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Research Article

Carbon and nitrogen signaling regulate *FLOWERING LOCUS C* and impact flowering time in Arabidopsis

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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 (https://academic.oup.com/plphys/pages/General-Instructions) is Vanessa Wahl.

Abstract

The timing of flowering in plants is modulated by both carbon (C) and nitrogen (N) signaling pathways. In a previous study, we established a pivotal role of the sucrose-signaling trehalose 6-phosphate pathway in regulating flowering under N-limited short-day conditions. In this work, we show that both wild-type Arabidopsis (*Arabidopsis thaliana*) plants grown under N-limited conditions and knock-down plants of *TREHALOSE PHOSPHATE SYNTHASE 1* induce *FLOWERING LOCUS C* (*FLC*) expression, a well-known floral repressor associated with vernalization. When exposed to an extended period of cold, a *flc* mutant fails to respond to N availability and flowers at the same time under N-limited and full-nutrition conditions. Our data suggest that SUCROSE NON-FERMENTING 1 RELATED KINASE 1-dependent trehalose 6-phosphate-mediated C signaling and a mechanism downstream of N signaling (likely involving NIN-LIKE PROTEIN 7) impact the expression of *FLC*. Collectively, our data underscore the existence of a multi-factor regulatory system in which the C and N signaling pathways jointly govern the regulation of flowering in plants.

Introduction

Owing to their sessile nature, plants adapt to environmental changes by modifying their development and growth. These processes require substantial amounts of energy. Plants are in constant feedback with the environment and their nutrient status, especially carbon (C) and nitrogen (N), that serve as crucial bases for energy production and biomass generation. Low levels of C or N in the cells suppress development and growth in plants and trigger the onset of senescence. To balance energy-intensive developmental processes with endogenous nutrient availability, plants have evolved intricate signaling networks (Fernie et al. 2020).

Flowering is an important developmental process in the life cycle of plants with correct timing being essential for reproductive success. It is regulated by a sophisticated genetic network that integrates various environmental and endogenous signals to regulate the expression of the floral integrator genes such as the florigen, *FLOWERING LOCUS T* (*FT*), and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) (Srikanth and Schmid 2011; Romera-Branchat et al. 2014; Song et al. 2015). FT integrates signals perceived in the leaves and conveys this information to the shoot apical meristem (SAM) to induce flowering (Corbesier et al. 2007; Jaeger and Wigge 2007; Mathieu et al. 2007). At the SAM, FT interacts with the bZIP transcription factor FLOWERING LOCUS D (FD) to form a complex that directly activates *SOC1* along with floral meristem identity genes such as *APETALA 1* (*AP1*) (Abe et al. 2005; Wigge et al. 2005).

In addition to other stimuli, temperature impacts greatly the time of flowering. Increased ambient temperature results in earlier flowering due to decreased SHORT VEGETATIVE PHASE (SVP) protein stability (Lee et al. 2013, 2014). SVP forms a temperature-dependent flowering repressor complex with partners such as FLOWERING LOCUS M (FLM)/MADS AFFECTING FLOWERING 1 (MAF1), an orthologue of FLOWERING LOCUS C (FLC) (Pose et al. 2013; Sureshkumar et al. 2016), resulting in earlier flowering when plants are exposed to warmer conditions (Pose et al. 2013). SVP was also shown to interact with FLC in a flowering repressor complex (Fujiwara et al. 2008; Li et al. 2008). This delays floral transition by directly reducing the expression of *FT*, *FD*, and *SOC1* (Hepworth et al. 2002; Helliwell et al. 2006; Searle et al. 2006; Lee Yoo, et al. 2007; Li et al. 2008). In winter-annual accessions of *Arabidopsis thaliana* (Arabidopsis) flowering is suppressed due to active *FRIGIDA* (*FRI*) resulting in promoted expression of *FLC*, unless the plants are exposed to a long period of cold (vernalization process) (Sheldon et al. 2000). This regulation involves a plethora of proteins and complexes acting in many layers of gene regulation, ranging from RNA structures, epigenetic modification to transcriptional and mRNA processing control

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(reviewed by Whittaker and Dean 2017; and Sharma et al. 2020; Xu Fang, et al. 2021; Xu Wu, et al. 2021; Yang et al. 2022).

Organic C and N supply is essential in particular for vegetative growth and plant development (Sulpice et al. 2013). It is known that nutrients are essential for developmental transitions (Fernie et al. 2020), but the underlying mechanisms continue to be subject to active investigation. Interestingly, *FLC* expression was observed to increase in NITRATE TRANSPORTER 1.1 (NRT1.1) and NRT1.13 defective mutant plants (Teng et al. 2019; Chen et al. 2021). While NRT1.13 is suggested to be a nitrate transporter, NRT1.1 is a key component of nitrate signaling functioning as both a transporter and a sensor in roots (Li et al. 2021). This suggests a nitrate signaling-dependent control of *FLC* as proposed by Kant and colleagues (Kant et al. 2011). This is supported by the introduction of an *flc-3* mutation into the late-flowering NRT1.1 deficient plant background which restored wild-type flowering (Teng et al. 2019).

Previous studies have identified multiple factors that influence N-regulated flowering, which often vary and depend on the cultivation systems used (Lin and Tsay 2017). We are using a soil-based N-limited system developed by Tschoep et al. (2009), which allows plant adaptation and the investigation of flowering time without stress-related symptoms (Olas et al. 2019, 2021). With this system, we previously reported that nitrate-regulated flowering depends on SAM factors. Notably, in N-limiting conditions, nitrate-responsive gene expression is affected and nitrate assimilation is reduced in the SAM (Olas et al. 2019). The early nitrate response involves the NIN-LIKE PROTEIN (NLP) transcription factors NLP6 and NLP7. They accumulate in the nucleus in the presence of nitrate, regulating gene expression through nitrate-responsive cis-elements (NRE) (Konishi and Yanagisawa 2013; Marchive et al. 2013; Liu et al. 2017, 2022). Limited nitrate availability delays flowering due to decreased expression of *SOC1*, likely through NLP6/NLP7-regulated expression of the SQUAMOSA PROMOTER-BINDING PROTEIN-LIKE transcription factors encoding genes *SPL3* and *SPL5* (Olas et al. 2019).

The sucrose signal trehalose 6-phosphate (T6P) regulates a plethora of developmental and physiological responses (reviewed by Fichtner and Lunn 2021). In Arabidopsis, T6P is synthesized by TREHALOSE PHOSPHATE SYNTHASE1 (TPS1) (Vandesteene et al. 2010; Yang et al. 2012), and it acts mainly by modulating the SUCROSE NON-FERMENTING 1-RELATED KINASE 1 (SnRK1) activity. Moreover, T6P was suggested to be able to bind directly to the SnRK1 upstream activating kinases and inhibit their activity (Zhai et al. 2018). SnRK1 is a key sensor of energy status, and it is required for both normal growth and plant responses to stresses that impact plant fitness and survival (Polge and Thomas 2007; Baena-Gonzalez and Sheen 2008). Although single mutants of SnRK1 catalytic subunits resemble wild-type plants (Baena-Gonzalez et al. 2007; Jeong et al. 2015), *tps1* mutants (*tps1-2*) are embryo-lethal (Eastmond et al. 2002). This can be bypassed by ectopically expressing dexamethasone-inducible *TPS1* (*GVG::TPS1*) during the seed set (van Dijken et al. 2004). However, plants grown from these seeds remain in the vegetative phase for a highly extended period or fail to flower entirely (van Dijken et al. 2004; Wahl et al. 2013). T6P signaling induces flowering in leaves via *FT* and also acts at the SAM through microRNA156 (miR156) and its target transcripts, *SPL3-5* (Wahl et al. 2013), at least partially via the modulation of the SnRK1 complex activity (Zacharaki et al. 2022). This was supported by the observation that loss of SnRK1 activity in the *tps1-2,GVG::TPS1* plants led to early induction of *FT* in the leaves, reduced *miR156* levels and strong induction of *SPL3* in the SAM during bolting (Zacharaki et al. 2022). Taken together, these findings indicate that both C and N signaling can target the same components of the flowering network at the SAM (Wahl et al. 2013; Olas et al. 2019; Zacharaki et al. 2022), underscoring their joint importance for the proper timing of flowering.

Even though the current understanding implies a straightforward output downstream of nutrient signaling, our data now indicate a more complex relationship between nutrient signaling and developmental programs. Here, we demonstrate that the T6P pathway, which controls flowering under N limitation in short days (Olas et al. 2019), impacts on the expression of *FLC* in addition to *FLC* being differentially expressed upon exposure to contrasting N levels. Our findings suggest that both C- and N-dependent pathways regulate Arabidopsis flowering time by modulating *FLC* expression, implying a role in the composition and timing of the FLC-SVP repressor complex within a developmental context.

Results

Sucrose signaling represses *FLC*

We have previously reported that plants grown under N-limited conditions and short days (SD) accumulate both sucrose and T6P towards the end of the vegetative growth phase. Importantly, *TPS1* knock-down plants (*35S::amiRTPS1*) did not flower under these conditions (Olas et al. 2019). To understand this phenomenon, we analyzed a developmental series of rosette samples from both Col-0 and *35S::amiRTPS1* plants, focusing on candidate genes, which specifically change their expression before the floral transition. This analysis included multiple flowering time genes assessed by RT-qPCR. Apart from positive regulators of flowering that have been previously investigated (Olas et al. 2019), we found that in 4- to 6-d-old *35S::amiRTPS1* plants, *FLC*, a key flowering repressor, displayed a strong upregulation (Fig. 1A). Considering that *FLC* expression has previously been suggested to be modulated in response to N availability (Kant et al. 2011), it is an interesting candidate for further investigation. Floral transition occurs 10 d after germination (DAG) in Col-0 wild-type plants and 19 DAG in *35S::amiRTPS1* grown in long days (LD) and full-nutrition soil as shown before (Wahl et al. 2013). We observed that *FLC* expression declines before the floral transition (Fig. 1A). This suggests that the T6P pathway fundamentally contributes to the full repression of *FLC* in young seedlings. While we initially did not anticipate that the T6P pathway could affect *FLC* expression at later stages, we found that when the *flc-3* mutation is introduced into the *tps1-2 GVG::TPS1* background, it partially rescues the late flowering and delayed vegetative phase transition observed in this *tps1-2 GVG::TPS1* (Fig. 1, B and C; Supplementary Fig. S1; Supplementary Tables S1 and S2). Our data, therefore, suggest that the T6P pathway is involved in *FLC* regulation to promote flowering and facilitate the vegetative phase change.

FLC integrates N-signaling into the flowering network

It has been previously shown that flowering is delayed in wild-type plants grown in the limited N (LN) soil (Olas et al. 2019). Furthermore, some data suggest that *FLC* expression may be influenced by N availability (Kant et al. 2011). To investigate the potential regulation of *FLC* expression by N status, we grew wild-type Col-0 plants in a soil-based growth system (Tschoep et al. 2009), consisting of soil with optimal N (ON) and one with LN source. We observed elevated *FLC* expression levels in LN in both rosettes and apices of Col-0 plants grown continuously in SD conditions

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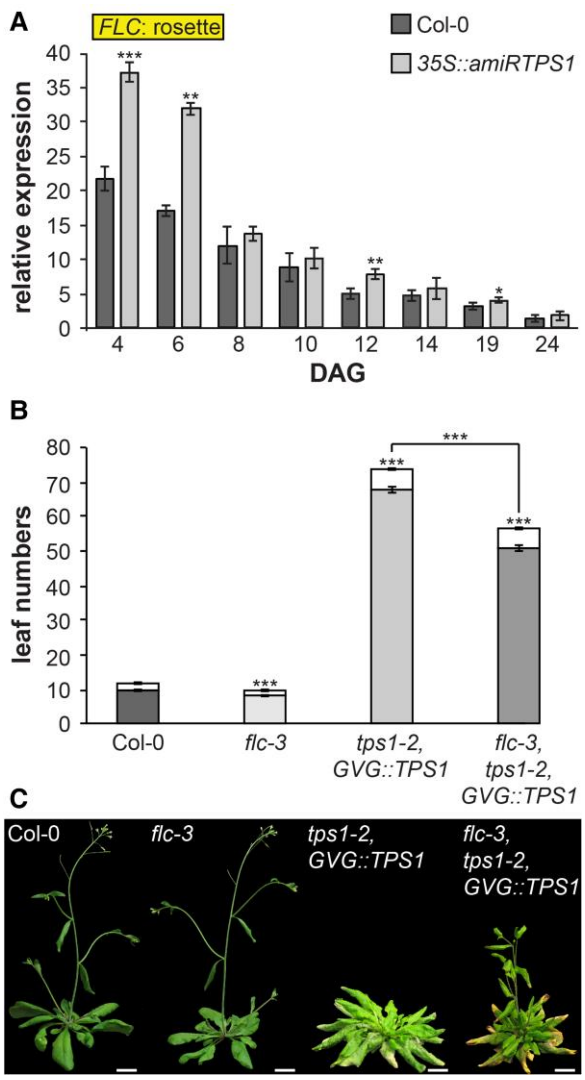

Figure 1. The trehalose 6-phosphate pathway impacts on *FLOWERING LOCUS C*. **A)** Expression of *FLOWERING LOCUS C* (*FLC*) measured by RT-qPCR in rosettes of Col-0 and *35S::amiRTPS1* plants grown in full-nutrition soil under long days (16 h light/8 h darkness). $n = 4$. **B)** Flowering time measured as leaf numbers (rosette leaves in gray; cauline leaves in white). $n \geq 15$ individual plants per genotype. **C)** Representative photographs of the plants analyzed in (B). Images were digitally extracted for comparison. Abbreviations: days after germination (DAG). Data represent mean, error bars are standard deviations (s.d.), statistically significant difference compared to Col-0 wild type (Student *t*-test, $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$).

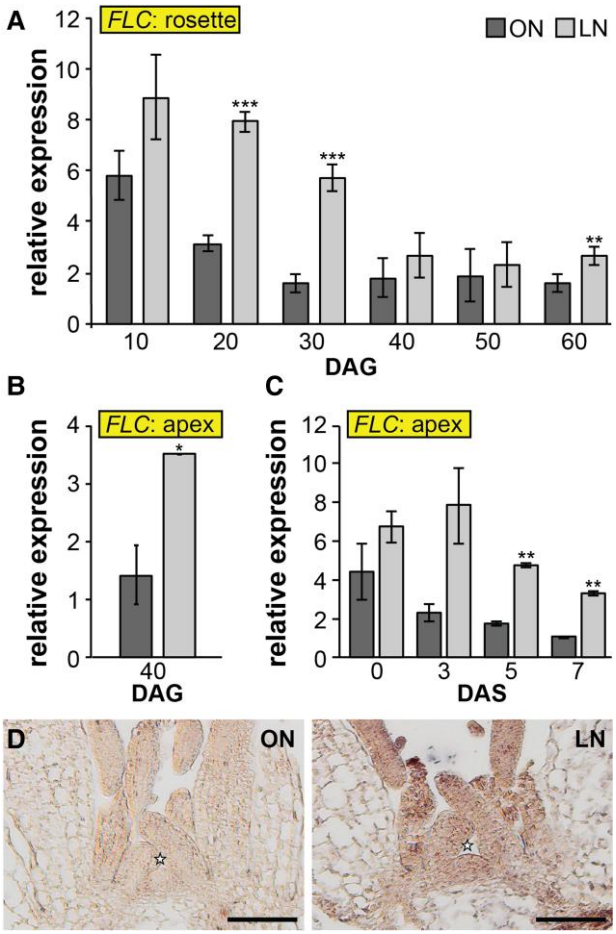

Figure 2. *FLOWERING LOCUS C* in response to nitrogen limitation. **A, B)** Expression of *FLOWERING LOCUS C* (*FLC*) measured by RT-qPCR in rosettes **A)** and apices **B)** of Col-0 plants grown in optimal nitrogen (ON) and limited-nitrogen (LN) conditions under short days (8 h light/16 h dark). **C)** *FLOWERING LOCUS C* expression measured by RT-qPCR in apices of plants initially grown under short days (30 d) and then transferred to long days to initiate the floral transition for 3, 5, and 7 d. **D)** RNA in situ hybridization using *FLOWERING LOCUS C* specific probe on longitudinal sections through vegetative apices of Col-0 plants grown in ON and LN soils (also see Supplementary Fig. S4). Abbreviations: days after germination (DAG); days after shift (DAS). Data represent mean, error bars are standard deviations (s.d.), $n = 3$, statistically significant difference between ON and LN (Student's *t*-test, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). Star indicates apex summit. Representative plant pictures are in Supplementary Figs. S2 and S3.

(Fig. 2, A and B; Supplementary Fig. S2), and in apices of plants that were initially grown in SD conditions and subsequently transferred to LD conditions (Fig. 2C; Supplementary Fig. S3). To obtain information on the expression pattern at higher spatial resolution, we used *FLC* as a probe and performed RNA in situ hybridization (Fig. 2D; Supplementary Fig. S4). *FLC* transcript was detectable at the SAM and in young leaves of LN-grown plants, confirming our previous observations that limited N availability enhances *FLC* expression. This finding suggests that FLC plays a crucial role in the regulation of flowering time in response to N availability.

It is well established that exposure to low temperatures decreases *FLC* expression in plants (Searle et al. 2006). Therefore, we grew wild-type plants at 4 °C in SD for 8 wk, followed by a transfer to 22 °C until flowering (Supplementary Fig. S5). This treatment resulted in wild-type plants flowering at the same time in both N regimes, suggesting that FLC contributes to the delayed flowering time observed in plants grown in the LN soil (Fig. 3A; Supplementary Table S1).

FLC is known to form a flowering repressor complex with SVP to suppress *SOC1* at the SAM (Li et al. 2008). Unlike *FLC*, *SVP* was not differentially expressed in either LN-grown plants or *TPS1* knockdown plants (Supplementary Figs. S6 and S7). Importantly, neither *flc-3* nor *svp-32* mutant plants responded to the reduced N content in the LN soil (Fig. 3B; Supplementary Figs. S8 and S9; Supplementary Table S1), flowering at the same time in ON and LN conditions. This indicates that both FLC and SVP play a role in the N-dependent regulation of flowering time.

N-signaling modulates *FLC* via NLP7

NLPs are key regulators of nitrate sensing and signaling, with NLP6 and NLP7 being 2 of the most well-characterized members

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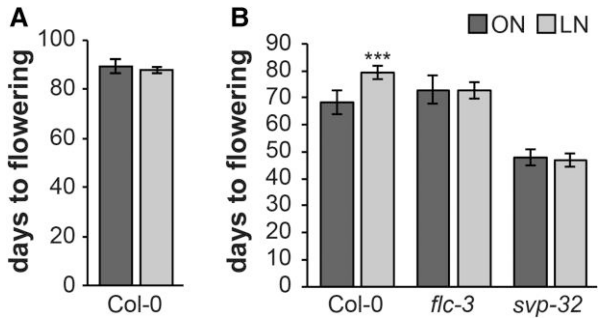

Figure 3. FLOWERING LOCUS C and SHORT VEGETATIVE PHASE are required for the limited nitrogen-dependent flowering response. **A)** Flowering time of Col-0 wild-type plants treated with an 8-week period of cold. Note that afterwards plants were transferred to 22 °C until flowering. **B)** Flowering time of Col-0, *flc-3*, and *svp-32* mutant plants grown under short-day (8 h light/16 h darkness) conditions. Data represent mean, error bars are standard deviations (s.d.), $n \geq 15$ individual plants per genotype, statistically significant difference between optimal nitrogen (ON) and limited-nitrogen (LN) (Student's *t*-test, $^{***}P < 0.001$). Representative plant pictures are in Supplementary Figs. S5, S8, and S9.

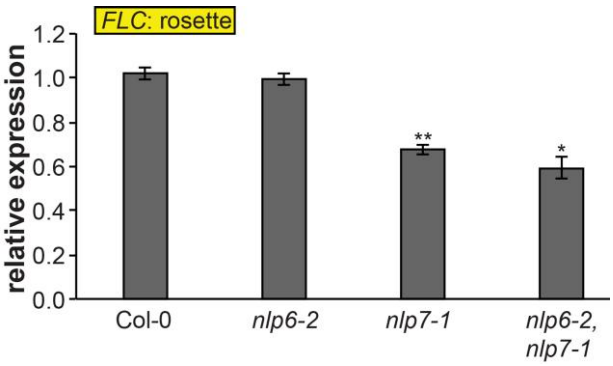

Figure 4. *FLOWERING LOCUS C* expression downstream of NIN-LIKE PROTEIN 6 (NLP6) and NIN-LIKE PROTEIN 7 (NLP7). Expression of *FLOWERING LOCUS C* (*FLC*) measured by RT-qPCR at 10 d after germination (DAG) in rosettes of Col-0, *nlp6-2*,*nlp7-1*, and *nlp6-2*,*nlp7-1* plants grown under full nutrition in short-day conditions. Data represent mean, error bars are standard deviations (s.d.), $n = 3$, statistically significant difference compared to Col-0 wild type (Student's *t*-test, $^{*}P < 0.05$, $^{**}P < 0.01$). Representative plant pictures are in Supplementary Fig. S10.

of this family in *Arabidopsis* (Fredes et al. 2019). In the presence of nitrate, NLP7 is retained in the nucleus through phosphorylation, where it binds to NREs present in N-responsive genes to promote their expression (Konishi and Yanagisawa 2013). Interestingly, we observed a significant reduction of *FLC* expression in the *nlp7-1* mutant, indicating that an active NLP7 modulates *FLC* expression when N is not limited (Fig. 4; Supplementary Fig. S10).

Since the *FLC* gene does not carry an NRE in its promoter, genomic or downstream sequences, we expanded our analysis to include other flowering time genes that regulate *FLC* (Supplementary Table S3). Notably, *FRI*, a key regulator upstream of *FLC*, has 4 putative NREs (Supplementary Table S3). However, the Col-0 accession carries a non-functional *FRI* allele that encodes a truncated protein (Schmalenbach et al. 2014). To exclude the possibility that a truncated FRI controls *FLC* expression downstream of N signaling or the T6P pathway, we quantified transcript abundance of *FRI* in Col-0 ON- and LN-grown plants (Supplementary Fig. S11) as well as in 35S::*amiRTPS1* plants (Supplementary Fig. S12). Our data demonstrated that the expression of *FRI* is largely unaffected, indicating that the FRI is not responsible for elevated *FLC* expression observed in this study. It will be interesting to investigate if this is the case in other accessions, such as those that are not rapid-cycling like Col-0. Additionally, we identified other genomic loci encoding *FLC* regulators with putative NREs (Supplementary Table S3), but their expression was also unaffected under N-limited conditions (Supplementary Fig. S11) and in 35S::*amiRTPS1* plants (Supplementary Fig. S12). Taken together, this suggests that *FLC* suppression in ON plants involves an as yet unknown transcription factor(s), whose activity is regulated by NLP7 and the T6P pathway.

Sucrose and N-signals interconnect at the level of *FLC* for coordinated flowering time regulation

We have previously demonstrated that the T6P pathway and sufficient nitrate levels are necessary for floral induction in SD (Olas et al. 2019). The fact that 35S::*amiRTPS1* plants fail to flower when N is limited and that *FLC* expression is modulated by N availability prompted us to test whether *FLC* is a target of both N signaling and the T6P pathway.

We observed that *FLC* transcription was elevated in rosettes of wild-type plants grown under SD with limited N which was even more pronounced in 35S::*amiRTPS1* plants (Fig. 5A; Supplementary Fig. S13). This suggests an additive effect between N signaling and the T6P pathway, both converging on the *SPL3-5* node at the SAM (Wahl et al. 2013; Olas et al. 2019). To test whether *FLC* could be regulated through SPL3-5, we measured its expression in *spl3/4/5* mutants (Xie et al. 2020). However, *FLC* expression in rosette leaves of *spl3/4/5* mutants was comparable to that of wild-type plants (Supplementary Fig. S14A), indicating that both pathways regulate *FLC* expression *via* another mechanism. Similarly to *FLC*, we did not observe any difference in *SVP* expression in *spl3/4/5* compared to Col-0 plants (Supplementary Fig. S14B).

The T6P pathway is known to function by directly modulating SnRK1 activity (Zhang et al. 2009). Loss of SnRK1 activity restores flowering of *tps1* (*GVG::TPS1*) mutants in LD by initial induction of *FT* in the leaves and subsequent suppression of miR156 followed by SPLs induction in the SAM (Zacharaki et al. 2022). Thus, we tested whether *FLC* regulation in *tps1* (*GVG::TPS1*) mutants is also mediated by SnRK1. We found that indeed *FLC* expression was increased in the *tps1* (*GVG::TPS1*) mutant where SnRK1 is fully active. Interestingly, introducing non-catalytically active mutations in SnRK1 within the *tps1* (*GVG::TPS1*) background restores *FLC* expression to wild-type levels in both rosette leaves and apex tissue (Fig. 5, B and C). The suppression of *FLC* in the double mutant is more pronounced in the apex than the rosette leaves, underscoring the critical role of the T6P pathway in controlling developmental transitions. Our data suggest that *FLC* expression is regulated by both nitrate and sugar availability *via* NPLs and the T6P pathway through the SnRK1 complex, respectively (Fig. 6).

Discussion

C and N are essential for plant growth and development, and the ability of plants to properly sense their availability is crucial due to their sessile nature. C in the form of sucrose is produced via photosynthesis in the leaves, while N can be taken up in both inorganic forms (nitrate and ammonia) and organic forms (amino acids).

In *Arabidopsis*, a key sugar sensor is the T6P pathway which functions *via* SnRK1 activity. The T6P pathway has a key role in

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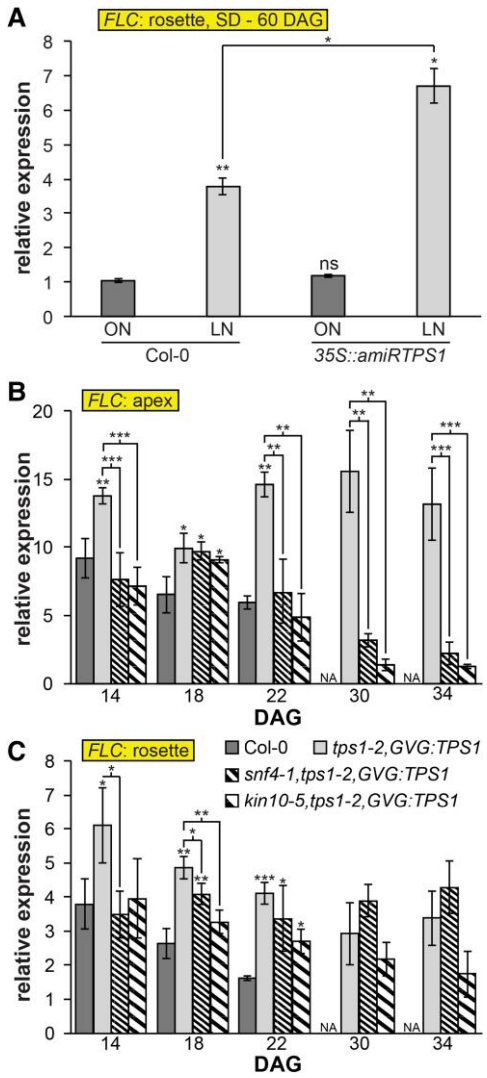

Figure 5. Trehalose 6-phosphate pathway and nitrogen-signaling converge at *FLOWERING LOCUS C*. **A, B C)** Expression of *FLOWERING LOCUS C* (*FLC*) measured by RT-qPCR in **A)** rosettes of wild-type Col-0 and 35S::*amiRTPS1* plants grown in optimal nitrogen (ON) and limited-nitrogen (LN) conditions under short days (8 h light/16 h dark) at 60 d after germination (DAG), in **B)** apices and **C)** rosettes of wild-type Col-0, *tps1-2,GVG:TPS1*, *snf4,tps1-2,GVG:TPS1* and *kin10,tps1-2,GVG:TPS1* plants grown in standard soil under long days (16 h light/8 h dark). Data represent mean, error bars are standard deviations (s.d.), statistically significant difference compared to Col-0 wild-type (Student *t*-test, $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$). Representative plant pictures are in Supplementary Fig. S13.

plants' developmental transitions, such as flowering. It has been shown that both *miR156* and *FT* regulation in the SAM and leaves, respectively, are required for *tps1* plants to complete their transition to flowering (Wahl et al. 2013; Ponnu et al. 2020). Here, we found that *FLC*, a repressor of flowering, is also regulated by the T6P pathway (Fig. 1A) and that loss of functional *FLC* partially restores flowering in *tps1* plants (Fig. 1, B and C). Although we do not expect that *FLC* regulation is the primary target of the T6P pathway under normal growth conditions, it could represent an additional mechanism to prevent flowering under non-optimal growth conditions.

Plants experiencing a sudden shift to colder temperatures have increased amounts of sucrose, which has been proposed to serve as a freezing protectant with concomitant rising T6P levels (reviewed by Stitt and Hurry 2002; Carillo et al. 2013). During long cold exposure, *FLC* is suppressed through several mechanisms, ranging from RNA structures to epigenetic control. *FLC* suppression allows the induction of *FT* and *SOC1*, initiating flowering (Whittaker and Dean 2017). In this scenario, when plants experience cooler temperatures, nutrients that provide plants with C and N, are transported and stored to serve as a basis for rapid growth for when conditions become optimal again or used and metabolically transformed into cryoprotectants to protect the cells from freezing damage (Kaplan et al. 2007). Thus, in sub-optimal growth conditions, the T6P pathway might contribute to the suppression of *FLC* in response to the C status.

N availability is a key factor in the regulation of plants' developmental processes and phase transitions, including the timing of flowering (Klebs 1913; Dickens and Van Staden 1988; Bernier et al. 1993; Olas et al. 2019). Arabidopsis cultivated on synthetic substrates exhibit early flowering in response to reduced N levels (Castro Marin et al. 2011; Kant et al. 2011; Liu et al. 2013). Conversely, soil-grown plants subjected to N limitation flower later than those cultivated in soil without N limitation, which we previously linked to the induction of *SPL3* and *SPL5* by NLP6 and NLP7 (Olas et al. 2019). In this study, we discovered that this phenotype can additionally be explained by significantly elevated levels of *FLC* under N limitation (Fig. 2, A to D). Furthermore, we found that flowering time in plants with suppressed *FLC* due to the vernalization response or with a non-functional *flc-3* allele is independent of N availability (Fig. 3, A and B). Although *FLC* does not prominently impact flowering time under long-day conditions in rapid-cycling accessions, such as Col-0, *FLC* variability was found to affect reproductive success in field studies and thus is a major adaptive determinant in different climates (Hepworth et al. 2020). Here, we demonstrate that it is also required for fine-tuning the timing of floral transition downstream of N signaling. Similar to *flc-3*, *svp-32* mutants flower at the same time in the ON and LN soils (Fig. 3B), suggesting a role of *SVP* in N-dependent flowering time regulation. However, in contrast to *FLC*, *SVP* is not differentially expressed in plants grown in ON and LN soil (Supplementary Fig. S7). FLC and SVP proteins form a flowering repressor complex that delays floral transition by directly reducing the expression of *FT* and *SOC1* (Hepworth et al. 2002; Helliwell et al. 2006; Lee, Yoo, et al. 2007; Li et al. 2008). Given that both functional *FLC* and *SVP* loci are required for the adjustment of flowering time in response to N availability, it is likely that the N signal is integrated at the level of the FLC-SVP complex. In this scenario, the formation of the repressor complex would be tuned by the adjustment of *FLC* expression downstream of N-signaling. Several transcription factors that are transcriptionally responsive to the N status have been identified as prime responsive genes to N availability (Vidal et al. 2015). NLPs are transcription factors facilitating nitrate signaling in plants, with NLP6 and NLP7 representing the master regulators and the 2 most studied (Fredes et al. 2019). In the absence of nitrate, NLP7 localizes strictly to the cytosol, while exposure to nitrate triggers its localization into the nucleus where it binds directly to NREs of nitrate-regulated genes (Konishi and Yanagisawa 2013; Marchive et al. 2013). Since NREs are not present in the *FLC* locus (Supplementary Table S3), it is unlikely to be directly controlled by NLPs. Other examples of *FLC* regulation related to N availability, are the *nrt1.1* and *nrt1.13*, mutants of the nitrate sensor and transporter NRT1.1 and transporter NRT1.13 (Teng et al. 2019; Chen et al. 2021). Similar to our findings (Supplementary Fig. S11), expression of known upstream regulators of *FLC* was not changed in *nrt1.13*, suggesting that

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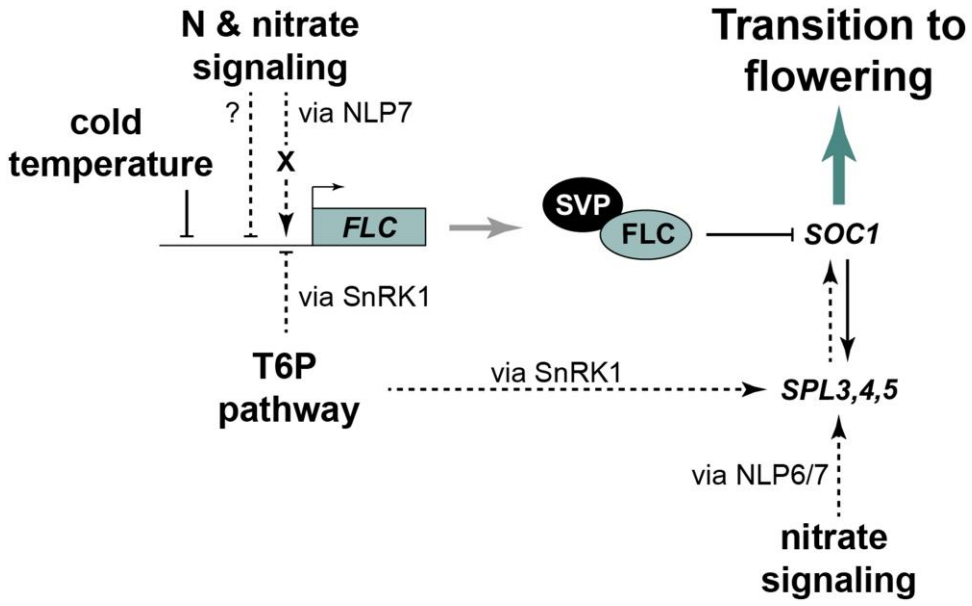

Figure 6. Carbon and nitrogen signaling target similar components of the flowering network in the shoot apical meristem for the proper timing of flowering. *FLOWERING LOCUS C* (*FLC*), a key repressor of flowering, is not only regulated by cold temperature as part of the vernalization process, but is also affected by nutrient availability. The Trehalose 6-phosphate (T6P) pathway negatively impacts *FLOWERING LOCUS C* via SUCROSE NON-FERMENTING 1 RELATED KINASE 1 (SnRK1). Nitrogen (N) signaling controls *FLOWERING LOCUS C* via a yet-to-identify mechanism (X) involving NIN-LIKE PROTEIN 7 (NLP7). The repressor complex composed of FLOWERING LOCUS C and SHORT VEGETATIVE PHASE (SVP) is eventually tuned by the adjustment of *FLOWERING LOCUS C* expression downstream of both carbon and nitrogen signaling to control *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) in the shoot apical meristem. Independently, both C and N pathways work via the age pathway (SQUAMOSA PROMOTER-BINDING PROTEIN-LIKE 3-5, SPL3-5) to induce flowering.

NRT1.13 regulates *FLC* expression and flowering time independently of these known pathways (Chen et al. 2021).

Interestingly, we found that *FLC* was significantly downregulated in the late-flowering *nlp7-1* and *nlp6-2 nlp7-1* mutants grown on standard soil (Fig. 4, Supplementary Fig. S10), indicating that NLP7 plays a role in the modulation of *FLC* expression. Given that NLP7 was found to control most of the nitrate-responsive genes (Marchive et al. 2013; Alvarez et al. 2020), the *nlp7-1* mutant is thought to mimic a low nitrate state. Hence, this result appears to contradict our observation of *FLC* accumulation in LN-grown plants (Fig. 2, A and B). This could be explained by the presence of an unknown NLP-independent mechanism responsible for *FLC* upregulation in LN conditions. However, it should be noted that in contrast to the mutant background, functional NLP7 is still present in wild-type plants exposed to limited N. Thus, *nlp7-1* might not entirely mimic the low-nitrate state after all and the absence of a functional NLP7 likely leads to compensation by other NLPs. Furthermore, NLP proteins contain a PB1 domain, which mediates protein-protein interactions influencing NLP activity (Konishi and Yanagisawa 2019). Given this, NLP7 might form a complex with an unknown *FLC* repressor, thereby preventing its nuclear localization under low-nitrate conditions. In the absence of NLP7 or when plants are grown under optimal N conditions, this potential repressor would localize into the nucleus, leading to a repression of *FLC* expression. It will be interesting to further dissect the mechanisms of *FLC* regulation downstream of N-signaling in the future.

Our data demonstrate that both the T6P and N-signaling pathways might affect *FLC* expression via different mechanisms. Previous studies have demonstrated that both pathways act through the miR156/SPLs node (Wahl et al. 2013; Olas et al. 2019; Ponnu et al. 2020; Zacharaki et al. 2022). In particular, the expression of *SPL3* and *SPL5* is reduced in plants grown in N-limited environment (Bi et al. 2007; Pant et al. 2009; Krapp et al. 2011; Liang et al. 2012; Fischer et al. 2013), suggesting a role for the miR156/SPL3/5 module in the regulation of flowering time when N is limited. Similarly, the T6P pathway acts via miR156 downregulation and *SPL3-5* upregulation to induce flowering and the vegetative phase change (Wahl et al. 2013; Ponnu et al. 2020; Zacharaki et al. 2022). Although both pathways converge on the miR156/SPLs module, *FLC* regulation appears to be independent (Supplementary Fig. S14A).

T6P plays a key role in promoting growth and development by suppressing SnRK1 complex activity through direct binding to the SnRK1 upstream kinases (Zhai et al. 2018). In a previous study, it was shown that *FT* was induced in the double *tps1-2 GVG::TPS1 kin10-5* and *tps1-2 GVG::TPS1 snf4* mutants as early as in wild-type plants (Zacharaki et al. 2022). Although this early *FT* induction promoted the floral transition in wild-type plants within a few days, this was not the case in both double mutants. The elevated expression of *FLC* in rosette leaves of these mutants (Fig. 5C) could thus at least partially explain this phenomenon. *FLC* downregulation directly coincides with early *FT* upregulation previously observed in the double mutants (Zacharaki et al. 2022). Interestingly, we observed that *FLC* was also downregulated in the double mutants in the apex (Fig. 5B) with more striking differences later on, coinciding with the timing of floral transition (Zacharaki et al. 2022). In addition, ectopic *FLC* expression in the SAM has been associated with delayed flowering and reduced *SOC1* and *FD* expression (Sheldon et al. 2002; Noh and Amasino 2003; Searle et al. 2006). This is also the case in *tps1-2 GVG::TPS1*, while gene expression is restored in the double mutants (Supplementary Fig. S15) (Zacharaki et al. 2022). Our data combined with the findings of Zeng et al. (2024) suggest that the regulation of SnRK1 activity is essential for T6P-dependent floral induction, which has several modes of action throughout the

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floral network to ensure that sufficient energy is available for this demanding developmental transition. Finally, our findings shed further light on the multifactorial aspects of C- and N-dependent regulation of flowering time.

Materials and methods

Plant material and growth conditions

Arabidopsis thaliana plants used for this study are of the Columbia (Col-0) ecotype. Mutant and transgenic lines such as *flc-3*, *svp-32*, *35S::amiRTPS1*, *tps1-2,GVG::TPS1*, *tps1-2,GVG::TPS1,kin10-5*, *tps1-2, GVG::TPS1,snf4-1*, *nlp6-2*, *nlp7-1*, *nlp6-2,nlp7-1*, and *spl3/4/5* were previously described (Michaels and Amasino 1999; Lee, Yoo, et al. 2007; Wahl et al. 2013; Olas et al. 2019; Xie et al. 2020; Zacharaki et al. 2022). The *flc-3,tps1-2,GVG::TPS1* double mutant lines were generated by crossing. Genotypes were confirmed by a genotyping PCR using the oligonucleotides listed in Supplementary Table S4.

Arabidopsis plants were grown in controlled growth chambers (Model E-36L, Percival Scientific Inc., Perry, IA, USA) at 22 °C in long-day (LD, 16 h light/8 h dark) or short-day (SD, 8 h dark/16 h light) conditions. Light intensity was approximately 160 μmol/m²s. Controlled induction of flowering was performed by transferring the plants from non-inductive (SD) to inductive conditions (LD) as described (Schmid et al. 2003).

A previously established, almost natural, soil-based N-limited growth system consisting of ON and LN soil was used to grow plants (Tschoep et al. 2009). Briefly, the growth system consists of 2 types of peat-based soil mixtures with either an optimal level of N (ON, ~850 mg (N)/kg) or a limited level of N (LN, ~40 mg (N)/kg). Soil mixtures were prepared as described (Olas et al. 2019).

Phenotypic analyses

Flowering time was defined as days to flowering (DTF), which describes the days after germination to the day of bolting (inflorescence length, 0.5 cm), and by the total number of leaves (TLN). At least 16 plants were used to determine flowering time of each genotype. For vegetative phase change, juvenile leaf numbers were recorded, and the leaf shape was digitally documented as described (Ponnu et al. 2020). A student's *t*-test was used to test the significance of the phenotypic differences.

Reverse transcription quantitative PCR (RT-qPCR)

Sampling, RNA extraction, and RT-qPCR analysis of *FLC* in the *tps1-2,GVG::TPS1*, *tps1-2,GVG::TPS1,kin10-5* and *tps1-2,GVG::TPS1, snf4-1* were performed as described (Zacharaki et al. 2022). RNA extraction and RT-qPCR analyses of all the other genes were performed according to Wahl et al. (2013). Relative expression values were calculated with the 2^DDCt method using Ct values of a housekeeping gene index of *TUB2* (At5g62690), *SAND* (At2g28390), *UBQ10* (At4g05320), and *PDF2* (At1g13320). RT-qPCR analyses were performed in 3 or 4 biological replicates ($n = 3$ or 4). A Student's *t*-test was used to test for statistical significance. All oligonucleotides used in this study can be found in Supplementary Table S4.

RNA in situ hybridization

Wax embedding, sectioning, RNA in situ hybridization, and imaging were performed as described (Wahl et al. 2013; Gramma and Wahl 2023). Probes were synthesized using the DIG RNA Labeling Kit (Roche, Mannheim, Germany) for CDS of the *FLC* gene cloned into the pGEM-T Easy vector (Promega, Madison, Wisconsin, USA). Oligonucleotides are listed in Supplementary Table S4.

Accession numbers

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers: TPS1 (At1g78580), FLC (At5g10140), SVP (At2g22540), FCA (At2g19520), EMF1 (At5g11530), PIE1/SNF2 (At3g12810), NLP6 (At1g64530), NLP7 (At4g24020), FRI (At4g00650), SUF4 (At1g30970), ELF7 (At1g79730), SEF (At5g37055), VRN1 (At3g18990), VRN2 (At4g16845), EMF2/CYR1 (At5g51230), TFL2 (At5g17690), FVE (At2g19520), HUA2 (At2g19520), SNF4 (At1g09020), KIN10 (At3g01090), SPL3 (At2g33810), SPL4 (At1g53160), and SPL5 (At3g15270).

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Author contributions

V.W. conceived and designed the experiments and prepared the figures. All authors performed essential experiments and analyzed data: J.J.O. and V.W. performed the RNA in situ hybridizations; V.G., J.J.O., V.Z., and M.M.L. the RT-qPCRs; J.J.O. and J.P. performed phenotypic analyses. V.G., V.Z., and V.W. wrote the manuscript with contributions from the other authors. All authors have read and commented on the text and figures within this manuscript.

Supplementary data

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

Supplementary Figure S1. *flc-3* partially suppresses the delayed vegetative phase change phenotype of *tps1-2,GVG::TPS1* plants.

Supplementary Figure S2. Phenotype in response to nitrogen (N) limitation of Col-0 plants continuously grown in short days (8 h light, 16 h dark).

Supplementary Figure S3. Phenotype in response to nitrogen (N) limitation of Col-0 plants transferred from short-day (SD) to long-day (LD) conditions.

Supplementary Figure S4. Additional pictures of RNA in situ hybridization with *FLOWERING LOCUS C* (*FLC*)-specific probe.

Supplementary Figure S5. Phenotype of vernalized Col-0 plants in response to nitrogen (N) limitation.

Supplementary Figure S6. *SHORT VEGETATIVE PHASE* expression in *35S::amiRTPS1* plants.

Supplementary Figure S7. *SHORT VEGETATIVE PHASE* expression in response to nitrogen limitation.

Supplementary Figure S8. Phenotype of *flc-3* mutant plants in response to nitrogen (N) limitation.

Supplementary Figure S9. Phenotype of *svp-32* mutant plants in response to nitrogen (N) limitation.

Supplementary Figure S10. Phenotype of *nlp6* and *nlp7* mutant plants.

Supplementary Figure S11. Regulators upstream of *FLOWERING LOCUS C* in response to nitrogen (N) limitation.

Supplementary Figure S12. Regulators upstream of *FLOWERING LOCUS C* in *35S::amiRTPS1* plants.

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Supplementary Figure S13. Phenotype of 35S::*amiRTPS1* plants in response to nitrogen (N) limitation.

Supplementary Figure S14. *FLOWERING LOCUS C* and *SHORT VEGETATIVE PHASE* expression in *spl3/4/5* mutant plants.

Supplementary Figure S15. *FLOWERING LOCUS D* expression in *snrk1,tps1-2,GVG::TPS1* mutants.

Supplementary Table S1. Flowering time data of experiments described in this study (Supplementary Fig. S2).

Supplementary Table S2. Vegetative phase change data of experiments described in this study.

Supplementary Table S3. Putative nitrate responsive *cis*-elements (NREs) in regulators upstream of *FLOWERING LOCUS C*.

Supplementary Table S4. Oligonucleotides used in this study.

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Conflict of interest statement. None declared.

Data availability

The data supporting the findings of this study are included in this manuscript or the supplemental information and material can be obtained from the corresponding author upon reasonable request.

References

Abe M, Kobayashi Y, Yamamoto S, Daimon Y, Yamaguchi A, Ikeda Y, Ichinoki H, Notaguchi M, Goto K, Araki T. FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. *Science*. 2005:309(5737):1052–1056. https://doi.org/10.1126/science.1115983

Alvarez JM, Schinke AL, Brooks MD, Pasquino A, Leonelli L, Varala K, Safi A, Krouk G, Krapp A, Coruzzi GM. Transient genome-wide interactions of the master transcription factor NLP7 initiate a rapid nitrogen-response cascade. *Nat Commun*. 2020:11(1):1157. https://doi.org/10.1038/s41467-020-14979-6

Baena-Gonzalez E, Rolland F, Thevelein JM, Sheen J. A central integrator of transcription networks in plant stress and energy signalling. *Nature*. 2007:448(7156):938–942. https://doi.org/10.1038/nature06069

Baena-Gonzalez E, Sheen J. Convergent energy and stress signaling. *Trends Plant Sci*. 2008:13(9):474–482. https://doi.org/10.1016/j.tplants.2008.06.006

Bernier G, Havelange A, Houssa C, Petitjean A, Lejeune P. Physiological signals that induce flowering. *Plant Cell*. 1993:5(10):1147–1155. https://doi.org/10.2307/3869768

Bi YM, Wang RL, Zhu T, Rothstein SJ. Global transcription profiling reveals differential responses to chronic nitrogen stress and putative nitrogen regulatory components in Arabidopsis. *BMC Genomics*. 2007:8(1):281. https://doi.org/10.1186/1471-2164-8-281

Carillo P, Feil R, Gibon Y, Satoh-Nagasawa N, Jackson D, Blasing OE, Stitt M, Lunn JE. A fluorometric assay for trehalose in the picomole range. *Plant Methods*. 2013:9(1):21. https://doi.org/10.1186/1746-4811-9-21

Castro Marin I, Loef I, Bartetzko L, Searle I, Coupland G, Stitt M, Osuna D. Nitrate regulates floral induction in Arabidopsis, acting independently of light, gibberellin and autonomous pathways. *Planta*. 2011:233(3):539–552. https://doi.org/10.1007/s00425-010-1316-5

Chen HY, Lin SH, Cheng LH, Wu JJ, Lin YC, Tsay YF. Potential transceptor AtNRT1.13 modulates shoot architecture and flowering time in a nitrate-dependent manner. *Plant Cell*. 2021:33(5):1492–1505. https://doi.org/10.1093/plcell/koab051

Corbesier L, Vincent C, Jang S, Fornara F, Fan Q, Searle I, Giakountis A, Farrona S, Gissot L, Turnbull C, *et al.* FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. *Science*. 2007:316(5827):1030–1033. https://doi.org/10.1126/science.1141752

Dickens CWS, Van Staden J. The in vitro flowering of Kalanchöe blossfeldiana Poellniz: I. ROLE OF CULTURE CONDITIONS AND NUTRIENTS. *J Exp Bot*. 1988:39(4):461–471. https://doi.org/10.1093/jxb/39.4.461

Eastmond PJ, van Dijken AJ, Spielman M, Kerr A, Tissier AF, Dickinson HG, Jones JD, Smeekens SC, Graham IA. Trehalose-6-phosphate synthase 1, which catalyses the first step in trehalose synthesis, is essential for Arabidopsis embryo maturation. *Plant J*. 2002:29(2):225–235. https://doi.org/10.1046/j.1365-313x.2002.01220.x

Fernie AR, Bachem CWB, Helariutta Y, Neuhaus HE, Prat S, Ruan YL, Stitt M, Sweetlove LJ, Tegeder M, Wahl V, *et al.* Synchronization of developmental, molecular and metabolic aspects of source-sink interactions. *Nat Plants*. 2020:6(2):55–66. https://doi.org/10.1038/s41477-020-0590-x

Fichtner F, Lunn JE. The role of trehalose 6-phosphate (Tre6P) in plant metabolism and development. *Annu Rev Plant Biol*. 2021:72(1):737–760. https://doi.org/10.1146/annurev-arplant-050718-095929

Fischer JJ, Beatty PH, Good AG, Muench DG. Manipulation of microRNA expression to improve nitrogen use efficiency. *Plant Sci*. 2013:210:70–81. https://doi.org/10.1016/j.plantsci.2013.05.009

Fredes I, Moreno S, Diaz FP, Gutierrez RA. Nitrate signaling and the control of Arabidopsis growth and development. *Curr Opin Plant Biol*. 2019:47:112–118. https://doi.org/10.1016/j.pbi.2018.10.004

Fujiwara S, Oda A, Yoshida R, Niinuma K, Miyata K, Tomozoe Y, Tajima T, Nakagawa M, Hayashi K, Coupland G, *et al.* Circadian clock proteins LHY and CCA1 regulate SVP protein accumulation to control flowering in Arabidopsis. *Plant Cell*. 2008:20(11):2960–2971. https://doi.org/10.1105/tpc.108.061531

Gramma V, Wahl V. RNA in situ hybridization on plant tissue sections: expression analysis at cellular resolution. *Methods Mol Biol*. 2023:2686:331–350. https://doi.org/10.1007/978-1-0716-3299-4_17

Helliwell CA, Wood CC, Robertson M, Peacock WJ, Dennis ES. The Arabidopsis FLC protein interacts directly in vivo with SOC1 and FT chromatin and is part of a high-molecular-weight protein complex. *Plant J*. 2006:46(2):183–192. https://doi.org/10.1111/j.1365-313X.2006.02686.x

Hepworth J, Antoniou-Kourounioti RL, Berggren K, Selga C, Tudor EH, Yates B, Cox D, Collier Harris BR, Irwin JA, Howard M, *et al.* Natural variation in autumn expression is the major adaptive determinant distinguishing Arabidopsis FLC haplotypes. *Elife*. 2020:9:e57671. https://doi.org/10.7554/eLife.57671

Hepworth SR, Valverde F, Ravenscroft D, Mouradov A, Coupland G. Antagonistic regulation of flowering-time gene SOC1 by CONSTANS and FLC via separate promoter motifs. *EMBO J*. 2002:21(16):4327–4337. https://doi.org/10.1093/emboj/cdf432

Jaeger KE, Wigge PA. FT protein acts as a long-range signal in Arabidopsis. *Curr Biol*. 2007:17(12):1050–1054. https://doi.org/10.1016/j.cub.2007.05.008

Jeong EY, Seo PJ, Woo JC, Park CM. AKIN10 delays flowering by inactivating IDD8 transcription factor through protein

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phosphorylation in Arabidopsis. *BMC Plant Biol*. 2015:15(1):110. https://doi.org/10.1186/s12870-015-0503-8

Kant S, Peng M, Rothstein SJ. Genetic regulation by NLA and microRNA827 for maintaining nitrate-dependent phosphate homeostasis in Arabidopsis. *PLoS Genet*. 2011:7(3):e1002021. https://doi.org/10.1371/journal.pgen.1002021

Kaplan F, Kopka J, Sung DY, Zhao W, Popp M, Porat R, Guy CL. Transcript and metabolite profiling during cold acclimation of Arabidopsis reveals an intricate relationship of cold-regulated gene expression with modifications in metabolite content. *Plant J*. 2007:50(6):967–981. https://doi.org/10.1111/j.1365-313X.2007.03100.x

Klebs G. Über das Verhältnis der Außenwelt zur Entwicklung der Pflanze. *Sitz-Ber Akad Wiss Heidelberg Ser B*. 1913:5:3–47. https://doi.org/10.11588/diglit.37628

Konishi M, Yanagisawa S. Arabidopsis NIN-like transcription factors have a central role in nitrate signalling. *Nat Commun*. 2013:4(1):1617. https://doi.org/10.1038/ncomms2621

Konishi M, Yanagisawa S. The role of protein-protein interactions mediated by the PB1 domain of NLP transcription factors in nitrate-inducible gene expression. *BMC Plant Biol*. 2019:19(1):90. https://doi.org/10.1186/s12870-019-1692-3

Krapp A, Berthome R, Orsel M, Mercey-Boutet S, Yu A, Castaings L, Elftieh S, Major H, Renou JP, Daniel-Vedele F. Arabidopsis roots and shoots show distinct temporal adaptation patterns toward nitrogen starvation. *Plant Physiol*. 2011:157(3):1255–1282. https://doi.org/10.1104/pp.111.179838

Lee JH, Ryu HS, Chung KS, Pose D, Kim S, Schmid M, Ahn JH. Regulation of temperature-responsive flowering by MADS-box transcription factor repressors. *Science*. 2013:342(6158):628–632. https://doi.org/10.1126/science.1241097

Lee JH, Sook Chung K, Kim SK, Ahn JH. Post-translational regulation of SHORT VEGETATIVE PHASE as a major mechanism for thermoregulation of flowering. *Plant Signal Behav*. 2014:9(4):e28193. https://doi.org/10.4161/psb.28193

Lee JH, Yoo SJ, Park SH, Hwang I, Lee JS, Ahn JH. Role of SVP in the control of flowering time by ambient temperature in Arabidopsis. *Genes Dev*. 2007:21(4):397–402. https://doi.org/10.1101/gad.1518407

Li D, Liu C, Shen L, Wu Y, Chen H, Robertson M, Helliwell CA, Ito T, Meyerowitz E, Yu H. A repressor complex governs the integration of flowering signals in Arabidopsis. *Dev Cell*. 2008:15(1):110–120. https://doi.org/10.1016/j.devcel.2008.05.002

Li L, Liu KH, Sheen J. Dynamic nutrient signaling networks in plants. *Annu Rev Cell Dev Biol*. 2021:37(1):341–367. https://doi.org/10.1146/annurev-cellbio-010521-015047

Liang G, He H, Yu D. Identification of nitrogen starvation-responsive microRNAs in Arabidopsis thaliana. *PLoS One*. 2012:7(11):e48951. https://doi.org/10.1371/journal.pone.0048951

Lin YL, Tsay YF. Influence of differing nitrate and nitrogen availability on flowering control in Arabidopsis. *J Exp Bot*. 2017:68(10):2603–2609. https://doi.org/10.1093/jxb/erx053

Liu KH, Liu M, Lin Z, Wang ZF, Chen B, Liu C, Guo A, Konishi M, Yanagisawa S, Wagner G, *et al.* NIN-like protein 7 transcription factor is a plant nitrate sensor. *Science*. 2022:377(6613):1419–1425. https://doi.org/10.1126/science.add1104

Liu KH, Niu Y, Konishi M, Wu Y, Du H, Sun Chung H, Li L, Boudsocq M, McCormack M, Maekawa S, *et al.* Discovery of nitrate-CPK-NLP signalling in central nutrient-growth networks. *Nature*. 2017:545(7654):311–316. https://doi.org/10.1038/nature22077

Liu T, Li Y, Ren J, Qian Y, Yang X, Duan W, Hou X. Nitrate or NaCl regulates floral induction in Arabidopsis thaliana. *Biologia (Bratisl)*. 2013:68(2):215–222. https://doi.org/10.2478/s11756-013-0004-x

Marchive C, Roudier F, Castaings L, Brehaut V, Blondet E, Colot V, Meyer C, Krapp A. Nuclear retention of the transcription factor NLP7 orchestrates the early response to nitrate in plants. *Nat Commun*. 2013:4(1):1713. https://doi.org/10.1038/ncomms2650

Mathieu J, Warthmann N, Kuttner F, Schmid M. Export of FT protein from phloem companion cells is sufficient for floral induction in Arabidopsis. *Curr Biol*. 2007:17(12):1055–1060. https://doi.org/10.1016/j.cub.2007.05.009

Michaels SD, Amasino RM. FLOWERING LOCUS C encodes a novel MADS domain protein that acts as a repressor of flowering. *Plant Cell*. 1999:11(5):949–956. https://doi.org/10.1105/tpc.11.5.949

Noh YS, Amasino RM. PIE1, an ISWI family gene, is required for FLC activation and floral repression in Arabidopsis. *Plant Cell*. 2003:15(7):1671–1682. https://doi.org/10.1105/tpc.012161

Olas JJ, Apelt F, Watanabe M, Hoefgen R, Wahl V. Developmental stage-specific metabolite signatures in Arabidopsis thaliana under optimal and mild nitrogen limitation. *Plant Sci*. 2021:303:110746. https://doi.org/10.1016/j.plantsci.2020.110746

Olas JJ, Van Dingenen J, Abel C, Dzialo MA, Feil R, Krapp A, Schlereth A, Wahl V. Nitrate acts at the Arabidopsis thaliana shoot apical meristem to regulate flowering time. *New Phytol*. 2019:223(2):814–827. https://doi.org/10.1111/nph.15812

Pant BD, Musialak-Lange M, Nuc P, May P, Buhtz A, Kehr J, Walther D, Scheible WR. Identification of nutrient-responsive Arabidopsis and rapeseed microRNAs by comprehensive real-time polymerase chain reaction profiling and small RNA sequencing. *Plant Physiol*. 2009:150(3):1541–1555. https://doi.org/10.1104/pp.109.139139

Polge C, Thomas M. SNF1/AMPK/SnRK1 kinases, global regulators at the heart of energy control? *Trends Plant Sci*. 2007:12(1):20–28. https://doi.org/10.1016/j.tplants.2006.11.005

Ponnu J, Schlereth A, Zacharaki V, Dzialo MA, Abel C, Feil R, Schmid M, Wahl V. The trehalose 6-phosphate pathway impacts vegetative phase change in Arabidopsis thaliana. *Plant J*. 2020:104(3):768–780. https://doi.org/10.1111/tpj.14965

Pose D, Verhage L, Ott F, Yant L, Mathieu J, Angenent GC, Immink RG, Schmid M. Temperature-dependent regulation of flowering by antagonistic FLM variants. *Nature*. 2013:503(7476):414–417. https://doi.org/10.1038/nature12633

Romera-Branchat M, Andres F, Coupland G. Flowering responses to seasonal cues: what's new? *Curr Opin Plant Biol*. 2014:21:120–127. https://doi.org/10.1016/j.pbi.2014.07.006

Schmalenbach I, Zhang L, Ryngajllo M, Jimenez-Gomez JM. Functional analysis of the Landsberg erecta allele of FRIGIDA. *BMC Plant Biol*. 2014:14(1):218. https://doi.org/10.1186/s12870-014-0218-2

Schmid M, Uhlenhaut NH, Godard F, Demar M, Bressan R, Weigel D, Lohmann JU. Dissection of floral induction pathways using global expression analysis. *Development*. 2003:130(24):6001–6012. https://doi.org/10.1242/dev.00842

Searle I, He Y, Turck F, Vincent C, Fornara F, Krober S, Amasino RA, Coupland G. The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in Arabidopsis. *Genes Dev*. 2006:20(7):898–912. https://doi.org/10.1101/gad.373506

Sharma N, Geuten K, Giri BS, Varma A. The molecular mechanism of vernalization in Arabidopsis and cereals: role of Flowering Locus C and its homologs. *Physiol Plant*. 2020:170(3):373–383. https://doi.org/10.1111/ppl.13163

Sheldon CC, Conn AB, Dennis ES, Peacock WJ. Different regulatory regions are required for the vernalization-induced repression of FLOWERING LOCUS C and for the epigenetic maintenance of repression. *Plant Cell*. 2002:14(10):2527–2537. https://doi.org/10.1105/tpc.004564

Sheldon CC, Rouse DT, Finnegan EJ, Peacock WJ, Dennis ES. The molecular basis of vernalization: the central role of FLOWERING

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LOCUS C (FLC). *Proc Natl Acad Sci U S A*. 2000:97(7):3753–3758. https://doi.org/10.1073/pnas.97.7.3753

Song YH, Shim JS, Kinmonth-Schultz HA, Imaizumi T. Photoperiodic flowering: time measurement mechanisms in leaves. *Annu Rev Plant Biol*. 2015:66(1):441–464. https://doi.org/10.1146/annurev-arplant-043014-115555

Srikanth A, Schmid M. Regulation of flowering time: all roads lead to Rome. *Cell Mol Life Sci*. 2011:68(12):2013–2037. https://doi.org/10.1007/s00018-011-0673-y

Stitt M, Hurry V. A plant for all seasons: alterations in photosynthetic carbon metabolism during cold acclimation in Arabidopsis. *Curr Opin Plant Biol*. 2002:5(3):199–206. https://doi.org/10.1016/S1369-5266(02)00258-3

Sulpice R, Nikoloski Z, Tschoep H, Antonio C, Kleessen S, Larhlimi A, Selbig J, Ishihara H, Gibon Y, Fernie AR, *et al.* Impact of the carbon and nitrogen supply on relationships and connectivity between metabolism and biomass in a broad panel of Arabidopsis accessions. *Plant Physiol*. 2013:162(1):347–363. https://doi.org/10.1104/pp.112.210104

Sureshkumar S, Dent C, Seleznev A, Tasset C, Balasubramanian S. Nonsense-mediated mRNA decay modulates FLM-dependent thermosensory flowering response in Arabidopsis. *Nat Plants*. 2016:2(5):16055. https://doi.org/10.1038/nplants.2016.55

Teng Y, Liang Y, Wang M, Mai H, Ke L. Nitrate transporter 1.1 is involved in regulating flowering time via transcriptional regulation of FLOWERING LOCUS C in Arabidopsis thaliana. *Plant Sci*. 2019:284:30–36. https://doi.org/10.1016/j.plantsci.2019.04.002

Tschoep H, Gibon Y, Carillo P, Armengaud P, Szecowka M, Nunes-Nesi A, Fernie AR, Koehl K, Stitt M. Adjustment of growth and central metabolism to a mild but sustained nitrogen-limitation in Arabidopsis. *Plant Cell Environ*. 2009:32(3):300–318. https://doi.org/10.1111/j.1365-3040.2008.01921.x

Vandesteene L, Ramon M, Le Roy K, Van Dijck P, Rolland F. A single active trehalose-6-P synthase (TPS) and a family of putative regulatory TPS-like proteins in Arabidopsis. *Mol Plant*. 2010:3(2):406–419. https://doi.org/10.1093/mp/ssp114

van Dijken AJ, Schluepmann H, Smeekens SC. Arabidopsis trehalose-6-phosphate synthase 1 is essential for normal vegetative growth and transition to flowering. *Plant Physiol*. 2004:135(2):969–977. https://doi.org/10.1104/pp.104.039743

Vidal EA, Alvarez JM, Moyano TC, Gutierrez RA. Transcriptional networks in the nitrate response of Arabidopsis thaliana. *Curr Opin Plant Biol*. 2015:27:125–132. https://doi.org/10.1016/j.pbi.2015.06.010

Wahl V, Ponnu J, Schlereth A, Arrivault S, Langenecker T, Franke A, Feil R, Lunn JE, Stitt M, Schmid M. Regulation of flowering by trehalose-6-phosphate signaling in Arabidopsis thaliana. *Science*. 2013:339(6120):704–707. https://doi.org/10.1126/science.1230406

Whittaker C, Dean C. The FLC locus: a platform for discoveries in epigenetics and adaptation. *Annu Rev Cell Dev Biol*. 2017:33(1):555–575. https://doi.org/10.1146/annurev-cellbio-100616-060546

Wigge PA, Kim MC, Jaeger KE, Busch W, Schmid M, Lohmann JU, Weigel D. Integration of spatial and temporal information during floral induction in Arabidopsis. *Science*. 2005:309(5737):1056–1059. https://doi.org/10.1126/science.1114358

Xie Y, Zhou Q, Zhao Y, Li Q, Liu Y, Ma M, Wang B, Shen R, Zheng Z, Wang H. FHY3 and FAR1 integrate light signals with the miR156-SPL module-mediated aging pathway to regulate Arabidopsis flowering. *Mol Plant*. 2020:13(3):483–498. https://doi.org/10.1016/j.molp.2020.01.013

Xu C, Fang X, Lu T, Dean C. Antagonistic cotranscriptional regulation through ARGONAUTE1 and the THO/TREX complex orchestrates FLC transcriptional output. *Proc Natl Acad Sci U S A*. 2021:118:e2113757118. https://doi.org/10.1073/pnas.2113757118

Xu C, Wu Z, Duan HC, Fang X, Jia G, Dean C. R-loop resolution promotes co-transcriptional chromatin silencing. *Nat Commun*. 2021:12(1):1790. https://doi.org/10.1038/s41467-021-22083-6

Yang HL, Liu YJ, Wang CL, Zeng QY. Molecular evolution of trehalose-6-phosphate synthase (TPS) gene family in Populus, Arabidopsis and rice. *PLoS One*. 2012:7(8):e42438. https://doi.org/10.1371/journal.pone.0042438

Yang M, Zhu P, Cheema J, Bloomer R, Mikulski P, Liu Q, Zhang Y, Dean C, Ding Y. In vivo single-molecule analysis reveals COOLAIR RNA structural diversity. *Nature*. 2022:609(7926):394–399. https://doi.org/10.1038/s41586-022-05135-9

Zacharaki V, Ponnu J, Crepin N, Langenecker T, Hagmann J, Skorzinski N, Musialak-Lange M, Wahl V, Rolland F, Schmid M. Impaired KIN10 function restores developmental defects in the Arabidopsis trehalose 6-phosphate synthase1 (tps1) mutant. *New Phytol*. 2022:235(1):220–233. https://doi.org/10.1111/nph.18104

Zeng L, Zacharaki V, Wang Y, Schmid M (2024) Mutations in HUA2 restore flowering in the Arabidopsis trehalose 6-phosphate synthase1 (tps1) mutant. bioRxiv 578264. https://doi.org/10.1101/2024.01.31.578264, 2 February 2024, preprint: not peer reviewed.

Zhai Z, Keereetaweep J, Liu H, Feil R, Lunn JE, Shanklin J. Trehalose 6-phosphate positively regulates fatty acid synthesis by stabilizing WRINKLED1. *Plant Cell*. 2018:30(10):2616–2627. https://doi.org/10.1105/tpc.18.00521

Zhang Y, Primavesi LF, Jhurreea D, Andralojc PJ, Mitchell RA, Powers SJ, Schluepmann H, Delatte T, Wingler A, Paul MJ. Inhibition of SNF1-related protein kinase1 activity and regulation of metabolic pathways by trehalose-6-phosphate. *Plant Physiol*. 2009:149(4):1860–1871. https://doi.org/10.1104/pp.108.133934

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