Mapping of *photoperiod-sensitive suppressor of elf3 21*

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Abstract:

Arabidopsis thaliana relies on day length or photoperiod to direct the transition from vegetative to reproductive development. The proper response to seasonal cues optimizes growth and reproduction. Many gene products are already known that regulate the photoperiodic control of flowering. Early flowering 3 (Elf 3) translates signals from photoreceptors to the circadian oscillator. Floral pathway integrators regulate flowering downstream from the circadian clock. One such integrator, Constans (Co), promotes flowering in long days by activating transcription of Flowering locus T (Ft), which results in floral induction. However, the molecular mechanism governing floral induction is not fully understood, and therefore the identification of unknown genes may provide a better understanding. In order to identify novel genes controlling the floral induction pathway, we are characterizing photoperiod-sensitive suppressors of *elf3-1.* We chose to work on *pse21* because it delays flowering of *elf3-1* and restores photoperiod sensitivity, but does not cause late flowering on its own. Segregation analysis suggests that there are two *pse 21* loci, one on chromosome II and the other on chromosome V. In attempts to understand how *pse 21* functions in the floral induction pathway, ongoing studies seek to narrow the location of *pse 21* on chromosome II and V.

Wild type → Flowering

elf3-1 single mutant → Flowering

elf3-1 pse21 → Flowering

Figure 2: Diagram comparing the flowering of wild type to the mutants *elf3-1* and *elf3-1 pse21.*

Introduction:

- Many plants optimize their growth and reproduction by sensing and responding to seasonal changes. Day length is one of the most reliable sources that optimize reproductive success in flowering plants
- Arabidopsis flowering is accelerated by conditions that reliably indicate the passage of winter and the onset of spring and summer (Simpson *et at*, 2002).
- *Arabidopsis thaliana* is a useful model for understanding the molecular pathway that regulates flowering in agricultural species.
- *A. thaliana* contains several known genes that regulate the photoperiodic control of flowering. However, the molecular mechanism governing floral induction is not fully understood and therefore the identification of unknown genes may provide a better understanding.
- In order to find novel genes involved in regulating flowering in Arabidopsis, a screen was done to look for mutations that suppress the *elf3-1* flowering time. We are characterizing *pse21* to better understand how this mutation alters *elf3-1* flowering time in response to day length.
- There are two *pse21* loci because we have observed 15:1 segregation from *elf3-1 pse21* x *elf3-3* crosses.
- The late flowering progeny that we obtained from the *elf3-1 pse21 (Co) x elf3-3 (Ws)* cross will help us determine the linkage relationship between *pse21* DNA and genetic markers of known locations.

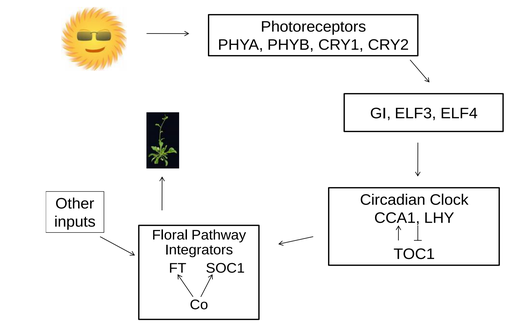

Figure 1: Model diagram of the photoperiod pathway regulating flowering in Arabidopsis thaliana.

Goal: We want to narrow the location of *pse21* on chromosome II and V.

Figure 3: Phenotypic analysis of F3 progeny from *elf3-1 pse21 (Co) x elf3-3 (Ws).* The plant on the left shows *elf3* early flowering phenotype; plant on right shows *elf3 pse21* suppressor phenotype.

Methods

Stratification, Planting, and Plant Growth
Seeds were stratified for 2-5 days at 4 ˚C to increase germination rate. For phenotypic analysis, seed was sown on 4" pots filled with moist Promix-BX and covered with domes. Plants were grown in short day conditions and remained covered until vegetative leaves began to form. Late flowering plants were transplanted to long day conditions to stimulate flowering and seed set.

Determination of flowering time
Flowering time was measured by counting the number of rosette leaves produced before stem elongation (bolting).

DNA Extraction
DNA was extracted as in the CTAB Mini-prep protocol.

PCR and Gel Electrophoresis
The PCR products were visualized using a 3% agarose gel to assess the genotype at molecular markers of known locations.

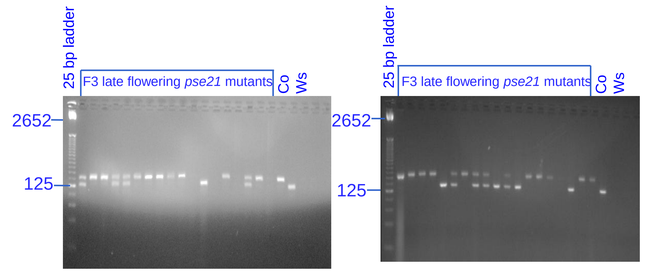

Figure 4: PCR products were run on a 3% agarose gel to assess the genotype of *pse 21* mutants with specific locations (left panel nga 139 and right panel LUGSSLP02). Co and Ws represent the controls used and N stands for the control with no DNA.

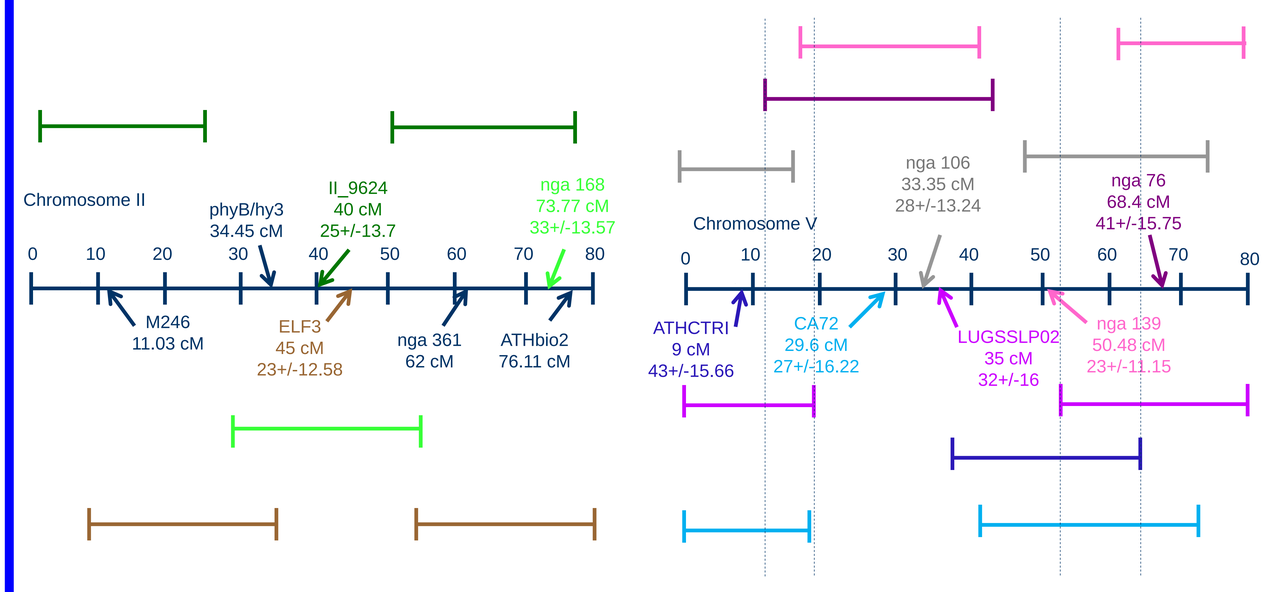

Figure 5: Genetic map of the regions containing *pse21* on chromosome II and Chromosome V. PCR-based markers are listed with marker or gene name and physical map positions. Recombination event frequencies are given with error +/- 95% confidence interval.

Results and Discussion

•We identified regions containing *pse21* on chromosomes II and V using primers of known locations. We measured recombination events between *pse21* and ELF3 and II_9624 on chromosome II; and measured recombination events using nga 106, nga76, ATHCTR1, CA72, nga139 and LUGSSLP02 on chromosome V.. On Chromosome II, the nga 139 interval overlaps the ELF3 interval suggesting that *pse21 is* between 27.2 cM and 34.58 cM. On chromosome V, nga 76 and LUGSSLP02 argue that *pse21* is located between 11.65 cM and 19 cM, respectively. LUGSSLP02 and ATHCTR1 argue that *pse21* is located between 51 cM and 67.66 cM, respectively (See dashed lines, Figure 5).

•In order to narrow the region containing *pse21* on chromosome II we want to use additional markers which include M246, nga 361, ATHbio2, and phyB/hy3. Further studies should increase the sample size of mapping population by obtaining different plant families.

Literature Cited
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