CHARACTERIZATION OF *pse12*, A MUTATION THAT RESTORES PHOTOPERIOD SENSITIVITY IN *elf3* MUTANT PLANTS
Characterization of $pse12$, a Mutation that Restores Photoperiod Sensitivity in $elf3$ Mutant Plants

By

Ansley Elizabeth Scott

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Many plants rely on environmental cues such as daylength, or photoperiod, to induce reproduction. The *ELF3* gene is vital to photoperiod sensitivity in *Arabidopsis thaliana*. In order to identify additional genes in this pathway, *elf3* mutants were subjected to EMS mutagenesis, and a few plants in the subsequent generations regained photoperiod sensitivity. These double mutants suppress the *elf3* mutant phenotype and have been named *photoperiod-sensitive suppressors of elf3* (*pse*). I am working to positionally clone *PSE12*, and I have discovered its location to be on chromosome 2 at 64 +/− 4 cM. In addition to discovering the position of *PSE12*, the *pse12* mutation was initially characterized and it was found to be a single recessive mutation. Eventually, genes such as *PSE12* that are involved in regulating flowering time could be used to genetically alter crop plants, increasing agricultural yields and productivity.
**Introduction**

Light, photoperiod, and circadian rhythms are intimately involved in the regulation of flowering in plants. Although much is known about the physiology of flowering time, the structure of the genetic pathway responsible for flowering is still a mystery. A key to breaking the mystery is to study flowering-time mutations. Mutations have been found that affect flowering time through the alteration of circadian rhythms, the light induction pathway, and photoperiod sensitivity. Some mutations affect all three processes. It is not known if these mutations alter these processes through one pathway, or if the wild type gene has multiple functions that are disrupted in the mutant. The study of additional mutations that affect one or more of these processes can begin to indicate if the processes are linked. As more flowering time mutants are characterized, the flowering time model becomes refined and the overall mechanism behind flowering is made clearer. This paper will attempt to analyze other researchers’ pioneering work in flowering time mutants, and to discuss my own findings about gene

**PHOTOPERIOD –SENSITIVE SUPPRESSOR OF elf3-1 12 (PSE12).**

**Light**

Light is necessary for the survival and reproduction of plants, and in order for plants to detect light, they must be able to absorb it. The proteins involved in the absorption of light are called photoreceptors. There are two known types of photoreceptors, phytochromes and cryptochromes, and they each
absorb different wavelengths of light. Phytochromes are sensitive to the red/far-red region of light, and cryptochromes are sensitive to blue light (Reviewed in Lin, 2000).

After absorption, light can regulate processes such as development in plants. The development of shape in plants due to light is known as photomorphogenesis. One example of photomorphogenesis is the inhibition of hypocotyl growth in the presence of light (Kendrick and Kronenburg, 1994).

Another process regulated by light is flowering. Flowering can be initiated or inhibited by light. In fact, the actual length of light during a day, called the photoperiod, is crucial for flowering time in many plants.

Photoperiod

Some plants are photoperiod (daylength) sensitive, which means that they respond to the length of light they receive in a day. Obligate plants require a specific daylength threshold to induce the transition from vegetative to reproductive growth. If this daylength is not reached, the plant will not flower. On the other hand, facultative plants flower precociously when a specific daylength is reached, but they will flower eventually even if the critical length of day is not reached (Samach and Gover, 2001).

In addition to being obligate or facultative, there can be either short day (SD) or long day (LD) plants. SD plants flower when the daylength is less than or equal to a critical daylength, but flowering is inhibited in these plants under LD conditions. On the other hand, LD plants flower when daylength is more
than or equal to a critical daylength. In LD plants, flowering is inhibited, but not prevented if the plants are placed in SD conditions (Samach and Gover, 2001).

In order to study the effects of photoperiod on plants more easily, a model organism can be used. One model system is *Arabidopsis thaliana*. *Arabidopsis*, also known as mouse ear cress, belongs to the mustard family and is commonly found in Europe and in the United States. Many genetic researchers use *Arabidopsis* because of its hardiness, ease of growth, short generation time, sequenced genome, and its many known molecular markers (Meyerowitz, 1994). Additionally, *Arabidopsis* can be used to study photoperiodic regulation (Thomas and Vince-Prue, 1997).

*Arabidopsis* is a facultative LD plant. It only takes about three weeks for wild type *Arabidopsis* plants to flower with 10-15 leaves in LD conditions, whereas it takes 7-10 weeks and the formation of about 40 leaves for *Arabidopsis* to flower in SD conditions. Different wild-type ecotypes of *Arabidopsis*, Columbia (Col), Landsberg erecta (Ler), and Wassilewskija (Ws), flower at slightly different times with different numbers of leaves (Thomas and Vince-Prue, 1997).

Light is not alone in affecting flowering time in plants. Many processes that are under the control of light are also under the control of an internal timekeeper called the circadian clock (Millar, 1998).
Circadian clock

Within many organisms, including plants, lie biological processes that cycle periodically and are controlled by an endogenous circadian clock (Murtas and Millar, 2000). The circadian clock is entrained by light and has a 24-hour cycle. In plants the clock controls levels of chemicals, flowering, and even leaf movement (Somers, 1999).

Many processes controlled by the circadian clock were once thought to be exclusively under the control of external environmental cues such as light. For example, leaf movement at specific times during the day was thought to be controlled by the sun. This was found to be untrue after researchers removed plants from a dark/light cycle and placed them into complete darkness. In these plants, leaf movements that were observed in daylight continued in the absence of light cues and followed a 24-hour cycle of movement (Somers, 1999).

Although the circadian clock can regulate processes in the absence of light, the clock must be entrained to its environment to allow processes in plants to remain synchronized to the environment. The clock relies on light cues for entrainment, which permits plants to adapt to changing daylengths and seasons (Millar and Kay, 1996).

The clock itself contains three components: the input pathway, the central oscillator, and the output pathway (See Figure 1). The input pathway is also known as the entrainment pathways. Light is thought to be carried through the input pathway by way of the photoreceptors, phytochromes (PHY) and cryptochromes (CRY), in order to reset the oscillator. The central oscillator
then creates a 24-hour period whereby processes cycle. Lastly, the output pathways function to carry signals from the central oscillator to various processes within a plant, such as flowering, which are called overt rhythms (Millar, 1998).

![Figure 1: A model of the circadian clock. A, B, C, and D are part of the central oscillator. E, F, and G are overt rhythms (Figure reprinted from Somers, 1999).](image)

**Photomorphogenic / Photoperiod / Circadian Clock mutants**

Although a model representing the components of the circadian clock exists, the actual mechanism and many of the specific genes involved with the components of the clock are still unknown. Analysis of mutant plants that are dysfunctional in some part of the circadian clock has allowed researchers to build an initial mechanism. In addition, many of the clock mutants have displayed characteristics that are also present in light reception and photoperiod insensitive mutants. These data suggest that these three processes (light reception, photoperiod sensitivity, and the circadian clock) are linked.

In addition to mutational studies, knowledge about the mechanism behind flowering grew considerably after the development of a reporter gene that was easily detectable in living plants. This reporter gene construct consists of the
promoter of the chlorophyll a/b-binding protein (cab2) driving the expression of the luminescent firefly gene luciferase (luc). The reporter gene allows the monitoring of cab2 expression, which cycles in a periodic fashion and is controlled by the circadian clock in plants (Millar et al, 1992).

The genes described in this paper will be discussed relative to their position in the flowering time model. Genes that are believed to be involved in the entrainment pathway, such as photoreceptors, will be discussed first, followed by genes thought to be involved with the central oscillator, and lastly, genes that are thought to function between the central oscillator and output pathways (See Figure 1, above).

Mutations in the Light Input Pathway

There are five known phytochrome genes in Arabidopsis, PHYA-PHYE, that encode the proteins PHYA- PHYE (Quail et al, 1995). Several of these genes, such as PHYA, PHYB, and PHYD, seem to be involved in flowering time (see figure 2).

Figure 2. The role of photoreceptors in floral initiation. Arrows show positive effects and lines that end with a bar represent negative effects (Figure reprinted from Lin, 2000).
PHYA is thought to be involved in flowering time because plants that over-express the PHYA gene flower earlier than wild type in SD and LD conditions (Johnson et al, 1994; Neff et al, 1998). Other researchers claim that phyA mutants flower late in pseudo LD conditions (SD with night breaks) compared to wild type, but high variability causes the data to be inconclusive (Reed et al, 1994).

PHYA seems to also be involved with the circadian clock. Evidence for this was observed in experiments using the cab::luc reporter gene. Plants with the phyA mutation experienced a reduced amplitude of cab2::luc expression although it was in phase with wild type cab2::luc expression (Anderson et al, 1997).

Although PHYA is involved with both flowering time and the circadian clock, PHYA does not seem to greatly affect photomorphogenesis of the hypocotyl. Hypocotyl length in plants with the mutant phyA gene is not different than wild type in all wavelengths of light except in far red light (Reed et al, 1994).

In contrast to phyA, the phyB mutation causes hypocotyl elongation in both white and red light as compared to wild type. In addition, phyA phyB double mutants have a more pronounced long hypocotyl phenotype than either mutant alone. This suggests that PHYA and PHYB function in separate pathways to inhibit hypocotyl elongation. The data also suggests that PHYB is the main hypocotyl inhibitor (Reed et al, 1994).
phyB mutants have a much more pronounced flowering phenotype than phyA mutants. phyB mutants flower with half as many leaves as wild type in both LD and SD conditions (Reed et al, 1994). Plants with the phyB mutation are still photoperiod sensitive; they flower later in SD than in pseudo LD conditions (Reed et al 1994).

Mutations in a third phytochrome gene, PHYD, cause no change in flowering time compared to wild type, but plants homozygous for both phyB and phyD flower significantly earlier than wild type (Aukerman et al, 1997). This suggests that phyD works in tandem with phyB to promote flowering. Devlin et al (1999) found similar results under continuous high red/far light and continuous low red/far red light conditions. Additionally, plants homozygous for both the phyB and phyD mutation flower earlier than plants homozygous for just the phyB mutation (Devlin et al, 1999).

A second set of photoreceptors, the cryptochromes, are also vital in light reception. The genes CRY1 and CRY2 have been discovered in Arabidopsis and seem to be involved in flowering (see Figure 2). Plants with a mutant cry1 gene flower slightly later than wild type (Lin et al, 1996). In addition to the flowering time phenotype, cry1 mutants cannot inhibit hypocotyl elongation in blue light (reviewed in Fankhauser and Chory, 1997).

A second cryptochrome gene, CRY2, is thought to be involved in the promotion of flowering. cry2 mutants flower late in LD conditions but not in SD conditions as compared to wild type. Guo et al (1998) claims that the cry2 mutants flower a bit earlier than Col in SD. However, the difference between
cry2 mutants and wild type is not significant with respect to error (see Figure 3, A and B).

Figure 3. Flowering time in cry mutants. A and B represent SD and C and D represent LD (Reprinted from Guo et al, 1998).

Plants that over-express CRY2 flower earlier than wild type in SD conditions, but there is no difference in flowering in LD conditions (Figure 3, C and D). This suggests that CRY2 promotes flowering, but wild type plants possess more than the threshold level of CRY2, thus over-expression will not induce floral initiation (Guo et al, 1998).

In addition to the photoreceptors, mutations in genes that encode proteins such as transcription factors affect flowering time through photoperiod sensitivity, light perception, and/or the circadian clock. A few of the most important genes, including GIGANTEA (GI), LATE ELONGATED HYPOCHOTYL (LHY), CIRCADIAN CLOCK ASSOCIATED 1 (CCA1), EARLY FLOWERING3 (ELF3), and CONSTANS (CO), will be described (see Figure 4).
The GI nuclear protein is thought to function in the light input pathway because it interacts with *PHYB*. The interaction with *PHYB* explains the lack of hypocotyl inhibition in *gi* mutants grown in continuous red light conditions (Huq et al, 2000).

In addition to lack of hypocotyl elongation, some plants containing *gi* mutations are photoperiod insensitive. Plants with *gi* mutations flower much later in LD conditions (Fowler et al, 1999), and some *gi* mutants can produce at least three times as many leaves in continuous light as compared to wild type (Araki and Komeda, 1993).

*gi* also disrupts the circadian period and amplitude (Araki and Komeda, 1993; Park et al, 1999). The circadian period decreases in homozygous *gi*
mutant plants from 24 hours to 22 hours. In addition to the shortened period, the amplitude of cab2::luc, CCA1, and LHY expression in homozygous gi-1 mutants was decreased as compared to wild type (Schaffer et al 1998, Wang and Tobin, 1998).

Similar to GI, ELF3 is involved in photoperiod sensitivity, photomorphogenesis, and the circadian clock. ELF3 may encode a transcription factor, and this gene product is thought to function in the light input pathway and/or in conjunction with the central oscillator (Hicks et al, 2001).

ELF3 is believed to function in the light input pathway because elf3-1 mutants lack hypocotyl inhibition in LD, SD, and constant light conditions. In fact, elf3-1 mutants are less sensitive than wild type to most wavelengths of light, especially blue and green wavelengths. Because elf3-1 mutants particularly lack sensitivity to blue and green light, ELF3 may be involved in the blue light transduction pathway (Zagotta et al, 1996).

One of the most striking features of elf3-1 mutants is their lack of photoperiod sensitivity. elf3 mutants flower after making about 5 leaves in both LD and SD (Zagotta, 1992).

In addition to disrupted photoperiod sensitivity, elf3 mutants have altered circadian rhythms. In fact, elf3-1 mutants do not display circadian rhythms in constant light conditions, but they do retain some rhythmic cycling in constant dark conditions (Hicks et al, 2001).

LHY is a third gene that seems to be involved with the central oscillator and to affect photoperiod sensitivity, light reception, and the circadian clock.
Like both GI and ELF3’s gene products, LHY is localized in the nucleus where it is believed to function as a DNA binding protein (Schaffer et al, 1998).

\( lhy \) mutants flower late and produce similar numbers of leaves in LD and SD conditions. Thus, these plants are photoperiod insensitive. \( lhy \) mutant plants also possess elongated hypocotyls, which is a sign of a disruption in the light reception pathway (Schaffer et al, 1998). Additionally, \( lhy \) mutants disrupt the circadian clock. Shaffer et al (1998) measured circadian rhythms in \( lhy \) mutants and found that the regular rhythmic movement of the cotyledons and the primary leaves decreased compared to wild type. In addition to leaf movement, the mRNA level of the gene COLD CIRCADIAN RHYTHM 2 (\( CCR2 \)) was monitored and \( CCR2 \) expression in \( lhy \) plants is arrhythmic in constant light conditions (Schaffer et al, 1998).

The LHY protein and gene sequence are very similar to the CCA1 protein and gene sequence. Resembling \( lhy \) mutants, \( cca1 \) mutants flower late, have arrhythmic cycling of \( cab \) and \( ccr2 \) expression, and exhibit elongated hypocotyls (Schaffer et al, 1998). These data suggest that the two genes share a similar, if not the same, function.

\( CONSTANS (CO) \) has been placed downstream of LHY, ELF3, and GI in the flowering time pathway. \( CO \) is placed downstream of the central oscillator because experiments show that the circadian clock controls \( CO \). \( CO \) is also placed downstream of several oscillator genes because its expression is disrupted in \( gi \) and \( lhy \) mutants. In \( gi \) mutants, the amplitude of \( CO \) mRNA is decreased as compared to wild type while the \( lhy \) mutation alters the rhythm of \( CO \) expression
and causes CO mRNA to decrease in amplitude as compared to wild type (Suarez-Lopez et al, 2001).

CO is thought to encode a transcription factor that is involved in flowering time. Plants with mutations at the CO locus flower later in LD conditions than wild type but resemble wild type in SD conditions. This suggests that CO is needed in the LD flowering promotion pathway, but not in the SD pathway (Suarez-Lopez et al, 2001). These data also show that mutations downstream of the central oscillator can affect flowering time.

In order to flesh out the flowering time pathway, gene expression and double mutants need to be studied. These experiments allow researchers to place genes in the flowering time model by determining if they function in an input pathway, the central oscillator, or an output pathway. Additionally, gene expression and double mutants reveal interactions between genes. For instance, researchers believe that ELF3 functions upstream of many known genes CO, GI, and LHY, which are all involved in the circadian clock and/or photoperiod sensitivity (see Table 1). The data that led to this assumption came from double mutants and from monitoring the gene expression in a mutant background (Hicks et al, 2001, Schaffer et al, 1998, Suarez-Lopez et al, 1998, Park et al, 1999). The genes PHYA and PHYD have been shown to interact with PHYB through similar experiments (see Table 1). Inevitably, many more interactions occur between known and undiscovered genes, and these interactions must be studied to refine the flowering time mechanism.
Table 1: Genes involved in photomorphogenesis, photoperiod sensitivity, and light reception.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Processes that mutants disrupt</th>
<th>Genes thought to be in same pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHYA</td>
<td>Circadian clock, light reception</td>
<td>PHYB</td>
</tr>
<tr>
<td>PHYB</td>
<td>Circadian clock, light reception, photoperiod sensitivity</td>
<td>PHYA, PHYD, ELF3</td>
</tr>
<tr>
<td>PHYD</td>
<td>Photoperiod sensitivity</td>
<td>PHYB</td>
</tr>
<tr>
<td>CRY1</td>
<td>Light reception</td>
<td></td>
</tr>
<tr>
<td>CRY2</td>
<td>Light reception, photoperiod sensitivity</td>
<td></td>
</tr>
<tr>
<td>CO</td>
<td>Photoperiod sensitivity</td>
<td>ELF3</td>
</tr>
<tr>
<td>GI</td>
<td>Circadian clock, photoperiod sensitivity</td>
<td>LHY, ELF3, CCA1</td>
</tr>
<tr>
<td>ELF3</td>
<td>Circadian clock, light reception, photoperiod sensitivity</td>
<td>PHYB, GI, LHY, CO</td>
</tr>
<tr>
<td>TOC1</td>
<td>Circadian clock, photoperiod sensitivity</td>
<td></td>
</tr>
<tr>
<td>LHY</td>
<td>Circadian clock, light reception, Photoperiod sensitivity</td>
<td>ELF3, GI</td>
</tr>
<tr>
<td>PSE12</td>
<td>Photoperiod sensitivity</td>
<td>ELF3</td>
</tr>
</tbody>
</table>

Although ELF3 is well studied, much is unknown about the mechanism by which it functions. To address this problem, more genes involved in flowering must be discovered and studied, and double mutants with ELF3 need to be found or produced. Our lab chose to find additional flowering time genes by using an EMS mutant screen. Our lab treated elf3-1 homozygous plants with EMS, self-crossed these plants, planted the offspring of the self-cross, and looked for offspring that contained mutations that suppressed the early flowering phenotype of elf3-1 in LD and SD conditions. The advantage of a second site modifier screen is that it may identify flowering time genes that would otherwise be overlooked. It addition, genes may be discovered in this screen that directly interact with ELF3 thus allowing for further study of the ELF3 gene. This screen led to the discovery of about a dozen suppressor mutations of elf3 called...
PHOTOPERIOD-SENSITIVE SUPPRESSORS OF elf3-1 (pse) (see Figure 5).

One of these suppressors, the mutant pse12, will be the topic of this thesis.

Figure 5. The flowering time of wild type, elf3 and, elf3 suppressors in LD (white bars) and SD (hatched bars) conditions (K.A. Hicks unpublished results).

Project

PSE12, which suppresses the phenotype of elf3-1, was initially mapped to chromosome II in Arabidopsis. Mutations in the PSE12 gene, among others, suppress the mutant phenotype of elf3 homozygous plants and restore wild type photoperiod sensitivity. PSE12 was one of many suppressors that were found in a genetic screen. PSE12 was chosen because it restored wild type photoperiodic sensitivity in both LD and SD conditions, and it had a more reproducible phenotype than other PSE mutations in LD conditions (see Figure 5).
Implications of the research

The photoperiod sensitivity, light reception, and circadian clock pathways in Arabidopsis have only begun to be elucidated, and only a few genes have been placed in a flowering time model. Additional genes must be discovered so the mechanism behind photoperiod sensitivity, light reception, and circadian clock regulation can be deduced.

Positionally cloning the PSE12 gene will allow for the study of the PSE12 gene product and further suggest a mechanistic role of PSE12 and ELF3 in the flowering time pathway. This knowledge could eventually be applied to help engineer transgenic plants with higher crop yields, which could allow us to be less dependent on herbicides and pesticides. In addition, these plants may be able to grow in more diverse climates and latitudes.
Materials and Methods

Plant material

Arabidopsis thaliana seeds containing the elf3-1 and elf3-3 mutations were previously described (Hicks et al, 1996, Zagotta et al, 1996). pse12 mutants were isolated in the elf3-1 background by Katie Jo Keppinger, Marta Doolittle, and K. A. Hicks (unpublished results).

Crosses

pse12 elf3-1 double mutants were crossed with elf3-1 mutants and seed was collected. F1 plants were allowed to self-cross and F2 plants were analyzed for flowering time in SD.

pse12 elf3-1 double mutants were crossed with wild type Columbia plants. F1 plants were then self-crossed and F2 plants were analyzed for flowering time in LD and SD.

Stratification

To increase germination rate, seeds were placed on damp filter paper in clean, plastic petri dishes (2” diameter). The dishes were labeled, sealed with Parafilm, and stored at 4°C for 2-7 days before planting.
**Planting/growth conditions**

Seeds were planted in moist soil. Five to nine seeds were planted with micro tweezers in each 2” square pot, and 36 pots were placed in a watertight flat. Flats were covered with plastic domes to keep the moisture level high until the first vegetative leaves were visible. The seals of the domes were then broken to allow the plants to acclimate to a less humid environment. The domes were completely removed two days after the seal was broken. Plants were watered every 2-4 days with tap water.

Plants were grown in two types of daylength. Short day (SD) conditions consisted of 8 hours continuous light followed by 16 hours of continuous dark. Long day conditions (LD) had light for 16 hours and dark for 8 hours. Light was provided by a series of Sylvania 120V (0.5 Amps, 60 Hz) cool white fluorescent bulbs. The temperature was maintained around 21°C.

**Flowering time**

Flowering time was measured by counting the number of vegetative leaves produced when the inflorescence reached a height of at least 1 cm.

**Photomorphogenesis**

Seed was sterilized with 30% bleach and 0.2% Triton X-100 (Sigma) for 20 minutes.

Seeds were then extensively washed with sterile water and placed onto plates containing 0.7% agar (Sigma), 0.5X MS medium (GibCo BRL), and 1% sucrose.
(Sigma). The plates were stored at 4°C for 7 days and then transferred into LD and SD growth conditions. Hypocotyl length was measured after the formation of two vegetative leaves.

**Mapping cross**

*pse12 elf3-1* double mutants (Columbia ecotype) were crossed with single *elf3-3* mutants (Wassilewskija ecotype) to form the F2 generation. DNA from the F2 generation was gathered and used in PCR reactions. The F2 plants were self-crossed and the offspring, F3 families, were grown and scored to determine the phenotype of the F2 parent.

**DNA Isolation**

Leaves from *A. thaliana* were collected, placed into labeled 1.5 ml microfuge tubes, flash frozen in liquid nitrogen, and then transferred to -80°C for storage. Frozen leaves were ground with a mini plastic pestle attached to a portable drill. 500µl of CTAB extraction buffer (140 mM sorbitol, 220mM Tris-HCl, 22mM EDTA, 800mM NaCl, 1.0% sarkosyl, 0.8% CTAB) was added and the solution was ground again to remove plant matter from the pestle (Reiter et al, 1992).

The solution was incubated at 60°C for 30-60 minutes. The tubes were chilled briefly on ice before extraction with 500 µl of Chloroform. Samples were centrifuged at 15,000 RPMs for 5 minutes, and the aqueous top layer containing the DNA was removed from the organic layer and interface, which contained proteins and carbohydrates.
DNA was precipitated by adding 340 µl of isopropanol, chilling on ice for at least 15 minutes, and centrifuging at 15,000 RPMs for 5 minutes. The supernatant was poured off and the DNA pellet was washed with 500 µl of 70% ethanol to remove salt. The tubes were then briefly centrifuged to attach the DNA pellet to the bottom of the microfuge tube. The remaining supernatant was pipetted off, and the pellet was air dried for at least five minutes.

DNA was resuspended in 50 µl of TE (10mM Tris-HCl pH8 and 1mM EDTA) and stored at –20°C.

**SSLP Analysis**

DNA was amplified via Polymerase Chain Reaction (PCR). The PCR cocktail for SSLP markers consisted of 1X PCR buffer (250 mM KCl, 50 mM Tris HCl pH 8, 0.5 g/L BSA, 0.5% Triton X-100, 60% sucrose, 200 µM Cresol Red), 1-3 mM MgCl₂ (Promega), 0.5 µM forward and reverse primers (RESGEN), 0.2mM dNTPs (Promega), 1U Taq DNA Polymerase (Promega), and water to adjust final volume in each tube to 10 µl. The following PCR program was used for all SSLP markers (Giraudat et al, 2000).

Amplification began with one cycle at 95°C for 3 minutes, followed by 9 cycles at 94°C for 15 seconds, annealing at 58°C for 30 seconds, and elongation at 72°C for 30 seconds. Next, 19 cycles at 94°C for 15 seconds, at 55°C for 30 seconds, and at 72°C for 30 seconds were completed and followed by one cycle at 72°C for 10 minutes. The temperature was brought to and held at 4°C until removal.
Amplified DNA was electrophoresed on a 3% or 4% 1:1 metaphor agarose gel. A 25 bp ladder (GibCo BRL) was used as a size reference and was loaded with the samples into the gel. Samples were electrophoresed at 70-80V for 1-2 hours to separate DNA. Data was visualized by exposure to UV light in the presence of ethidium bromide. The gel was placed in the digital photographic box, and the program “Chemi Imager” was used.

**CAPS Analysis**

The reaction mixture for CAPS contained 1X PCR buffer, 1-3 mM MgCl₂ (Promega), 0.5 µM forward and reverse primers (RESGEN), 0.2mM dNTPs (Promega), 2U Taq DNA Polymerase (Promega), and water to adjust final volume in each tube to 20 µl.

Amplification began with one cycle at 94°C for 4 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 1 minute, and elongation at 72°C for 45 seconds. Upon completion of the cycle, a final elongation step for 5 minutes at 72 °C was programmed and the temperature was brought down to 4°C until removal.

The restriction digest contained 5 µl of CAPS PCR product, 1X restriction buffer (NEB), 5 units of a restriction enzyme (NEB), 1X BSA (NEB) when needed, and water to bring the total volume up to 10 µl/ reaction. This mixture was then incubated at the optimal temperature for the restriction enzyme for at least 1.5 hours.
After incubation, the samples were run out on an agarose gel (< 2%). A 1-Kb ladder (NEB) was used for a size reference. The same loading, running, and visualizing procedures mentioned for SSLPs were also used for CAPS markers.
Results

Analysis of the pse12 mutation

Unlike wild type Arabidopsis plants, which are photoperiod sensitive (flower early in LD and late in SD), homozygous elf3-1 plants flower early in both LD and SD conditions. The photoperiod insensitivity observed in elf3-1 homozygotes is reduced in pse12 elf3-1 double mutants. We hypothesize that EMS led to the pse12 mutation and that this mutation suppressed the photoperiod insensitive phenotype of elf3-1 homozygotes.

Data from pse12 mutants can help elucidate the wild type function of the PSE12 gene. For instance, if the pse12 mutation is recessive, there is a high probability that pse12 is a loss-of-function mutation. On the other hand, if pse12 is a dominant mutation, it is likely that the mutation causes a gain of function.

To determine whether pse12 was dominant or recessive, pse12 elf3-1 double mutants were crossed to elf3-1 single mutants (see figure 6). This cross produced an F1 generation that was theoretically heterozygous at all loci except for the elf3-1 locus. The phenotype of the F1 generation was early flowering and this early flowering phenotype suggested that the pse12 mutation was recessive (Hicks, unpublished).
<table>
<thead>
<tr>
<th>Generation</th>
<th>Genotype</th>
<th>Flowering Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>$pse12\ elf3-1\ \times\ PSE12\ elf3-1$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$pse12\ elf3-1\ \times\ PSE12\ elf3-1$</td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>$PSE12\ elf3-1\ \times\ PSE12\ elf3-1$ (self cross)</td>
<td>Early Flowering</td>
</tr>
<tr>
<td>F2</td>
<td>$\frac{PSE12\ elf3-1}{PSE12\ elf3-1}$</td>
<td>Early Flowering</td>
</tr>
<tr>
<td></td>
<td>$\frac{PSE12\ elf3-1}{pse12\ elf3-1}$</td>
<td>Early Flowering</td>
</tr>
<tr>
<td></td>
<td>$\frac{pse12\ elf3-1}{pse12\ elf3-1}$</td>
<td>Wild Type Flowering</td>
</tr>
</tbody>
</table>

**Figure 6.** Expected genotype and flowering in F1 and F2 generations from crossing $pse12\ elf3-1$ double mutants to $elf3-1$ single mutants.

To substantiate the F1 data, the F1 plants were self-crossed to produce the F2 generation. The F2 generation had the flowering ratio of 1:2.7 (n=122) wild type: early flowering plants in SD (see Figure 7). These data supported the F1 data and suggested that $pse12$ was indeed a recessive mutation. If the $pse12$ mutation were dominant, every plant with one $pse12$ mutation would flower like wild type, thus all of the F1 plants would flower like wild type and three fourths of the F2 would flower like wild type.
Figure 7: Self cross of pse12/+ elf3-1/elf3nSD shows late flowering phenotype. Plants were grown for months and flowering time data was collected during this time. Bold arrows indicate flowering time range of elf3-1 and wild type.

The same cross that showed that the pse12 mutation was recessive allowed the determination that a single mutation, pse12, caused elf3-1 plants to regain photoperiod sensitivity. Results from this cross revealed that 26% (n=29) of all F2 offspring flowered like wild type, and 71% (n=76) of the plants flowered early (see Figure 7). If one recessive gene causes wild type flowering, then one fourth of the offspring would be expected to display wild type flowering and three fourths would flower early. On the other hand, the expected ratio for two genes acting together is 1:15 wild type to early flowering. The F2 data suggest that a mutation in only one gene, PSE12, is involved in suppressing the mutant phenotype.

Once it was determined that pse12 was recessive and that the phenotype was caused by only one mutation, the next step was to initiate the placement of
the \textit{PSE12} gene in the flowering time pathway. To place the gene, the
phenotype of \textit{pse12} was characterized outside of the \textit{elf3-1} background. \textit{pse12} \textit{elf3-1} double mutants flowered later in SD and LD than \textit{elf3-1} plants. This
effect could be the result of \textit{pse12} being a late flowering mutation.
Alternatively, \textit{pse12} might counteract only the \textit{elf3-1} mutant phenotype and have
no phenotype on its own. To discover which of these hypotheses may be true,
\textit{pse12} \textit{elf3-1} double mutants were crossed to wild type plants to produce
offspring that would be heterozygous for both the \textit{pse12} and \textit{elf3-1} mutations.
These heterozygotes were then self-crossed and 3/16 of offspring were expected
to be homozygous for the \textit{pse12} mutation, but wild type at the \textit{ELF3} locus (see
Table 2).

\textbf{Table 2.} Punnett square for the offspring of the self-cross of \textit{pse12} \textit{elf3-1}
heterozygotes. Green indicates the expected genotype of late flowering plants,
yellow shows expected early the flowering genotype, and white boxes designate
the expected genotype of wild type flowering. Plusses represent a wild type
allele.

\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{Parental} & \textbf{elf3} & \textbf{pse12} & \textbf{elf3} & \textbf{+} \\
\textbf{combinations} & \textbf{+} & \textbf{+} & \textbf{pse12} & \textbf{+} \\
\hline
\textit{elf3} & \textit{pse12} & \textit{elf3} & \textit{pse12} & \textit{elf3} \\
\textit{elf3} & \textit{pse12} & \textit{elf3} & \textit{pse12} & \textit{elf3} \\
\hline
\textit{elf3} & + & \textit{elf3} & + & \textit{pse12} \\
\textit{elf3} & + & \textit{elf3} & + & \textit{pse12} \\
\hline
+ & \textit{pse12} & \textit{elf3} & \textit{pse12} & \textit{elf3} \\
+ & \textit{pse12} & \textit{elf3} & \textit{pse12} & \textit{elf3} \\
\hline
+ & + & \textit{elf3} & \textit{pse12} & \textit{elf3} \\
+ & + & \textit{elf3} & \textit{pse12} & \textit{elf3} \\
\hline
\end{tabular}

If \textit{pse12} caused a unique late flowering phenotype by itself, then 3/16
(18.8\%) of the offspring from the \textit{pse12} \textit{elf3-1} heterozygous self cross would be
expected to flower later than wild type. On the other hand, if *pse12* did not cause late flowering by itself, there would be an expected ratio of 3:16 (19%: 81%) of early to wild type flowering plants and no late flowering plants. Figure 8 shows flowering data in both LD and SD conditions from this cross, which is summarized in table 3.
Figure 8 (A and B). Effects of self-crossing pse12 elf3-1 heterozygotes in LD (A) and SD (B) conditions. Plants were grown for three months and flowering time data was collected throughout this time. Bold arrows indicate range in flowering of elf3-1 and wild type control (Columbia).
Table 3. Number and percentage of self-crossed plants heterozygous for elf3-1 and pse12 that flower early, like wild type, or late in SD or LD conditions (early in SD is 4-6 leaves, wt in SD 21-52 leaves, late in SD > 52 leaves, early in LD 4-6 leaves, wt in LD 8-12 leaves and late in LD >12 leaves).

<table>
<thead>
<tr>
<th></th>
<th>% Early (n)</th>
<th>% Questionable (n)</th>
<th>% Wild type (n)</th>
<th>% Late (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD</td>
<td>8% (21)</td>
<td>4% (11)</td>
<td>75% (187)</td>
<td>11.6% (29)</td>
</tr>
<tr>
<td>SD</td>
<td>15% (18)</td>
<td>21% (26)</td>
<td>52% (64)</td>
<td>12% (15)</td>
</tr>
</tbody>
</table>

Flowering phenotypes were determined by using the range of flowering time found in the controls. For example, plants were scored as early flowering if they flowered within the flowering time range of elf3-1 that were planted at the same time. Plants were scored for wild type flowering if they were in the range of flowering for wild type plants that were grown at the same time. Plants that flowered later than wild type plants were scored as late flowering. Plants that did not fit into these three categories, but flowered between elf3 and Col, were named questionable. The questionable plants were probably a combination of wild type and early flowering plants, but since there was no clear boundary, these plants were not scored in either category.

Since pse12 likely delays flowering independently of elf3-1, then it is interesting to compare the late flowering plants in both LD and SD conditions to further characterize the pse12 mutation. The data from plants in LD and SD showed that the pse12 mutation did not result in photoperiod insensitivity. The late flowering plants in SD produced 49-65 leaves before flowering and the late flowering plants in LD produced 25-27 leaves (Figure 8).
**Photomorphogenic Analysis**

*ELF3* is involved in photoperiod sensitivity and light reception; *elf3* mutants flower early regardless of photoperiod and have elongated hypocotyls in light conditions. *pse12 elf3* double mutants regained photoperiod sensitivity in LD and SD conditions, but it is not known if the double mutants regained hypocotyl inhibition. To test if the *pse12* mutation caused *elf3* mutant plants also to regain wild type hypocotyl inhibition, offspring from a *pse12/+ elf3/elf3* self cross were planted in agar plates and grown under both LD and SD conditions until the first two vegetative leaves were visible. Only 12% of plants in LD (8/68) and SD (7/58) had short hypocotyls (Figure 9). This was lower than the expected 25% of offspring with short hypocotyls if *pse12* caused inhibition of hypocotyl elongation.

![Figure 9](image.png)

**Figure 9.** Some offspring from *pse12/+ elf3/elf3* self cross show short hypocotyl phenotype. Seeds were grown for 16 days in either SD or LD conditions. A total of 68 offspring from the *pse12/+ elf3/elf3* self cross were scored in LD and 58 in SD. *elf3*-1 and wild type Col were used as controls.
Genetic Mapping

In addition to elucidating the phenotype of the *pse12* mutation, finding and characterizing the actual gene product was important. To study the *PSE12* gene product, one must know the sequence of the *PSE12* gene. The gene sequence can be determined if the position of the gene is known. To find the location of a gene, genetic mapping was employed, which involves testing linkage with previously mapped markers.

During meiosis, recombination of genes between the maternal and paternal chromosomes occurs. One can measure the distance between genes by the number of recombination events between them. Recombination rate is related to chromosomal distance. In fact, percent recombination is used as a measurement of distance and has the unit of centimorgans (cM). One cM is equivalent to 1% recombination and represents about 100-1000 KBp in the *Arabidopsis* genome (300 KBp near *pse12*) ([The Arabidopsis Information Resource (TAIR) www.arabidopsis.org](http://www.arabidopsis.org)).

Molecular markers make use of recombination events and differences in genotype between different ecotypes. For example, the Columbia (Col) and Wassilewskija (Ws) ecotypes in *Arabidopsis* possess regions of DNA that differ in size or sequence. These differences, called polymorphisms, rarely affect the phenotype of the plant, but their unique features are useful for genetic mapping with PCR based genetic markers.

One type of genetic marker that exploits polymorphic sequences between two ecotypes is called Cleaved amplified polymorphic sequences (CAPS)
markers. More specifically, polymorphic DNA sequences that have a restriction site in one ecotype and not another can be distinguished using CAPS markers. Once the DNA is amplified and cut, it is run on an agarose gel. After gel visualization, one can see that the amplified region in one ecotype has one band and the other, with the restriction site, has two bands (Giraudat et al, 2000).

Simple sequence length polymorphisms (SSLPs) markers work in a slightly different manner than CAPS and take advantage of tandem repeats of 1-3 nucleotides called microsatellites. If two ecotypes differ in the number of microsatellite repeats at a locus, then the locus can be used as an SSLP marker detectable by the different lengths of amplified product.

Primers annealing with SSLP and CAPS markers were used in PCR reactions to amplify specific regions. Once the DNA was amplified, it was run on an agarose gel and ideally, the two ecotypes with polymorphisms at the marker were distinguished from each other (See Figure 10).

**Figure 10.** The SSLP markers nga76 and nga168 run out on a 3% (1:1 metaphor: agarose) TBE gel. Col and Ws are used as controls. The negative control, marked with a dashed line, contains no DNA. Col has more microsatellite repeats than Ws.
in both markers. Lanes with two bands are heterozygous Col/Ws at the \textit{PSE12} locus. A 25-bp DNA ladder (NEB) was used to gauge the size of the bands.

In the \textit{PSE12} mapping experiment, plants derived from two different ecotypes, Col and Ws, were crossed. \textit{pse12 elf3-1} double mutants in the Col background were crossed with \textit{elf3-3} single mutants in the Ws background. \textit{elf3-3}, like \textit{elf3-1}, is a null or greatly reduced function allele of \textit{elf3}. The offspring of this cross, the F1 generation, were self-crossed to produce an F2 generation. The F2 plants flowered in a ratio of 1:2.6 wild type: early. This is close to the expected ratio of 1:3 wild type: early.

To determine linkage to genetic markers, the genotype at the \textit{PSE12} locus was needed. In the F2 generation, only plants homozygous for \textit{pse12} could be genotyped because these plants flowered like wild type. Since \textit{pse12} was only in the Col ecotype, F2 plants that flowered like wild type were homozygous \textit{Col/Col} at the \textit{PSE12} locus.

On the other hand, plants with one or two copies of \textit{pse12} flowered early and could not be distinguished from each other in the F2 generation. To determine the genotype of early flowering F2 plants at the \textit{pse12} locus, these F2 plants were self-crossed to create an F3 generation. If about a quarter of the F3 offspring flowered like wild type, then the F2 parent was determined to be heterozygous (Col/Ws) at the \textit{PSE12} locus, and if all of the F3 offspring flowered early, the F2 parent was determined to be homozygous (Ws/Ws) at the \textit{PSE12} locus.
A total of 68 different F3 pse12 elf3-1 X elf3-3 families were phenotypically analyzed to determine the genotype of the F2 families. The mean number of plants per family was 32 ± 10 plants. An additional 108 F2 families were analyzed and 28 of these were determined to be wild type flowering plants and Col/Col at the PSE12 locus.

The genotype at CAPS and SSLP marker loci was determined and compared to the phenotypes of F3 plants, and this information was used to calculate percent recombination between PSE12 and a given marker. From table 4 it was clear that PSE12 was not linked to any markers on chromosomes I, III, IV, and V, since the smallest percent recombination was 34%. For unlinked genes the percent recombination was expected to be around 50%. The actual percent recombination for unlinked genes in this experiment was 30-40% because heterozygotes were used to increase the size of the mapping population.

**Table 4.** Results from PCR amplifications of genetic markers. The number of mismatches was calculated as the number of differences between the genotypic PSE12 data from F3 plants and each individual marker. The number of genomes was the total number of chromosomes analyzed for a particular marker (two per plant). The percent recombination was calculated by dividing the number of mismatches by the number of chromosomes. The 95% confidence interval (CI) was used to gauge error.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Chrom</th>
<th># Chrom</th>
<th># mismatches</th>
<th>% recombination</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>nga 248</td>
<td>I</td>
<td>36</td>
<td>15</td>
<td>41.6</td>
<td>6.2</td>
</tr>
<tr>
<td>nga 280</td>
<td>I</td>
<td>56</td>
<td>19</td>
<td>34</td>
<td>6.9</td>
</tr>
<tr>
<td>nga 168</td>
<td>II</td>
<td>80</td>
<td>4</td>
<td>5</td>
<td>3.8</td>
</tr>
<tr>
<td>nga 162</td>
<td>III</td>
<td>60</td>
<td>27</td>
<td>45</td>
<td>7.5</td>
</tr>
<tr>
<td>GL1</td>
<td>III</td>
<td>98</td>
<td>36</td>
<td>36.7</td>
<td>9.4</td>
</tr>
<tr>
<td>G3883</td>
<td>IV</td>
<td>44</td>
<td>17</td>
<td>38.6</td>
<td>6.4</td>
</tr>
<tr>
<td>DHS1</td>
<td>IV</td>
<td>40</td>
<td>16</td>
<td>40</td>
<td>6.1</td>
</tr>
<tr>
<td>nga 76</td>
<td>V</td>
<td>46</td>
<td>18</td>
<td>39.1</td>
<td>6.5</td>
</tr>
<tr>
<td>nga129</td>
<td>V</td>
<td>58</td>
<td>24</td>
<td>41.4</td>
<td>7.4</td>
</tr>
</tbody>
</table>
One marker, nga 168, in the table showed linkage to \textit{pse12}. \textit{nga168} was the only marker analyzed that had a percent recombination less than 30% suggesting that \textit{PSE12} may be located on chromosome II. To confirm this assessment, additional markers on chromosome II, including \textit{veo17} and \textit{Athbio2}, were tested; these results (Table 5), confirm that \textit{PSE12} is located on chromosome II.

\textbf{Table 5.} Results from PCR amplifications of genetic markers on chromosome II.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Chrom</th>
<th># Chrom</th>
<th># mismatches</th>
<th>% recombination</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>veo17</td>
<td>II</td>
<td>74</td>
<td>3</td>
<td>4</td>
<td>3.3</td>
</tr>
<tr>
<td>nga 168</td>
<td>II</td>
<td>80</td>
<td>4</td>
<td>5</td>
<td>3.8</td>
</tr>
<tr>
<td>Athbio2</td>
<td>II</td>
<td>68</td>
<td>11</td>
<td>16.2</td>
<td>5.9</td>
</tr>
</tbody>
</table>

All three markers on chromosome II had a smaller percent recombination when compared to markers on other chromosomes. I estimated the position of \textit{PSE12} from this data by comparing the three markers. The percent recombination for \textit{Athbio2} was 16.2 percent, so \textit{PSE12} should be approximately 16 cM on either one side or the other of \textit{Athbio2}. The data from \textit{nga 168} indicated that this marker is much closer to \textit{PSE12} than \textit{Athbio2}, only 5 cM away. Additionally, the \textit{veo17} data suggested that \textit{PSE12} is on the left side of \textit{Athbio2}, because \textit{veo17} appeared to be only 4 cM away from \textit{PSE12}. The region between 64 and 68 cM had overlap from all three markers and is a likely location for \textit{PSE12} (Figure 11).
Figure 11. Overlap of marker data on chromosome II shows the probable location of PSE12. This 4 cM region contains approximately 1.2 MBp of DNA sequence and 9 genes (on the classical map 60-64 cM) (TAIR-www.Arabidopsis.org).

Prior to the experiment, it was not known if the CAPS marker veo17 was polymorphic between Col and Ws. Polymorphisms were found by empirically testing many restriction enzymes. Results from these experiments showed that veo17 DNA was cut by the enzyme PstI only in the Col ecotype. Thus, when veo17 was used and cut with PstI, Col had two bands and Ws had one band.

veo17 was the only successful marker found by randomly cutting PCR products with restriction enzymes. DNA sequencing was used for other markers. The DNA of three other markers was sequenced in the Ws ecotype (University of Maine Sequencing Center). The Ws sequences were then compared to Col sequences in order to find polymorphic restriction sites (K.A. Hicks). This analysis led to the discovery that T27e13 could be cut with the restriction enzyme Nla to produce Col bands of 160 bp, 100 bp, 310bp, and 380 bp and Ws bands of 160bp, 100 bp, and 700 bp.

A third marker, COP1, has known polymorphisms between Col and Ws, which can be detected with the restriction enzyme MSEI (Col- 400 bp, 290 bp;
Ws-380 bp, 325 bp) (TAIR-www.arabidopsis.org). COPI and T27e13 are currently in the initial stages of testing.
Discussion

Mapping PSE12 to Chromosome II

Mapping PSE12 will eventually lead to its characterization because discovering the exact map position is equivalent to knowing the gene sequence. The gene sequence can then be used to predict the function of the gene product based on homology with known proteins.

PSE12 was mapped to 61-69 cM on chromosome II. This 2.5 MBp region was determined by measuring the percent recombination data from the markers nga168, veo17, and Athbio2. Since the data from all three markers complement each other, the map position is believed to be accurate.

This initial map position provides too large an area to pinpoint a gene sequence for PSE12. One way to narrow down PSE12’s position would be to reduce error, and error decreases as the mapping population increases. Leaf tissue from 200 additional plants was collected to increase the mapping population. Another way to narrow the region is to find genetic markers that are closer to the gene. COP1 and T27e13 have been found in the area near PSE12 and initial testing was initiated.

Candidate Genes

There are three genes involved in flowering or hypocotyl elongation that are in the vicinity of PSE12 and could possibly be allelic to PSE12 (Figure 12). The first candidate gene, SUPPRESSOR OF phyA 1 (SPA1), is located at 74 cM
or about 18.9 MBp on chromosome II. From mutational analysis, *SPA1* is thought to be involved in light reception because *spa1 phyA* double mutants have shorter hypocotyls than *phyA* single mutants (Hoecker and Quail 1998). *SPA1* is the least likely candidate gene for *PSE12* because it is located 3 MBp from the projected location of *PSE12* and *spa1* mutants do not display a late flowering phenotype.

The second candidate gene, *DE-ETIOLATED-2 (DET-2)* is much closer to *PSE12* than *SPA1*. *DET2* is located at 68 cM (15.7 MBp) on chromosome II. *det2* mutants have short hypocotyls in dark conditions and express light regulated genes constitutively. *det2* mutants also affect photoperiod responses. *DET2* is not likely to be allelic to *PSE12* because the *det2* mutation does not seem to affect flowering time (Chory et al, 1991).

As its name suggests, *CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1)* is also involved in photomorphogenesis. The hypocotyls of dark grown *cop1* seedlings resemble those in light grown wild type plants (McNellis et al, 1994). *COP1* is located at 63 cM (13.94 MBp) on chromosome II (TAIR-www.Arabidopsis.org). This is very close to the projected area for *PSE12* and in fact, *COP1* is being used as a marker for further mapping of *PSE12*. Based on preliminary mapping data, *PSE12* and *COP1* do not appear to be allelic. Although there is not enough data to accurately determine the distance between *COP1* and *PSE12*, mapping data shows that *PSE12* and *COP1* have several recombination events in a population of only 40 chromosomes.
The most likely candidate gene, *FPA*, is located at 72 cM (17.9 MBp). *fpa* mutants cause late flowering in both LD and SD conditions (TAIR-www.arabidopsis.org). All known alleles have an extreme late flowering phenotype and can flower with 5 times as many leaves as wild type Ler or WS (Schomburg et al, 2001). There is a possibility that *FPA* and *PSE12* are the same gene because mutants of both flower late compared to wild type. On the other hand, *FPA* mutants have much more pronounced phenotypes than the *pse12* mutant. Additionally, *pse12* is believed to be located at least 4 cM away from *FPA*.

<table>
<thead>
<tr>
<th>63 cM</th>
<th>68 cM</th>
<th>72 cM</th>
<th>74 cM</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>COP1</em></td>
<td><em>Likely position of</em></td>
<td><em>DET2</em></td>
<td><em>FPA</em></td>
</tr>
</tbody>
</table>

**Figure 12.** Location of candidate genes in relation to *PSE12* on the bottom of chromosome II in *Arabidopsis*. The location of the genes was based on data from the Lister and Dean RI Map (TAIR-www.arabidopsis.org). The probable position of *PSE12* indicated by the red arrow.

Since no mutations in genes near *PSE12*’s projected region seem to fit the characteristics of *pse12*, we suggest that *PSE12* is a new gene. To strengthen this contention, the region where *PSE12* is located needs to be reduced considerably, and complementation tests with the four candidate genes need to be completed.
**Light reception Pathway**

Preliminary data showed that the *pse12* mutation may have inhibited hypocotyl elongation. 25% of the offspring from the self cross of *pse12/+ elf3-1 / elf3-1* should have had short hypocotyls if *pse12* inhibited hypocotyl elongation, but only 12% of plants in LD and SD had short hypocotyls (Figure 9). It is possible that these offspring with short hypocotyls were homozygous for the *pse12* mutation and that *pse12* caused *elf3* plants to regain hypocotyl inhibition. To determine if this was true, a larger population should be analyzed and all plants with short hypocotyls should be transferred into soil and placed in LD and SD conditions to determine if they flower late. Additionally, all plants with short hypocotyls should be self-crossed and the offspring examined to confirm the *pse12 elf3* double mutant phenotype.

Although the data suggest that *pse12* inhibited hypocotyl elongation, the small number of plants with short hypocotyls in LD and SD caused the data to be less convincing. The scarcity of plants with short hypocotyls could be caused by poor germination rate observed in *pse12* double mutants. Additionally, there were some plants in SD that displayed an intermediate hypocotyl length and these plants were not scored as either short hypocotyl or long hypocotyl. Some of these could in fact be homozygous for *pse12*. On the other hand, the plants with short hypocotyls could be contaminants or could represent an erratic control of hypocotyl elongation in *pse12* mutants. Lastly, two genes may be involved in the short hypocotyl phenotype and this would result in 5% of the offspring with
short hypocotyls. In the future, a larger hypocotyl experiment needs to be scored for more accurate data.

**Implications of a recessive mutation**

Two genetic crosses were used to deduce the nature of the \textit{pse12} mutation. The first cross showed that \textit{pse12} was a recessive mutation. This suggested that \textit{pse12} was a loss of function mutation that caused late flowering in an \textit{elf3-1} background. If the \textit{pse12} mutation caused late flowering, the wild type \textit{PSE12} gene most likely promotes flowering. These data indicate that \textit{PSE12} may work in opposition to the wild type function of \textit{ELF3}, which is to delay flowering. In the flowering time pathway \textit{ELF3} may function by inhibiting \textit{PSE12}, or the two genes could function in separate pathways. Results from the second cross indicated that \textit{pse12} may have a late flowering phenotype.

**\textit{PSE12} late flowering or not?**

The results from the \textit{pse12 elf3-1} heterozygous self cross suggested that \textit{pse12} might be a late flowering mutation. Although 18.75\% of the offspring were expected to flower late if \textit{pse12} promoted late flowering, there were only 11.6\% late flowering offspring in LD conditions and 12 \% late flowering offspring in SD conditions (Figure 8).

The observed ratio of late flowering to wild type flowering may be low for three reasons. The first reason was that \textit{pse12} homozygotes may have had a poor germination rate. This was consistently observed in control \textit{pse12} seed. The second reason for the small number of late flowering plants was that the late
flowering phenotype might be weak. Some plants that were classified as wild type flowering plants could have been homozygous for the \textit{pse12} mutation. To determine the genotype of these plants, an F3 generation must be planted out and scored for flowering. The third reason could be that growth conditions might have affected the flowering ratio. Growth conditions, such as temperature changes and power outages, could have influenced flowering time in both the experimental and wild type plants. For instance, wild type plants flowered earlier than the usual 10-15 leaves in LD and 40 leaves in SD (Thomas and Vince-Prue, 1997). Thus, \textit{pse12} homozygotes could have also flowered early.

\textit{Pse12 elf3} double mutants have been shown to flower like wild type (Figure 5), however, only 2\% of the offspring of the \textit{pse12/+ elf3/elf3} self cross displayed a late flowering phenotype (Figure 7). Given that there were so few late flowering offspring from this cross, these offspring may just be on the late end of wild type flowering. On the other hand, 6.25\% of the offspring would be expected to flower late if two mutations were involved in the late flowering phenotype. The results from the \textit{pse12/+ elf3/elf3} self cross indicated that either \textit{pse12} did not cause a late flowering phenotype in the \textit{elf3-1} background or that a second mutation was involved in the late flowering phenotype.

\textit{Three Flowering Time Models}

Based on my genetic analysis of the \textit{pse12} mutation, there are three possible models that can explain the flowering phenotypes. The first model incorporates the late flowering phenotype of putative \textit{pse12} single mutants and
shows how \textit{PSE12} could affect flowering independently of \textit{ELF3} (Figure 13A). In this model, in wild type plants, \textit{ELF3} and \textit{PSE12} counteract each other in separate pathways to affect flowering. If \textit{ELF3} is mutated, \textit{PSE12} promotes flowering and plants flower early. If both \textit{ELF3} and \textit{PSE12} are mutated, plants flower like wild type. However, when only \textit{PSE12} is mutated, plants flower late because of the inhibitory role of \textit{ELF3}.

The second model (Figure 13B) is identical to the first model, except it incorporates a second unknown mutation that causes late flowering in combination with the \textit{pse12} mutation. In wild type plants, \textit{GENE X} and \textit{PSE12} both promote flowering. In \textit{elf3-1} plants, the combination of \textit{PSE12} and \textit{GENE X} cause plants to flower early. When both \textit{gene X} and \textit{pse12} are mutated in the \textit{ELF3} background, plants flower late.

The third model, where \textit{pse12} does not cause a late flowering phenotype, places \textit{pse12} downstream of \textit{elf3-1} (Figure 13C). In this scenario, wild type \textit{ELF3} inhibits the floral promoting function of \textit{PSE12}. Thus, plants homozygous for the \textit{elf3-1} mutation would flower early since \textit{ELF3} no longer inhibits \textit{PSE12}. Plants that contain both the \textit{elf3-1} and \textit{pse12} mutations would flower like wild type since both genes are non-functional. Finally, in homozygous \textit{pse12} mutant plants, flowering time would be similar to wild type.
A.  
Genotype                  Pathway                                      Phenotype
Wild typ            \[ELF3 \rightarrow\text{Flowering} \leftarrow PSE12\]        Wild type
\textit{elf3}        \[ELF3 \rightarrow \text{X} \rightarrow \text{Flowering} \leftarrow PSE12\]    Early Flowering
\textit{pse12 e}     \[ELF3 \rightarrow \text{X} \rightarrow \text{Flowering} \leftarrow PSE12\]    Wild type
\textit{pse12}       \[ELF3 \rightarrow \text{Flowering} \leftarrow PSE12\]                        Late Flowering

B.  
Genotype                  Pathway                                      Phenotype
Wild type          \[ELF3 \rightarrow \text{Flowering} \leftarrow PSE12\]                                   Wild type
\textit{elf3}        \[ELF3 \rightarrow \text{X} \rightarrow \text{Flowering} \leftarrow PSE12\]\[GENE X\]    Early
\textit{PSE12}\[GENE X\]  
\textit{pse12elf3x}  \[ELF3 \rightarrow \text{X} \rightarrow \text{Flowering} \leftarrow PSE12\]  Wild type
\textit{pse12gene}   \[ELF3 \rightarrow \text{Flowering} \leftarrow PSE12\]                        Late Flowering
\[GENE X\]      

C.  
Genotype                  Pathway                                      Phenotype
Wild type          \[ELF3 \rightarrow \text{PSE12} \rightarrow \text{Flowering}\]    Wild type
\textit{elf3}        \[ELF3 \rightarrow \text{X} \rightarrow PSE12 \rightarrow \text{Flowering}\]        Early flowering
\textit{pse12 elf3}  \[ELF3 \rightarrow \text{X} \rightarrow PSE12 \rightarrow \text{X} \rightarrow \text{Flowering}\]    Wild type
\textit{pse12}       \[ELF3 \rightarrow \text{PSE12} \rightarrow \text{X} \rightarrow \text{Flowering}\]        Wild type

**Figure 13:** Three models representing the possible interactions between \textit{ELF3} and \textit{PSE12}. \textit{PSE12} is placed in a separate pathway from \textit{ELF3} in A and B. B also shows the interaction of an unknown gene, \textit{gene X}. In C, \textit{PSE12} is placed downstream of \textit{ELF3}. Lines with bars on the end represent inhibitors, arrows represent promoters, and X’s represent interrupted pathways.
Although all models have some experimental evidence to support them, two are less likely. The most unlikely model is model 2, where two mutations working in tandem cause late flowering. This is weakly supported based on the 2% late flowering plants in Figure 7. The third model, which suggests that pse12 does not have a late flowering phenotype, is unlikely due to the large number of late flowering plants observed in the pse12 elf3-1 heterozygous self cross in both LD and SD. Thus, the simplest model, which suggests that pse12 is a late flowering mutant, is thought to be most likely. Data from both crosses are consistent with this model.
Works Cited


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