

Utilization of a Sequenced EMS-Mutagenized Population to Characterize the Photoperiodic Response in Wheat

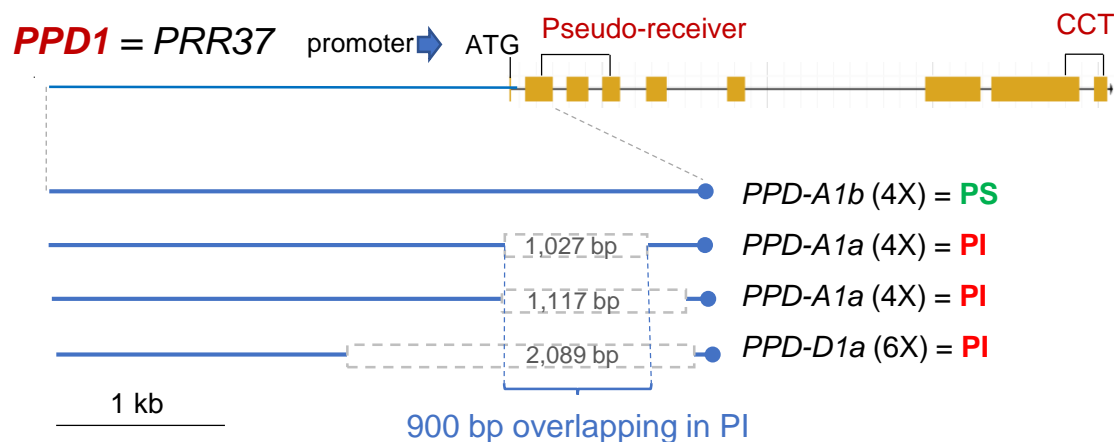


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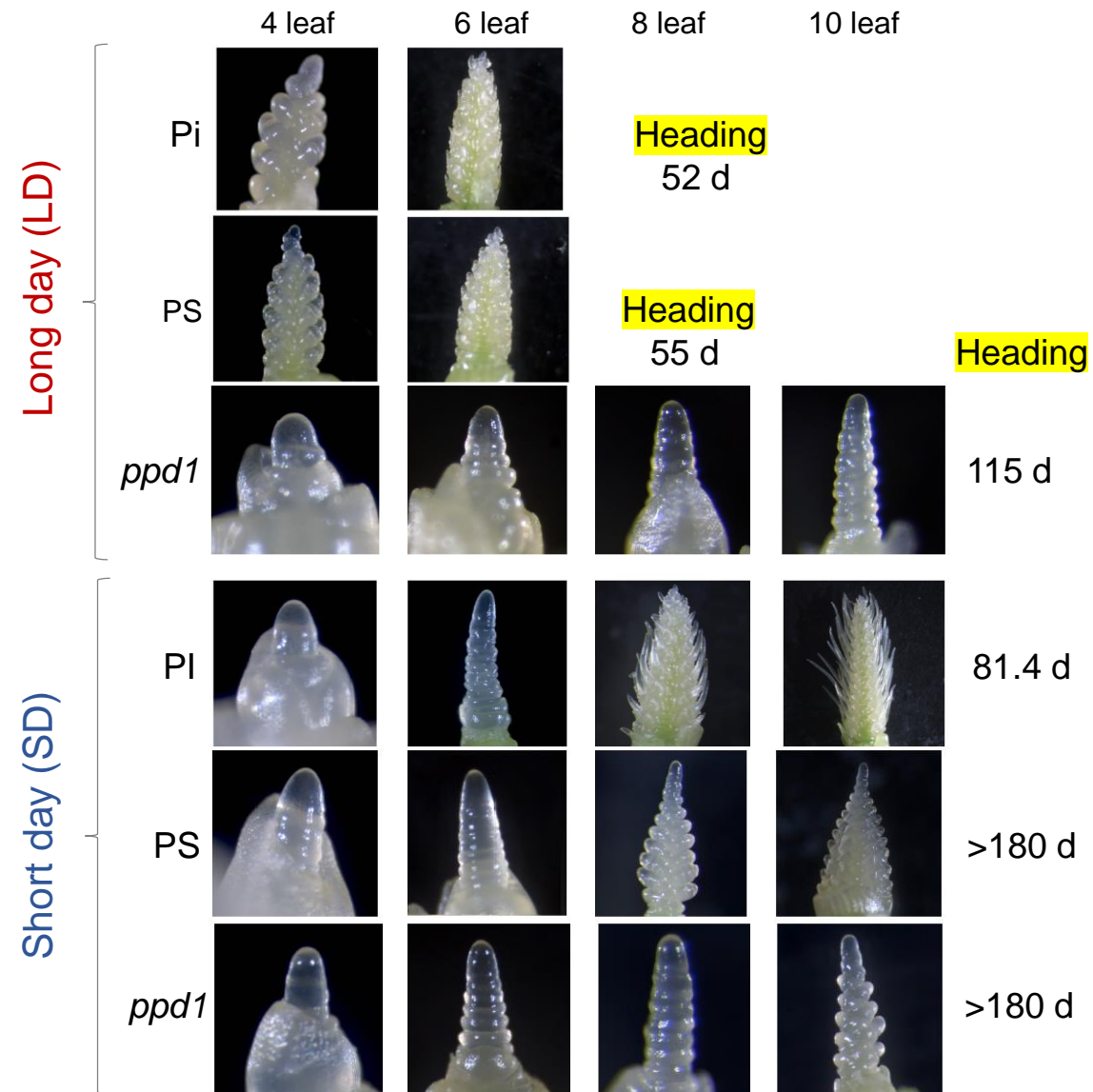
Photoperiod sensitive and insensitive wheats

Photoperiod sensitive (PS): Ancestral state.
Under SD it transitions at ~8 leaves
but heads very late (>180 d)

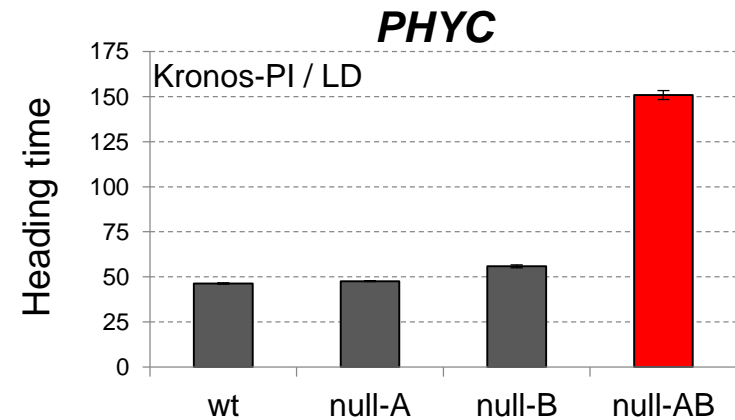
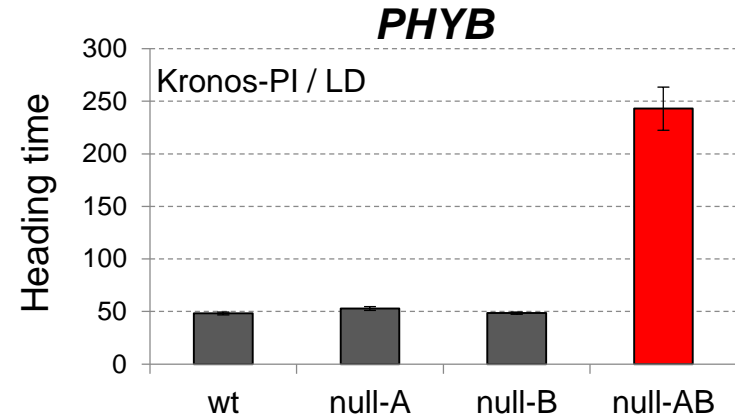
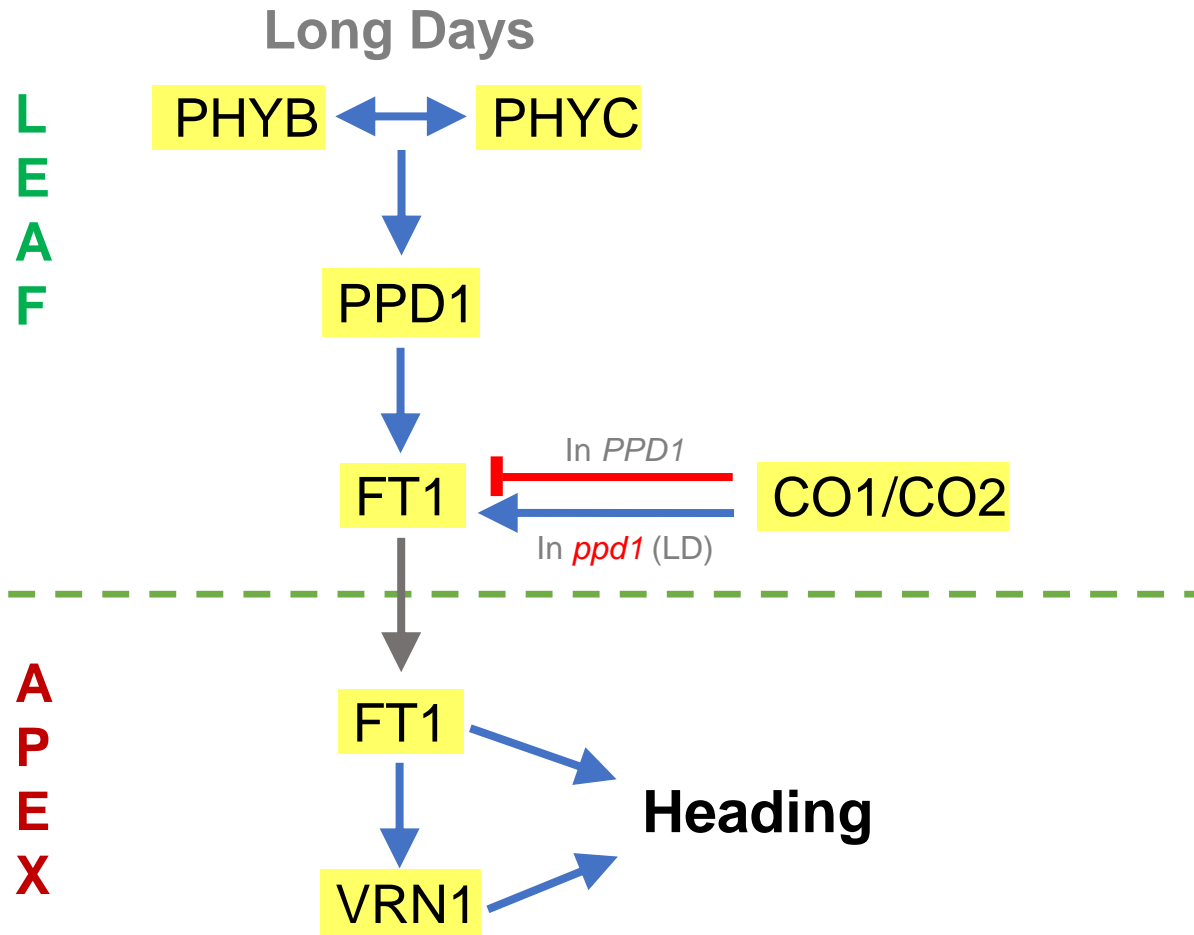
Photoperiod 'insensitive' (PI): Accelerated
heading in SD (still earlier in LD).
Deletions in *PPD1* promoter.



Turner et al. *Science* (2005) 310:1031
Wilhelm et al. *TAG* (2009) 118:2854

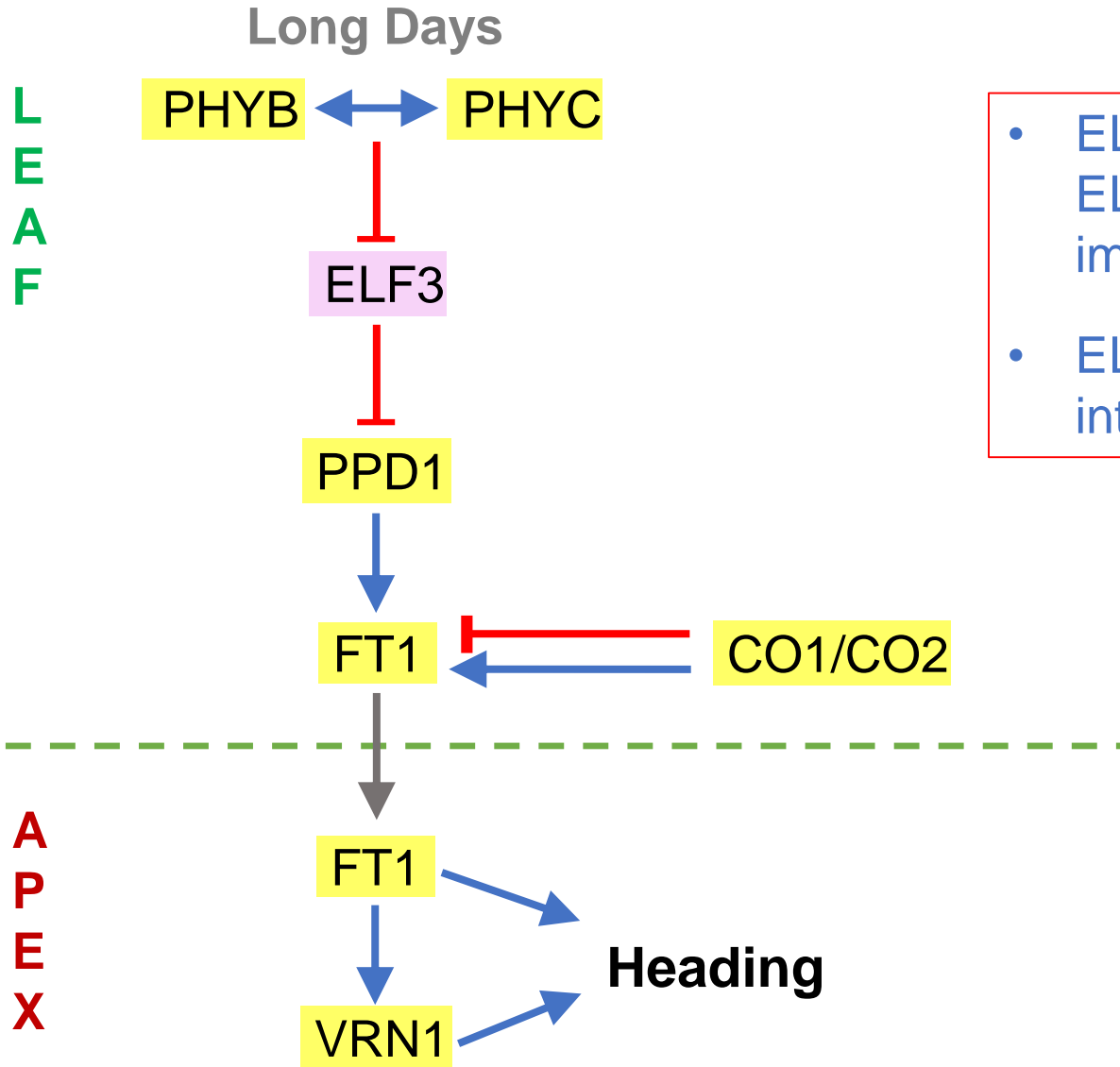


The wheat photoperiod pathway: previous knowledge



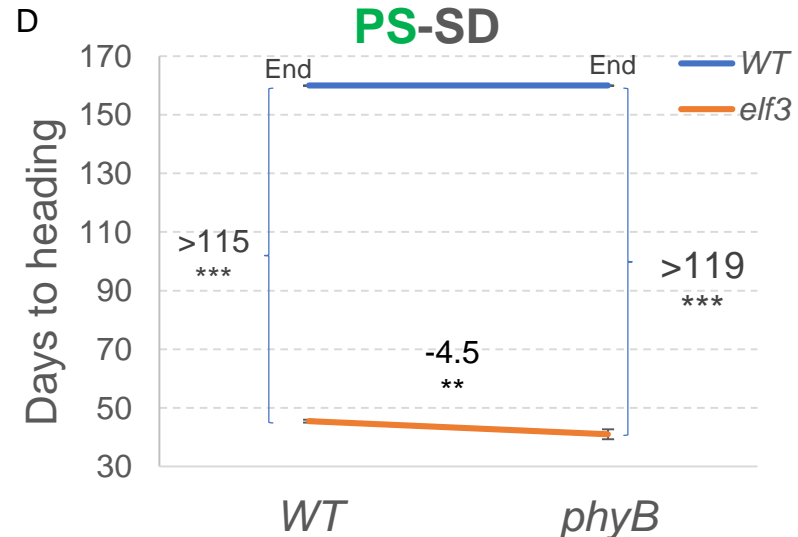
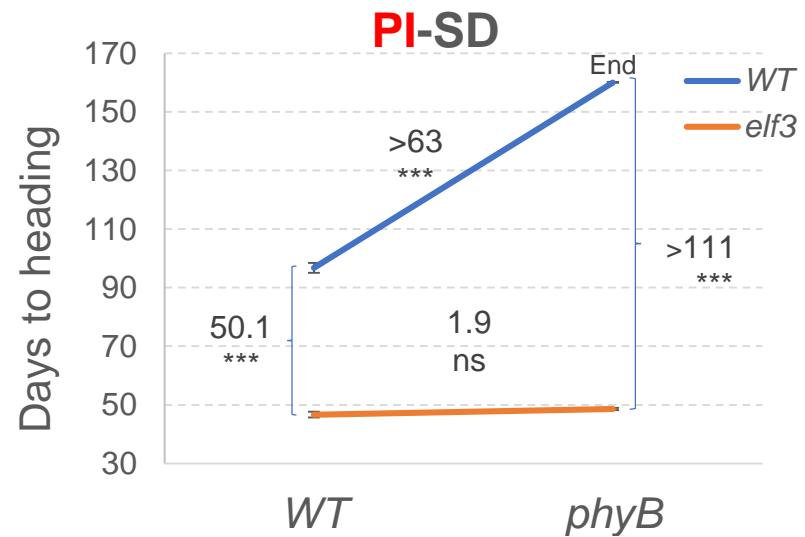
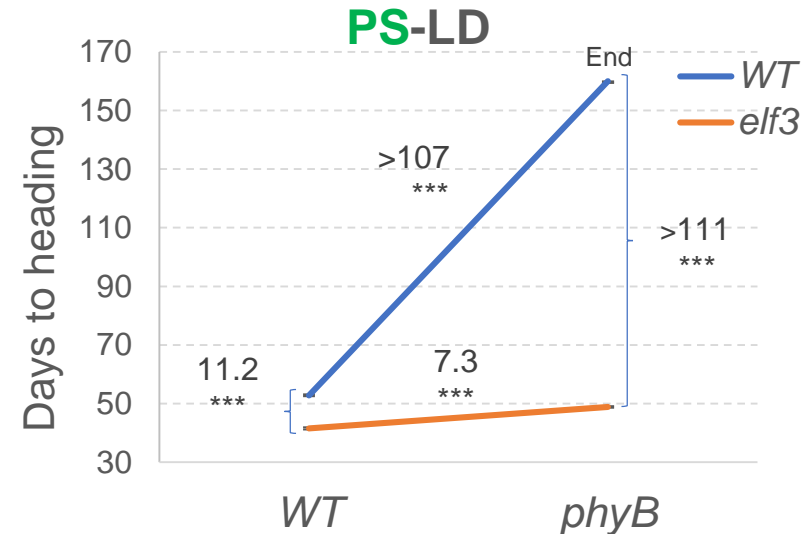
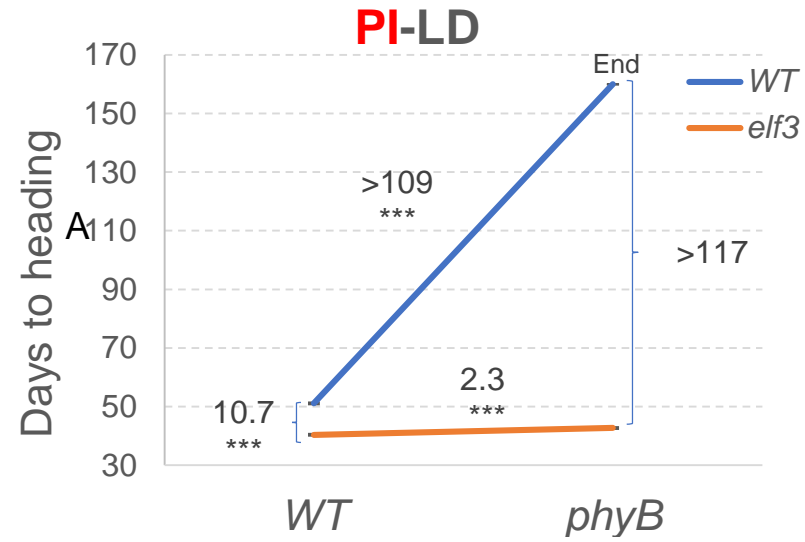
Loss-of-function mutations in either *PHYB* or *PHYC* result in late heading

The light activation of *PPD1* by *PhyB* is mediated by *ELF3*



- ELF3 functions as an adaptor between LUX and ELF4 to form the Evening Complex (EC), an important component of the circadian clock
- ELF3 also acts as a hub for multiple protein interactions

The large delay in heading time in *phyB* mostly disappears in *elf3 phyB*

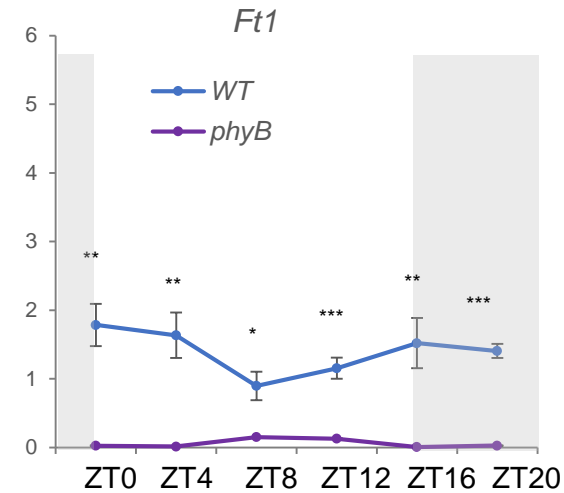
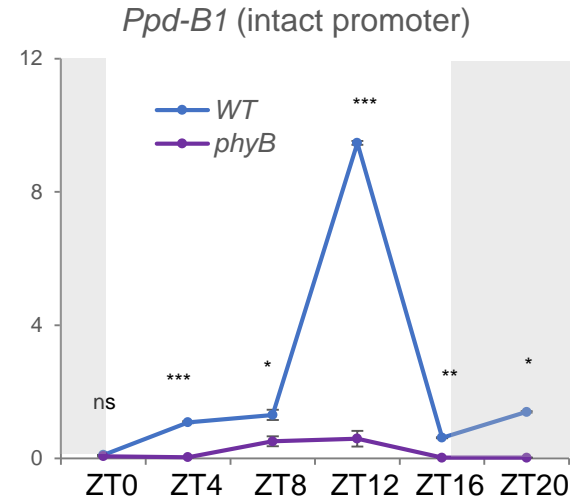
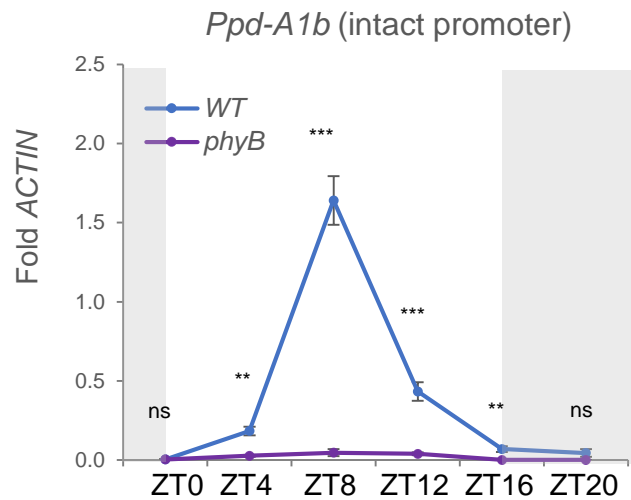


LD: Long day
SD: short day

PS= photoperiod sensitive

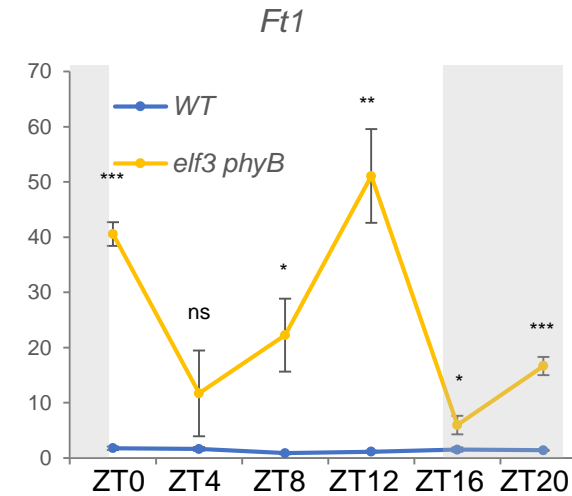
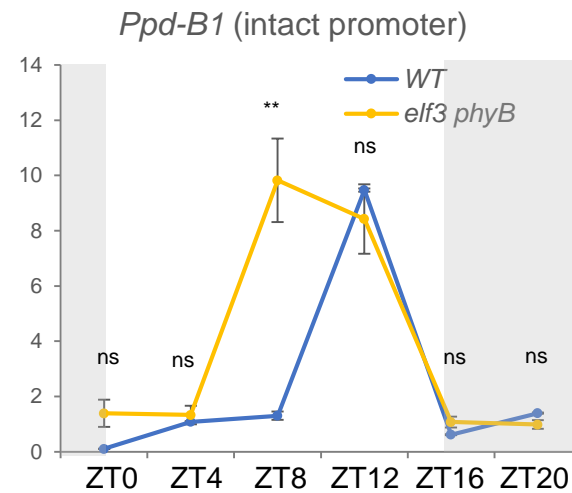
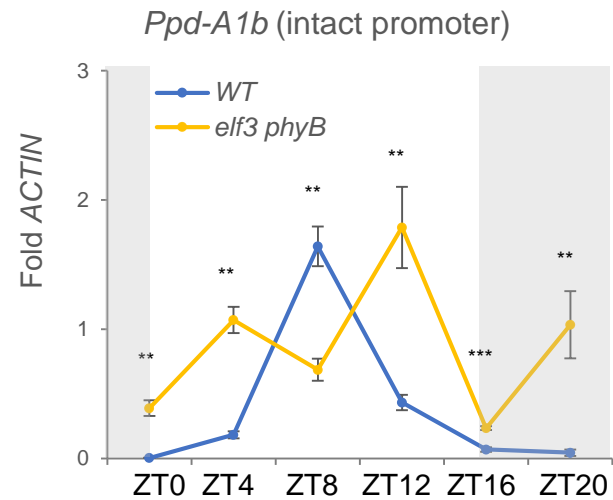
PI= reduced sensitivity (promoter deletion)

Effect of *phyB* and *elf3* mutations on *PPD1* and *FT1* expression under LD



In the *phyB* mutant, both *PPD1* & *FT1* are downregulated.

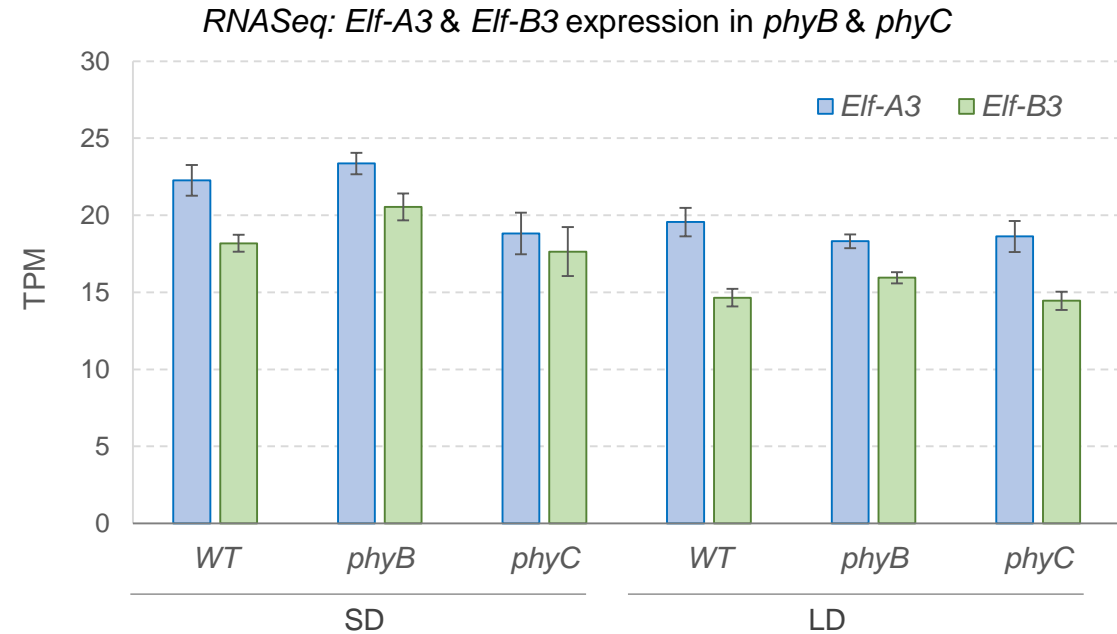
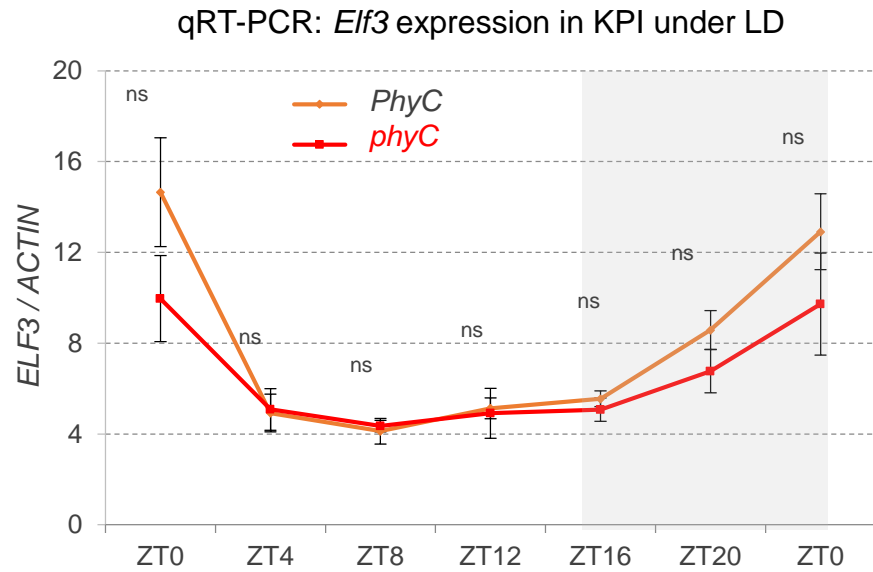
This correlates with the late heading time.



In the *phyB elf3* combined mutant, *PPD1* & *FT1* transcription is restored

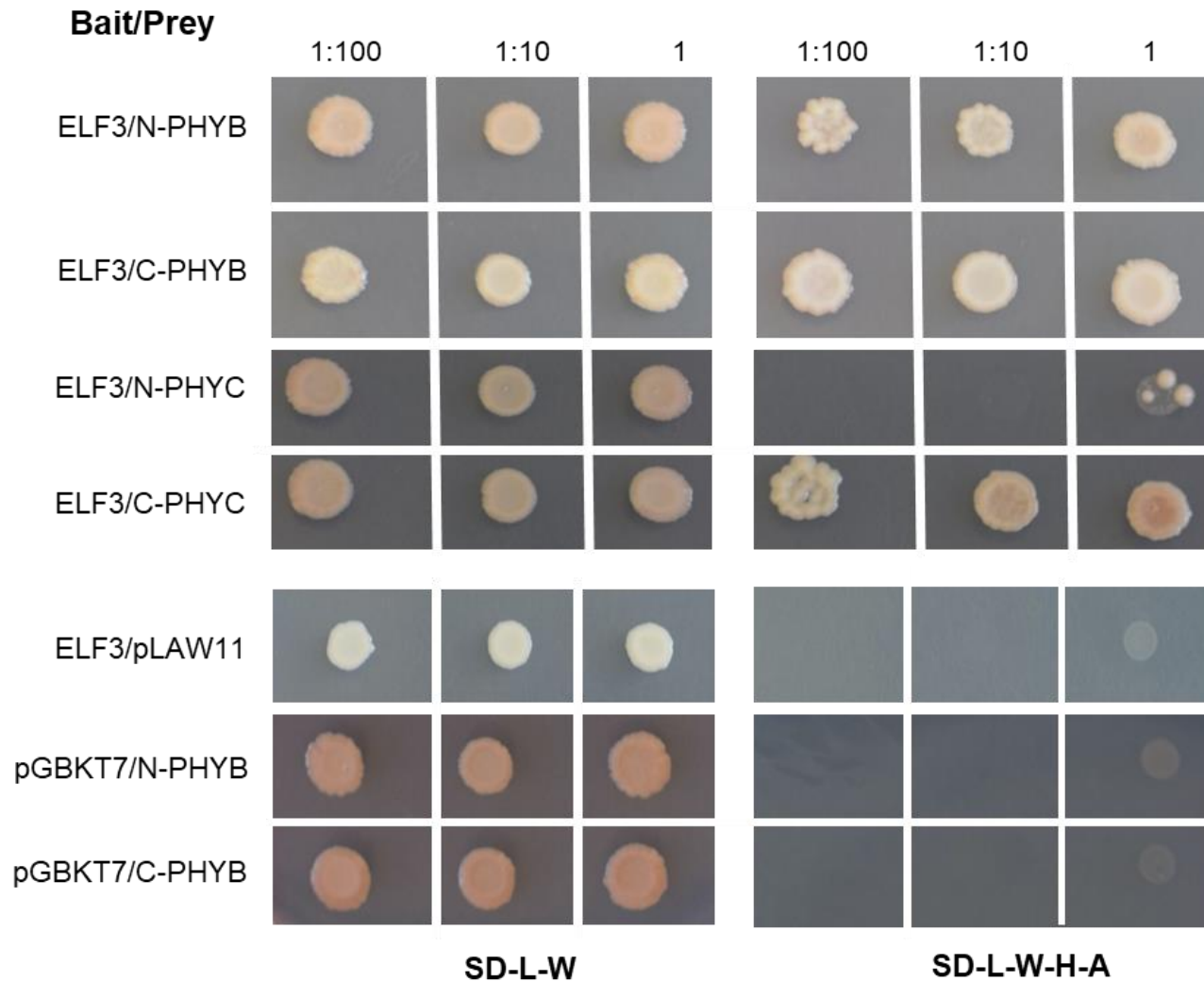
This correlates with the earlier heading time

phyB and *phyC* mutants do not affect the expression levels of *ELF3*



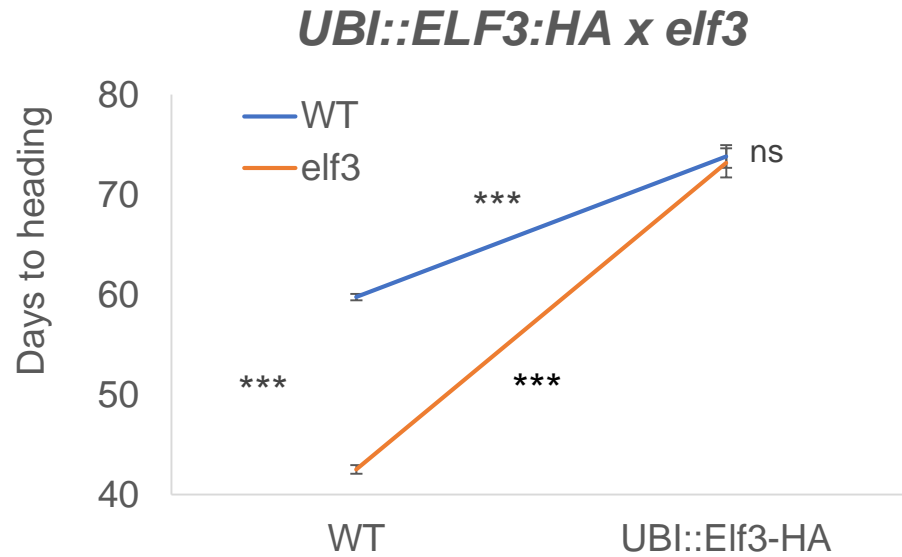
None of the differences between WT and *phyB* or WT and *phyC* were significant in LD and SD

ELF3 protein interacts with PHYB and PHYC in yeast-two-hybrid assays

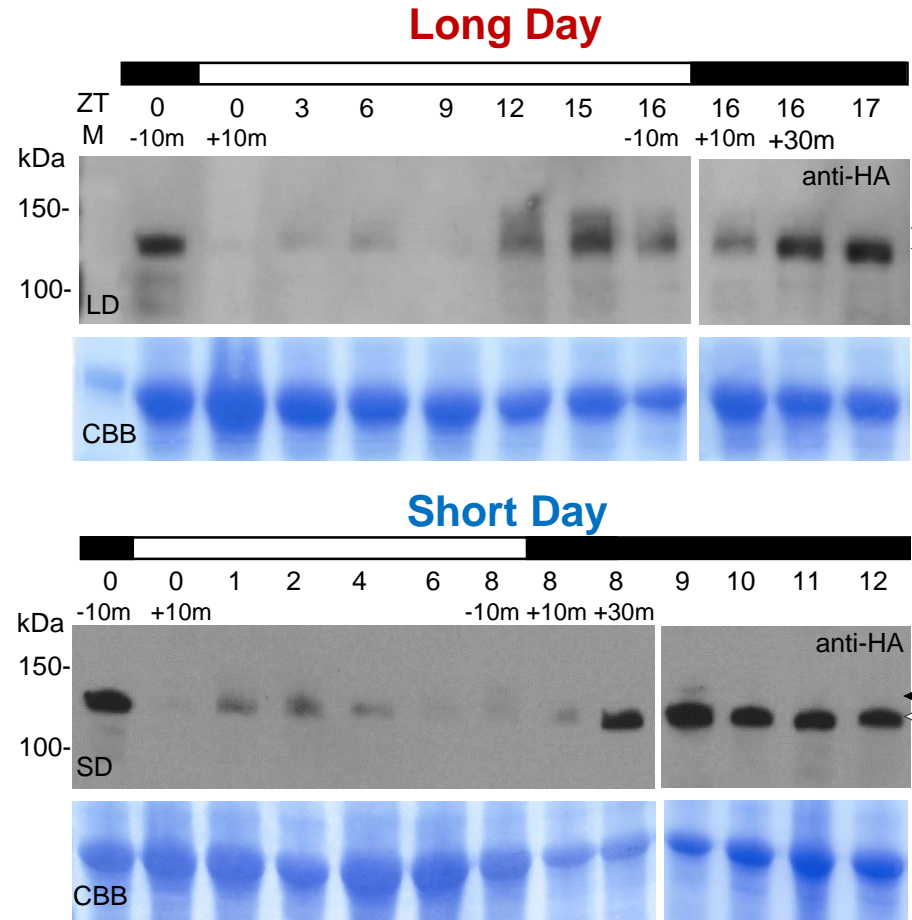


- ELF3 showed a strong interaction with the C-terminal portion of PHYB and PHYC.
- The interaction with the N-terminal part was strong in N-PHYB but weak in N-PHYC.
- Autoactivation tests showed no interaction with empty vectors for ELF3 and PHYB clones.
- We hypothesize that the protein interactions between phytochromes and ELF3 play an important role in the regulation of ELF3 activity.

UBI::ELF3:HA protein is modified by light

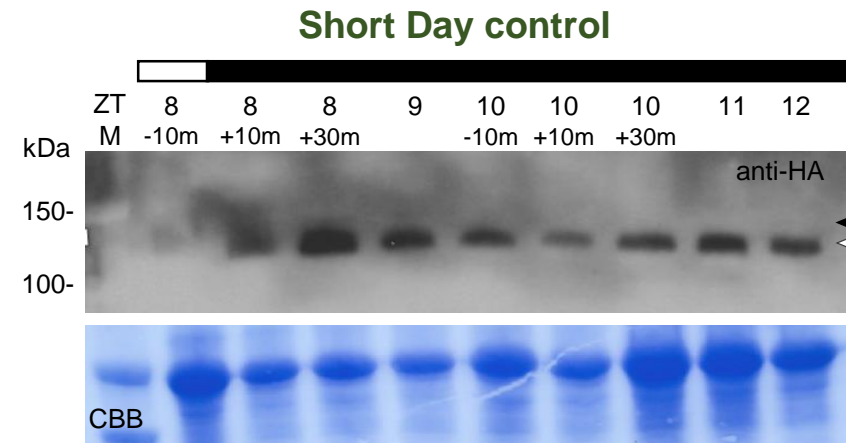
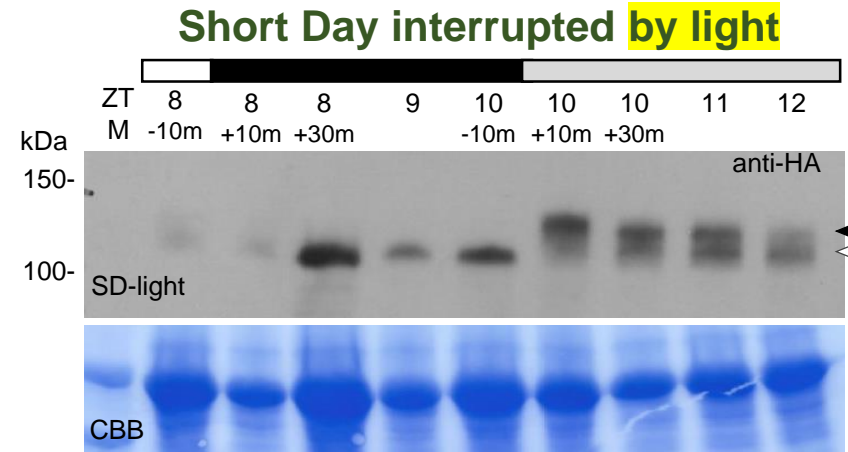
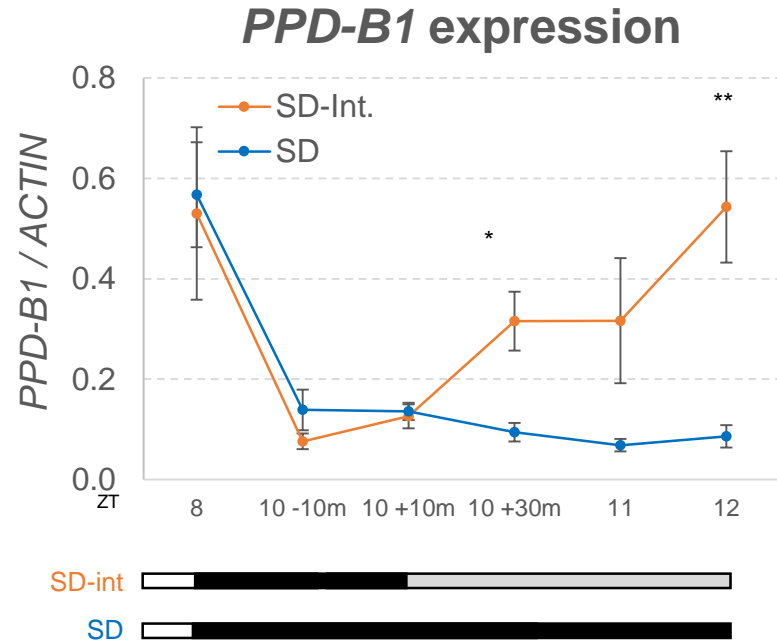


**UBI::ELF3:HA transgene
complements early heading of *elf3***



- ELF3 is degraded 10 m after lights are on in ZT0.
- A lower ELF3 band accumulates after lights are off.

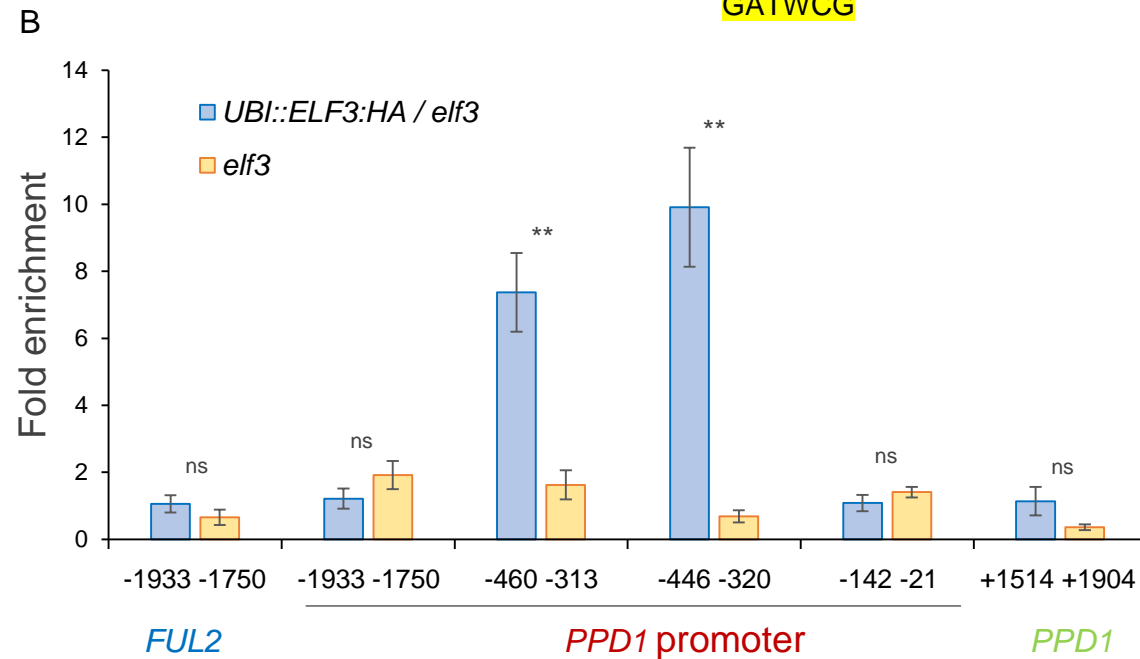
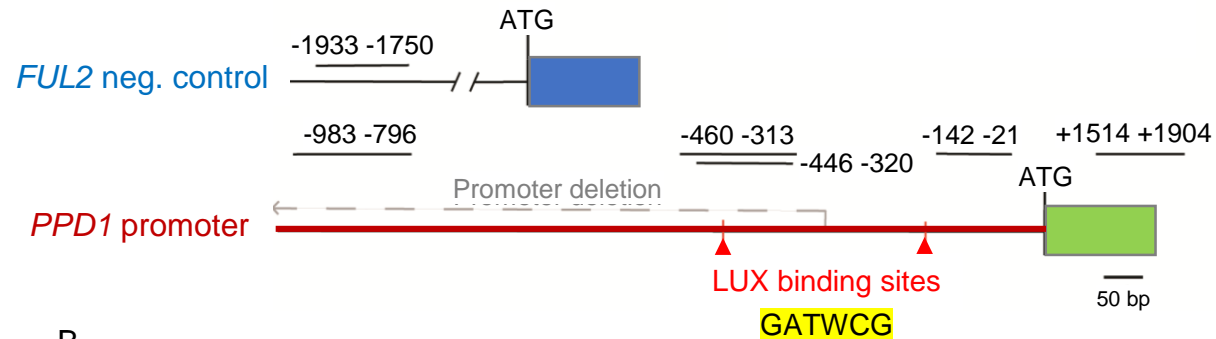
UBI::ELF3:HA protein is modified by light



- ELF3 lower band is modified 10 min after lights are turned on (ZT10).
- Then, even under the light, the low band forms again and the upper bands decreases.

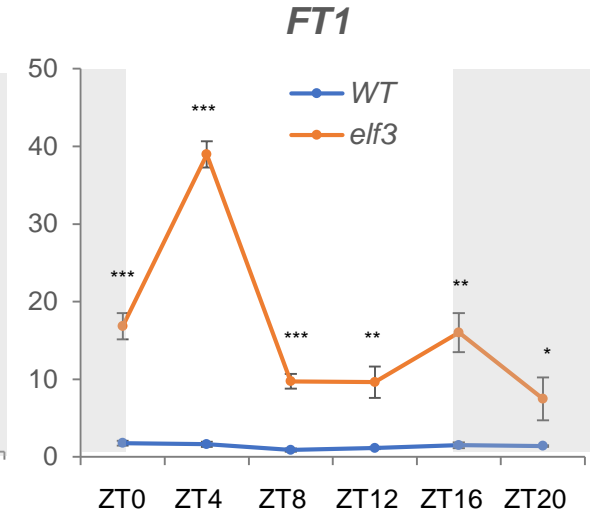
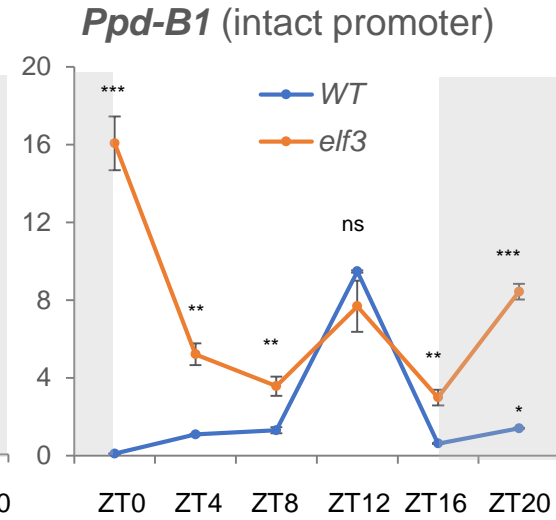
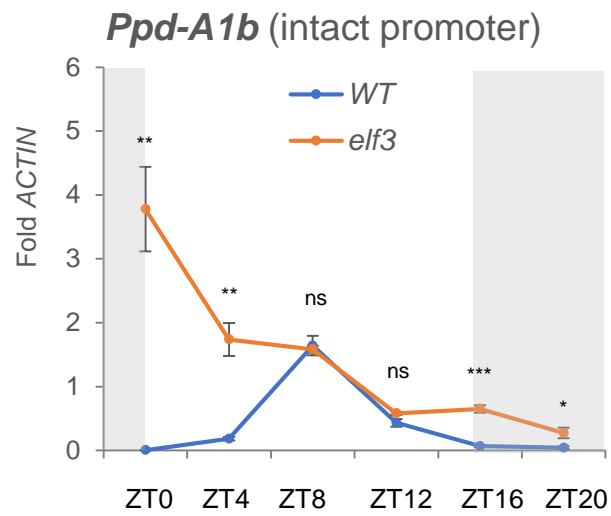
ELF3 directly represses *PPD1*

ChIP-PCR experiment using UBI::ELF3:HA/*elf3* plants

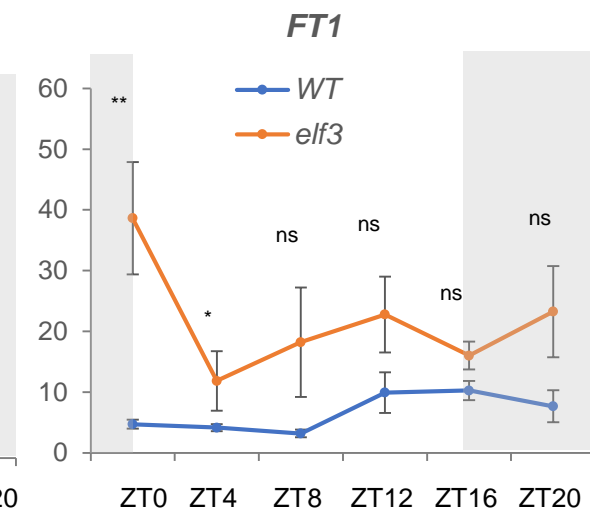
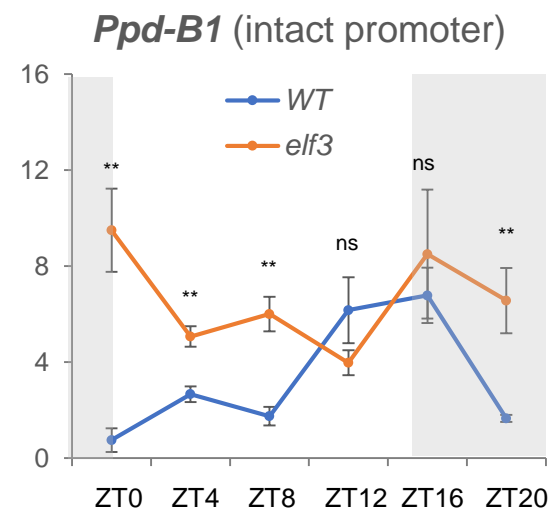
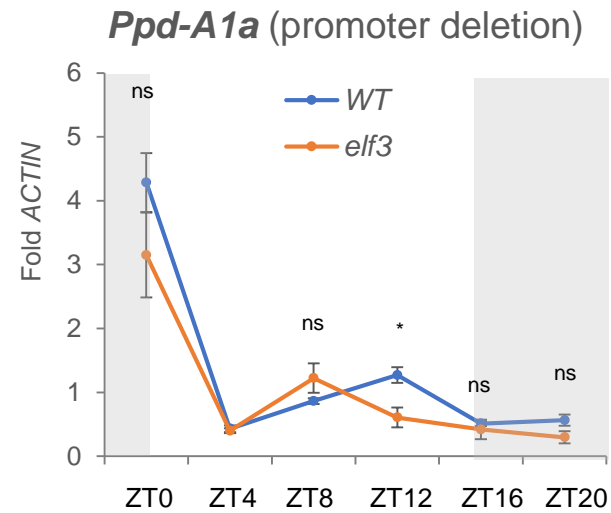


Effect of *elf3* mutations on *PPD1* and *FT1* expression under LD

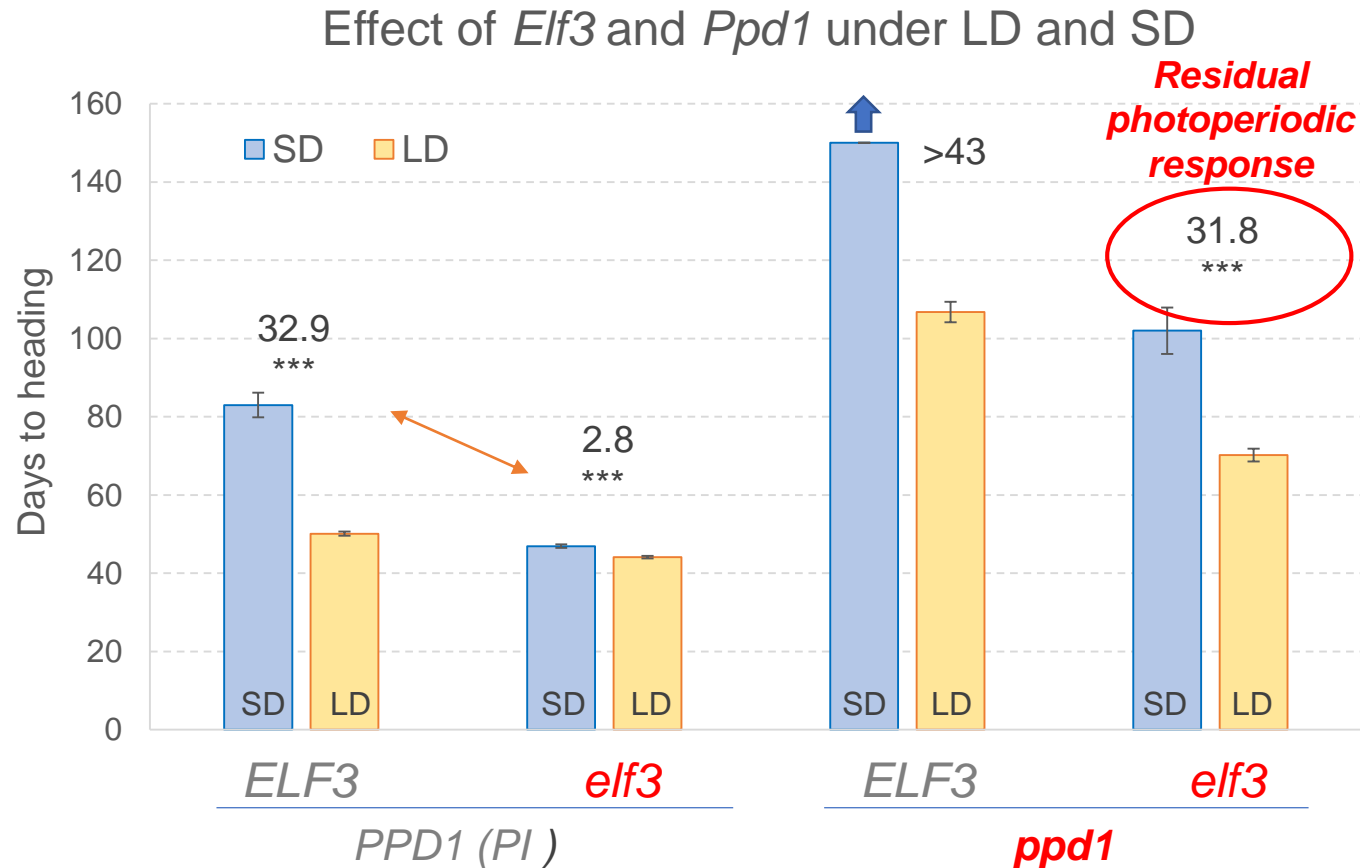
PS
Intact promoter



PI
Promoter deletion



Loss-of-function mutations in *ppd1* delay heading time in the absence of *elf3*



- *elf3* loss-of-function accelerates flowering both in the presence or absence of *Ppd1* (PI).
- The photoperiodic response (SD-LD) was reduced in absence of *ELF3*.
- The *elf3 ppd1* mutant headed significantly later than the single *elf3* mutant → *PPD1* can accelerate heading in the absence of *ELF3*, both under LD and SD.
- *elf3 ppd1* headed significantly earlier than the single *ppd1* mutant under both LD and SD → *ELF3* can delay heading time in a *PPD1*-independent manner.

SUMMARY

- ELF3 operates between the phytochromes and *PPD1*, and is an important part of the mechanism by which light activates *PPD1* transcription.
- ELF3 protein is modified by light.
- ELF3 is a direct repressor of *PPD1* particularly at the end of the night and dawn.
- In the absence of *ELF3* and *PPD1* wheat still has a photoperiodic response likely mediated by *CONSTANS*



THANK YOU



Jorge Dubcovsky



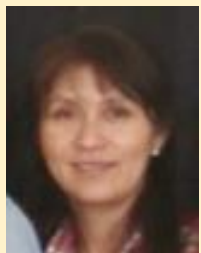
Chengxia Li



Huiqiong Liu



Anna Joe



Mariana Padilla



Daniel Woods