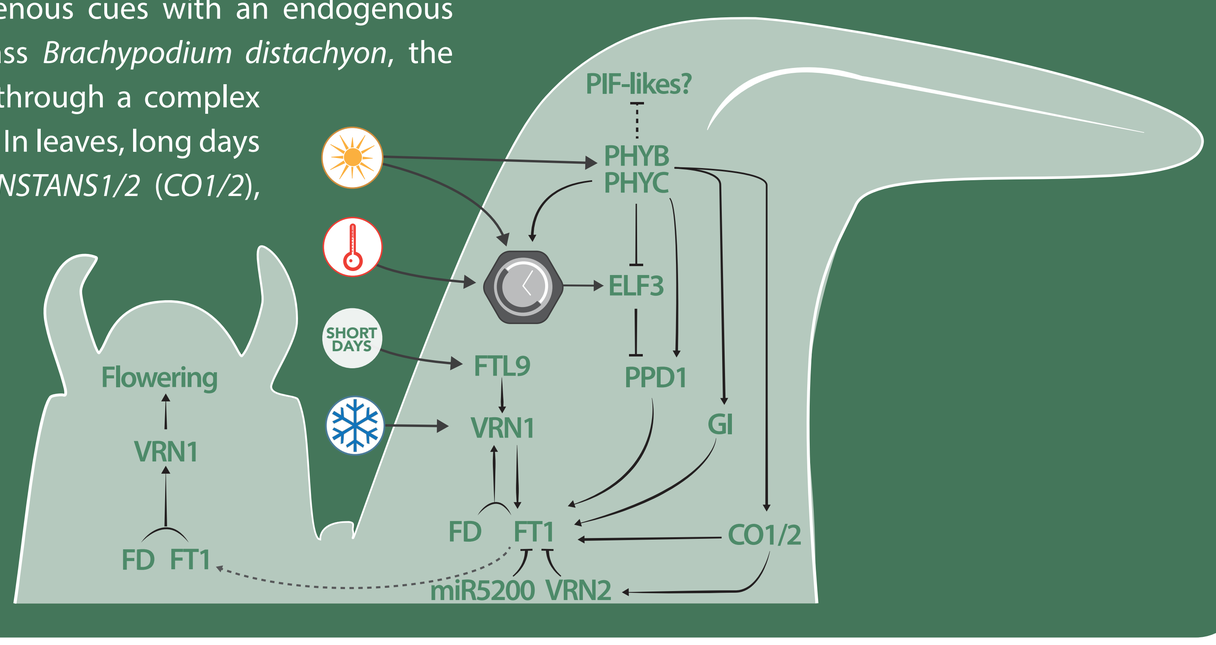
Exploring the mechanisms of photoperiod sensing in *Brachypodium distachyon*

Frédéric Bouché [1*], Daniel P. Woods[2,3], Julie Linden[1], Weiya Li[3], Kevin S. Mayer[3], Richard M. Amasino[3] and Claire Périlleux [1].

1 | Laboratory of Plant Physiology, InBioS-PhytoSYSTEMS, Department of Life Sciences, University of Liège, Liège, Belgium. 2 | Plant Sciences Department, University of California, Davis, CA, United States. 3 | Laboratory of Genetics, University of Wisconsin, Madison, WI, United States

OVERVIEW OF THE FLOWERING TIME CONTROL IN *BRACHYPODIUM DISTACHYON*

In crops, the proper timing of flowering, which relies on the coordination of exogenous cues with an endogenous developmental program, is crucial to maximize yields. In the model temperate grass *Brachypodium distachyon*, the perception of the increasing day lengths of the spring is key to promote flowering through a complex interplay between the photoperiodic pathway and circadian clock-controlled processes. In leaves, long days positively regulate the expression of *PHOTOPERIOD1* (*PPD1*), *GIGANTEA* (*GI*), and *CONSTANS1/2* (*CO1/2*), which are possible transcriptional activators of the florigen-encoding gene *FLOWERING LOCUS T1* (*FT1*) through yet unresolved pathways. When the levels of *FT1* overcome a threshold set by the repressing effects of VERNALIZATION2 and miR5200, a positive feedback loop with *VERNALIZATION1* is triggered in order to lock in floral induction. The expression of *VRN1* can also be directly activated by prolonged cold exposure and indirectly—through *FTL9*—by exposure to short days, ensuring that vernalization-requiring accessions are exposed to winter prior flowering. As observed across many plant species, the leaf-produced florigen migrates to the shoot apical meristem where it triggers the transition to reproductive development.



1 FORWARD GENETIC SCREEN FOR EARLY FLOWERING PLANTS

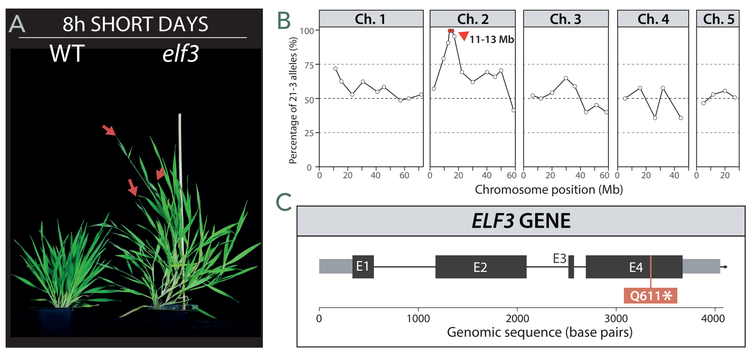


Figure 1. Identification of the mutation causing an early flowering phenotype under short days.
A. Phenotype of the *elf3* mutant and wild-type (WT) *Brachypodium distachyon* plants under 8 h SD (80 days). The red arrows indicate early emerging spikes. **B.** InDel mapping of the segregation of the early flowering phenotype in an M2 mapping population (Bd21-3 and Bd3-1). **C.** Schematic representation of the *ELF3* gene (E1 to E4 indicate exons), including the identified mutation.

In order to identify new flowering time genes in *Brachypodium distachyon*, we screened a randomly mutated Bd21-3 population for early flowering mutants under 8 h short days. We identified a mutant that displayed an early flowering phenotype (Fig. 1A). Using an M2 mapping population, we could locate the mutation on a 2 Mb interval of chromosome 2 (Fig. 1B), which encompasses a gene known as an important flowering time regulator across many species: *EARLY FLOWERING 3* (*ELF3*). The sequencing of the *ELF3* gene revealed a nonsense mutation leading to the apparition of a premature STOP codon in the fourth exon (Fig. 1C).

IDENTIFICATION OF A MUTANT ALLELE OF THE *EARLY FLOWERING 3* GENE

2 PHENOTYPIC CHARACTERIZATION OF THE MUTANT

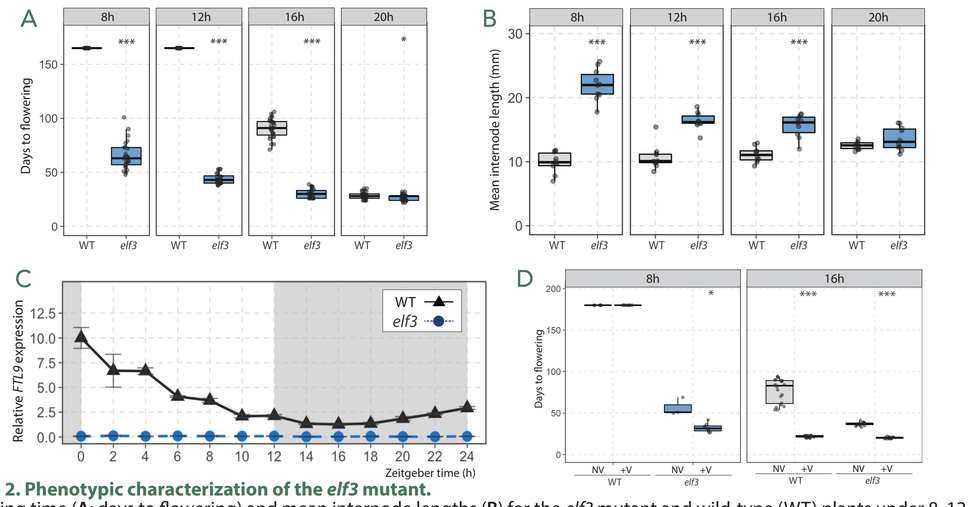


Figure 2. Phenotypic characterization of the *elf3* mutant.
Flowering time (**A**; days to flowering) and mean internode lengths (**B**) for the *elf3* mutant and wild-type (WT) plants under 8, 12, 16, and 20 h photoperiods (n = 10–25). **C.** Relative *FTL9* expression kinetics in WT (black triangles) and *elf3* (blue circles) plants under a 12 h photoperiod. Data were normalized using the geometric mean of *ACT3* and *UBC18* reference genes. Error bars represent the standard error of the mean. **D.** Flowering time (days to flowering) of *elf3* mutant and WT plants exposed (V) or not (NV) to a 3-week vernalizing treatment and subsequently grown under 8 or 16 h photoperiods. Student t-tests were used for pairwise comparisons (*** p <0.001; * p <0.05).

To further understand the roles played by *ELF3*, we characterized the mutant backcrossed twice under 8h, 12h, 16h, and 20h photoperiods. We found that the early flowering phenotype of *elf3* is conserved among most tested photoperiods (Fig. 2A). Under shorter photoperiods, the early flowering is accompanied by an increase in internode lengths compared to WT (Fig. 2B), a phenotype normally associated with long day exposure. We could corroborate these phenotypes with a constitutive repression of the *FTL9* gene—whose expression is a marker of short days—in the *elf3* mutant under shorter photoperiods (Fig. 2C). As expected, the *elf3* mutant was still sensitive to vernalization (Fig. 2D), suggesting that the contribution of ELF3 to the control of flowering acts mainly through the photoperiodic pathway.

THE MUTATION CAUSES A CONSTITUTIVE LONG DAY PHENOTYPE

3 QUANTIFICATION OF FLOWERING & CIRCADIAN CLOCK GENES

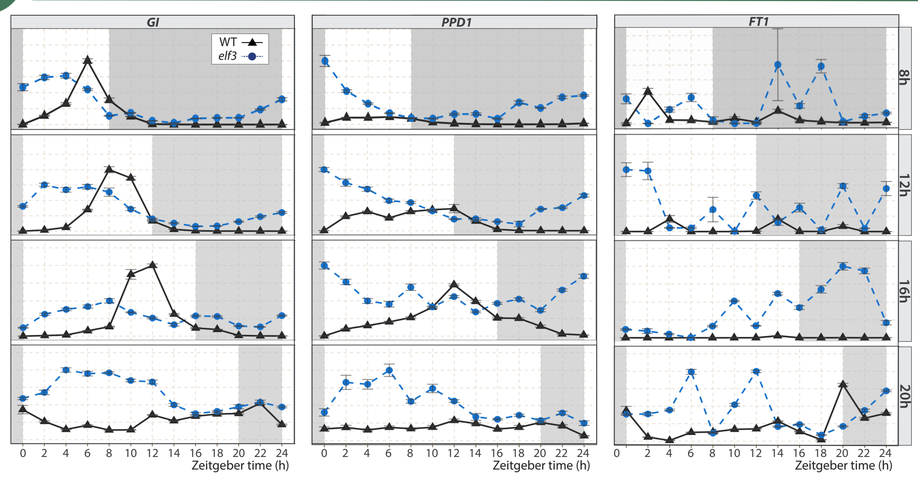


Figure 3. Expression kinetics of selected genes in the *elf3* mutant.
Relative expression of *GI* (left), *PPD1* (center), and *FT1* (right) in leaves during a 24 h time course for WT plants (black triangles) and *elf3* mutants (blue circle) cultivated under 8, 12, 16, and 20 h photoperiods. Error bars represent the standard error of the mean. Data were normalized using the geometric mean of *ACT3* and *UBC18*.

We then examined the time-course expression of several candidate genes involved in the photoperiodic induction of flowering, among which the floral inducers *GI*, *PPD1*, and *FT1* (Fig. 3). The expression of *GI*, which normally peaks late in the afternoon, was also detected in the morning and during the night in the mutant. The expression of *PPD1* and *FT1* were increased at most time points in *elf3*, which is consistent with its early flowering phenotype.

THE MUTATION OF *ELF3* LEADS TO THE DEREGULATION OF THE EXPRESSION OF BOTH CIRCADIAN CLOCK AND FLOWERING TIME GENES

4 NIGHT BREAKS AND FLOWERING INDUCTION

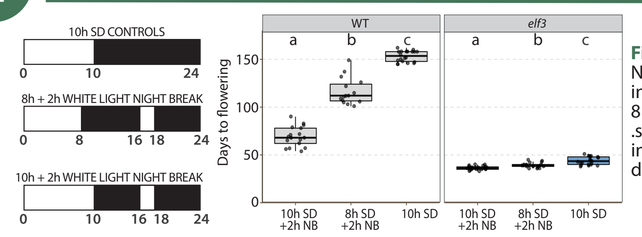


Figure 4. Effect of night breaks on flowering time.
Night break (NB) experiments performed by exposing wild-type (WT) and *elf3* plants growing in either 8 or 10 h photoperiods to a 2 h NB (NB; 150 µmol.m^{-2}.s^{-1}) from ZT16 h to ZT18h. Controls were maintained in 10 h SDs (n = 15–20). Letters indicate statistical difference (p <0.05) using Tukey's HSD tests.

To test whether the flowering time of the *elf3* mutant could be further accelerated by night breaks (NB), we exposed short day grown plants to 2 h of light provided between Zt16 and Zt18 (Fig. 4). We found that these treatments were sufficient to significantly accelerate flowering in WT plants. The flowering time of *elf3* mutants was only slightly accelerated, indicating that the *elf3* mutation attenuates the photoperiodic response to night breaks.

NIGHT BREAKS INDUCE FLOWERING IN *BRACHYPODIUM DISTACHYON*

5 EFFECT OF LIGHT QUALITY ON FLOWERING INDUCTION

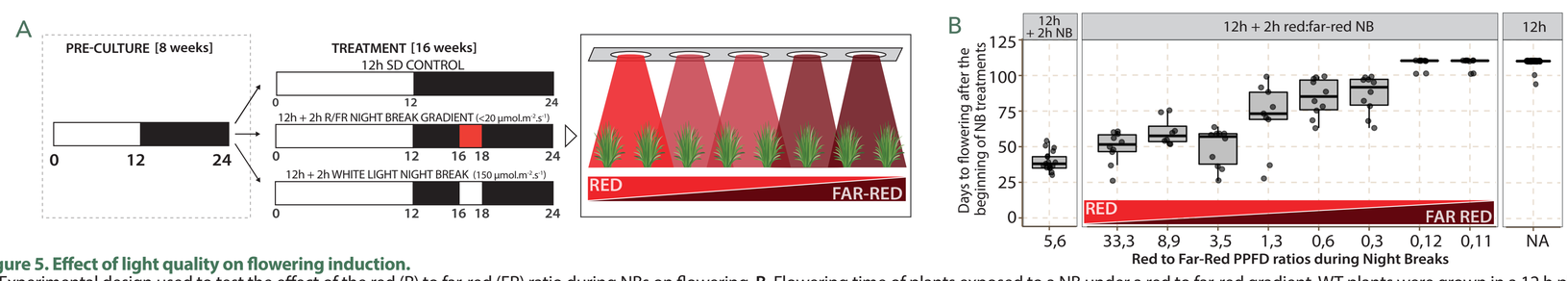


Figure 5. Effect of light quality on flowering induction.
A. Experimental design used to test the effect of the red (R) to far-red (FR) ratio during NBs on flowering. **B.** Flowering time of plants exposed to a NB under a red to far-red gradient. WT plants were grown in a 12 h photoperiod for 8 weeks before being transferred to a 12 h photoperiod supplemented with a 2 h NB (from ZT16h to ZT18h) under a red to far-red gradient (n = 8–15 per condition). Control plants were either maintained under a 12 h photoperiod (right panel) or exposed to a 2 h white light NB (left panel). **C.** Effect of far-red (FR) light on flowering time. WT and *elf3* plants grown under an 18 h photoperiod were exposed to 1 h of FR light (25 µmol.m^{-2}.s^{-1}). Control plants were exposed to an uninterrupted night (n = 15–20); t-tests revealed no statistically significant differences (ns).

To explore the effect of light quality on the florigenic effect of night breaks, we exposed 12 h short-day grown plants to 2 h night breaks provided as a mixture of varying red to far-red ratios (Fig. 5A) at low light intensities. We observed a strong correlation between higher flowering induction and higher red to far-red ratios (Figure 5B), indicating a robust effect of light quality on flowering time. However, far-red treatments provided at the end of 18 h long days did not affect flowering time (Fig 5C), suggesting that the flowering cascade triggered by longer photoperiods is initiated earlier during the day.

CONCLUDING REMARKS

Expanding the collection of flowering time mutants in *Brachypodium distachyon* through forward genetic screens is key to better understand the complex mechanisms controlling flowering in temperate grasses. In association with the modularity of new LED technologies, these mutants will provide essential tools to explore the mechanisms through which photoperiod and environmental conditions fine-tune the timing of flowering.

fbouche@uliege.be

WANT TO READ THE FULL STORY ?

Bouché et al., 2022
doi: 10.3389/fpls.2021.769194

WANT TO CONNECT ON TWITTER?