Mapping of *photoperiod-sensitive suppressor of elf3 21*

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Abstract:

Arabidopsis thaliana responds to day length or photoperiod by flowering earlier in long day conditions than short day conditions. The proper response to seasonal cues maximizes reproductive success. 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 find 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.

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 thaliana* is a facultative long-day plant, meaning that it flowers more rapidly in long days than during short days. Arabidopsis flowering is accelerated by conditions that reliably indicate the passage of winter and the onset of spring and summer (Simpson *et at*, 2002).
- The entire genome of Arabidopsis has been sequenced and orthologs of Arabidopsis genes that regulate flowering have been found in agriculturally important plants such as rice and maize (Samach and Gover, 2001). Arabidopsis can grow relatively quickly in small spaces. Due to these qualities, *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. Previously, we have found that *pse21* behaves in a recessive manner. *pse21* does not cause late flowering on its own but delays flowering of *elf3-1* and restores photoperiod sensitivity.
- 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.

Goal:

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

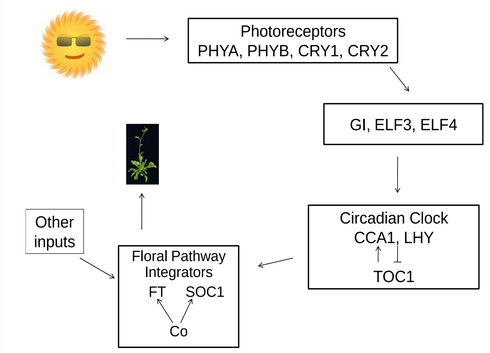

Figure 1: Model diagram of the photoperiod pathway regulating flowering in Arabidopsis thaliana.

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.*

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 22 ˚C short day condition with 8 hours light and 16 hours dark and remained covered until vegetative leaves began to form. Late flowering plants were transplanted to long day conditions with 16 hours and 8 hours dark 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). Early flowering plants were defined as having 0-10 rosette leaves, medium late plants as having 11-20 rosette leaves, and late plants as having 21 or more rosette leaves.

DNA Extraction

Rosette leaf tissue was collected and immediately frozen in liquid nitrogen. DNA was extracted with CTAB extraction buffer. Nucleic acids were separated to a new eppendorf tube using chloroform and then precipitated with isopropanol. Pelleted DNA was washed with 70% ethanol and resuspended in TE.

PCR and Gel Electrophoresis

DNA was amplified using Polymerase Chain Reaction (PCR). The PCR products were visualized using a 3% agarose gel to assess the genotype at molecular markers of known locations.

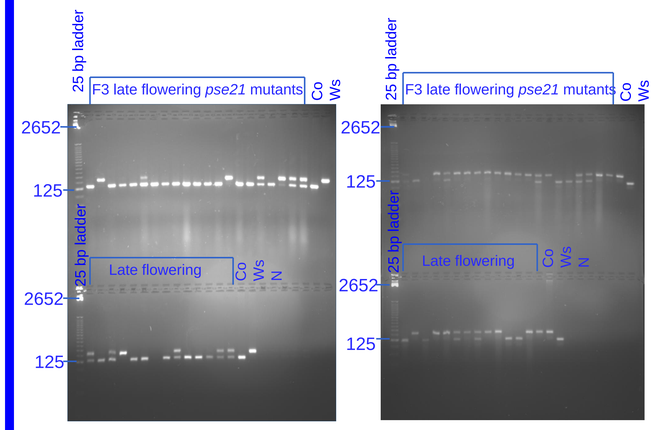

Figure 4: PCR products were run on a 3% agarose gel to assess the genotype of *pse 21* mutants with specific locations (left panel ELF3 and right panel nga 106). Co and Ws represent the controls used and N stands for the control with no DNA.

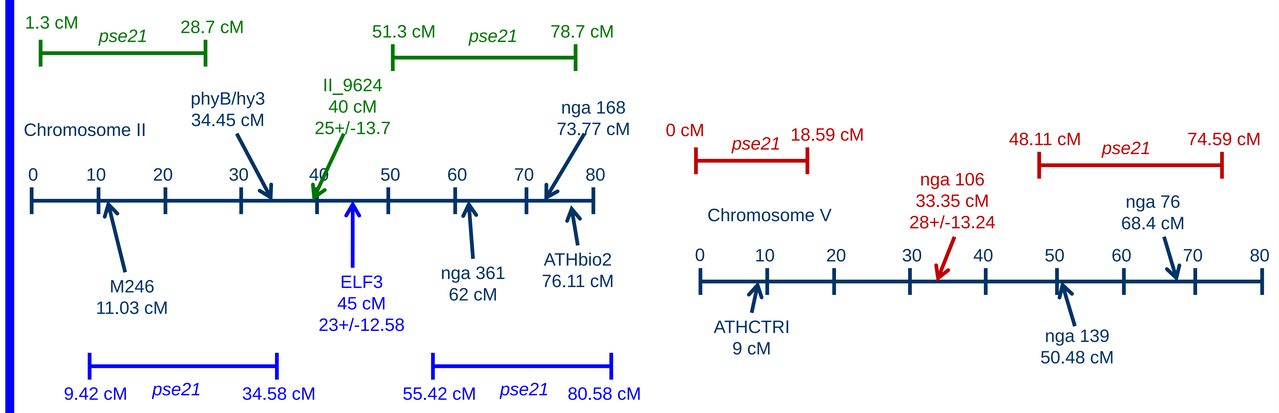

Figure 5: Genetic map of the regions containing *pse21* on chromosome II and Chromosome V. PCR-based markers are listed with marker or gene name and physical map positions. Recombination event frequencies are given with error +/- 95% confidence interval.

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 on chromosome V. We found that *pse21* is located 23 +/- 12.58 cM from ELF3 and 25 +/- 13.7 cM from II_9624 on chromosome II. On chromosome V, *pse21* is located 28 +/- 13.24 cM from nga 106. However, the location of pse21 could be on either side of each marker (Figure 5).
- In order to narrow the region containing *pse21* we want to use additional markers which include M246, nga 361, ATHbio2, phyB/hy3, ATHCTRI, nga 139, and nga 76.

Literature Cited

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