold response and flowering in *Arabidopsis* is mediated through the flowering-time gene *SOC1* and its upstream negative regulator *FLC*. Plant Cell **21:** 3185–3197

Seo M, Koshiba T (2011) Transport of ABA from the site of biosynthesis to the site of action. J Plant Res **124:** 501–507

Sheldon CC, Rouse DT, Finnegan EJ, Peacock WJ, Dennis ES (2000) The molecular basis of vernalization: the central role of FLOWERING LOCUS C (FLC). Proc Natl Acad Sci USA **97:** 3753–3758

Sherrard ME, Maherali H (2006) The adaptive significance of drought escape in Avena barbata, an annual grass. Evolution **60:** 2478–2489

Suzuki M, Ketterling MG, Li Q-B, McCarty DR (2003) Viviparous1 alters global gene expression patterns through regulation of abscisic acid signaling. Plant Physiol **132:** 1664–1677

Tao Z, Shen L, Liu C, Liu L, Yan Y, Yu H (2012) Genome-wide identification of SOC1 and SVP targets during the floral transition in Arabidopsis. Plant J **70:** 549–561

Valverde F, Mouradov A, Soppe W, Ravenscroft D, Samach A, Coupland G (2004) Photoreceptor regulation of CONSTANS protein in photoperiodic flowering. Science **303:** 1003–1006

Verslues PE, Juenger TE (2011) Drought, metabolites, and Arabidopsis natural variation: a promising combination for understanding adaptation to water-limited environments. Curr Opin Plant Biol **14:** 240–245

Wahl V, Ponnu J, Schlereth A, Arrivault S, Langenecker T, Franke A, Feil R, Lunn JE, Stitt M, Schmid M (2013) Regulation of flowering by trehalose-6-phosphate signaling in Arabidopsis thaliana. Science **339:** 704–707

Wigge PA, Kim MC, Jaeger KE, Busch W, Schmid M, Lohmann JU, Weigel D (2005) Integration of spatial and temporal information during floral induction in Arabidopsis. Science **309:** 1056–1059

Wilson RN, Heckman JW, Somerville CR (1992) Gibberellin is required for flowering in *Arabidopsis thaliana* under short days. Plant Physiol **100:** 403–408

Xi W, Liu C, Hou X, Yu H (2010) *MOTHER OF FT AND TFL1* regulates seed germination through a negative feedback loop modulating ABA signaling in *Arabidopsis*. Plant Cell **22:** 1733–1748

Xu JL, Lafitte HR, Gao YM, Fu BY, Torres R, Li ZK (2005) QTLs for drought escape and tolerance identified in a set of random introgression lines of rice. Theor Appl Genet **111:** 1642–1650

Yamaguchi A, Kobayashi Y, Goto K, Abe M, Araki T (2005) TWIN SISTER OF FT (TSF) acts as a floral pathway integrator redundantly with FT. Plant Cell Physiol **46:** 1175–1189

Yant L, Mathieu J, Dinh TT, Ott F, Lanz C, Wollmann H, Chen X, Schmid M (2010) Orchestration of the floral transition and floral development in *Arabidopsis* by the bifunctional transcription factor APETALA2. Plant Cell **22:** 2156–2170

Yant L, Mathieu J, Schmid M (2009) Just say no: floral repressors help Arabidopsis bide the time. Curr Opin Plant Biol **12:** 580–586

Yoo SK, Chung KS, Kim J, Lee JH, Hong SM, Yoo SJ, Yoo SY, Lee JS, Ahn JH (2005) *CONSTANS* activates *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* through *FLOWERING LOCUS T* to promote flowering in Arabidopsis. Plant Physiol **139:** 770–778

Yu J-W, Rubio V, Lee N-Y, Bai S, Lee S-Y, Kim S-S, Liu L, Zhang Y, Irigoyen ML, Sullivan JA, et al (2008) COP1 and ELF3 control circadian function and photoperiodic flowering by regulating GI stability. Mol Cell **32:** 617–630

Zheng Z, Xu X, Crosley RA, Greenwalt SA, Sun Y, Blakeslee B, Wang L, Ni W, Sopko MS, Yao C, et al (2010) The protein kinase SnRK2.6 mediates the regulation of sucrose metabolism and plant growth in Arabidopsis. Plant Physiol **153:** 99–113

Zuo Z, Liu H, Liu B, Liu X, Lin C (2011) Blue light-dependent interaction of CRY2 with SPA1 regulates COP1 activity and floral initiation in Arabidopsis. Curr Biol **21:** 841–847

Downloaded from https://academic.oup.com/plphys/article/162/3/1706/6110857 by U.S. Department of Justice user on 16 August 2022