The Effect of Overexpression of SKIP16 on *Arabidopsis thaliana* Reproductive Development

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Abstract: In order to gain a better understanding of the function of *SKIP16*, a gene encoding the F-box protein of the SKP1-CULLIN-F-BOX PROTEIN (SCF) complex, on the reproductive development of *Arabidopsis thaliana*, we conducted an experiment with transgenic plants containing the gene *ASK1:SKIP16*. We did this to see the effect of overexpression of *SKIP16* on the plants to further begin to understand the role of *SKIP16* on *Arabidopsis* development since the *ASK1* promoter ubiquitously drives high expression levels of *SKIP16*. While it is known that SCF complexes impact growth and development in plants, the function of SKIP16 is unknown. We observed that at the T_1 generation, two of the *ASK1:SKIP16* plants had only one terminal flower on each inflorescence stem while many other plants produced either infertile siliques or siliques with reduced fertility. These observations indicate that *SKIP16* plays an important role in Arabidopsis reproductive development. Further research on the function of *SKIP16* may yield crucial information about how SCF F-box protein regulates plant development in Arabidopsis and other plants.

Keywords: *Arabidopsis*, SKIP16, Overexpression, Reproduction, SCF

Introduction

Flowering time in *Arabidopsis thaliana* has been largely researched because it responds to many of the same environmental stimuli as other agriculturally significant plants such as corn and wheat (Mouradov, Cremer, & Coupland 2002). Reproductive development in the *Arabidopsis thaliana* is affected by many factors, such as temperature, environmental cues, and regulatory proteins. These factors all affect the flowering time and fertility of the reproductive buds that form. Regulatory proteins influence reproductive development by affecting gene expression and the abundance and ratio of proteins in the plant. As a major mechanism for protein degradation regulation, a group of enzymes called E3 ubiquitin ligases identifies and ubiquitinates proteins for degradation via the proteasomal pathway (Ardley & Robinson 2005). The types of proteins degraded at particular times are important for regulating many cell functions. One type of E3 ubiquitin ligase is the SKP1-CULLIN-F-BOX-PROTEIN (SCF) complex (Gagne et al. 2002). SCF complexes influence a number of plant functions, such as cell cycle regulation, hormone signaling, and reproductive development. Although it is generally known that SCF complexes regulate plant development, the exact functions of many SCF complex components remain unknown (Ni et al. 2004). *SKIP16* is an F-box protein in Arabidopsis that seemed to have a role in plant development according to our preliminary studies. In order to better understand the impact of *SKIP16* on Arabidopsis development, we created and analyzed transgenic plants that harbored the transgene *ASK1:SKIP16*. The *ASK1* promoter led to an overexpression of the

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SKIP16 gene in the plants. We found that there was a correlation between the overexpression and the reproductive phenotype. These findings suggest that the SCF complex containing the SKIP16 protein plays an important role in regulating plant flowering, number of siliques, and fertility. This could have implications for proteins homologous to SKIP16 in agriculturally significant plants such as corn and wheat.

Methods

A floral dip transformation was performed to insert the transgene into wild-type plants according to the protocol by Davis et al (2009). The seeds from these plants were harvested and dried to be later used in this experiment. The T1 seeds were sterilized in standard procedure using bleach, ethanol, and water in a sterile hood. After sterilization, we planted the seeds on an agar medium containing the MS salts, 1% sucrose, and the antibiotic gentamycin. Gentamycin was a selection agent for the transgenic plants. For effectively spreading the seeds on the surface of the medium, a top agar was used (Figure 1). 160 seeds that were antibiotic resistant were transplanted to soil two weeks after plating. The plants were allowed to grow until they finished reproductive growth, i.e., the apical meristem on the main stem had stopped producing new buds. The number of fertile and infertile siliques on the main stem were counted and compared to wild-type plants to identify differences in fertility.

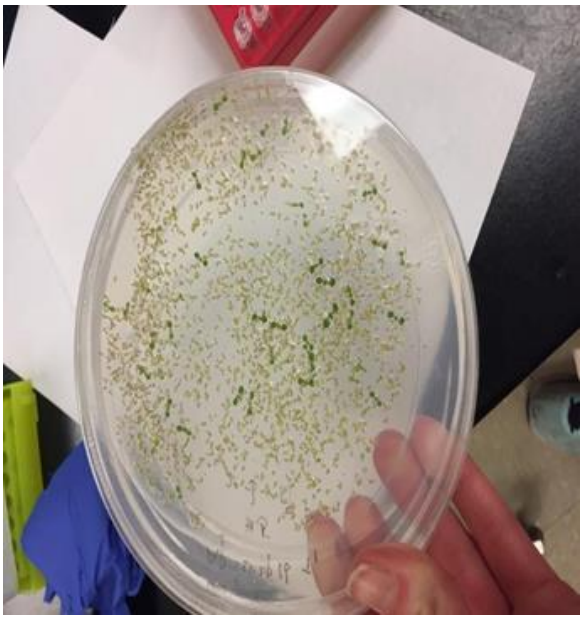

Figure 1 - One of the five gentamycin plates performed to determine insertion

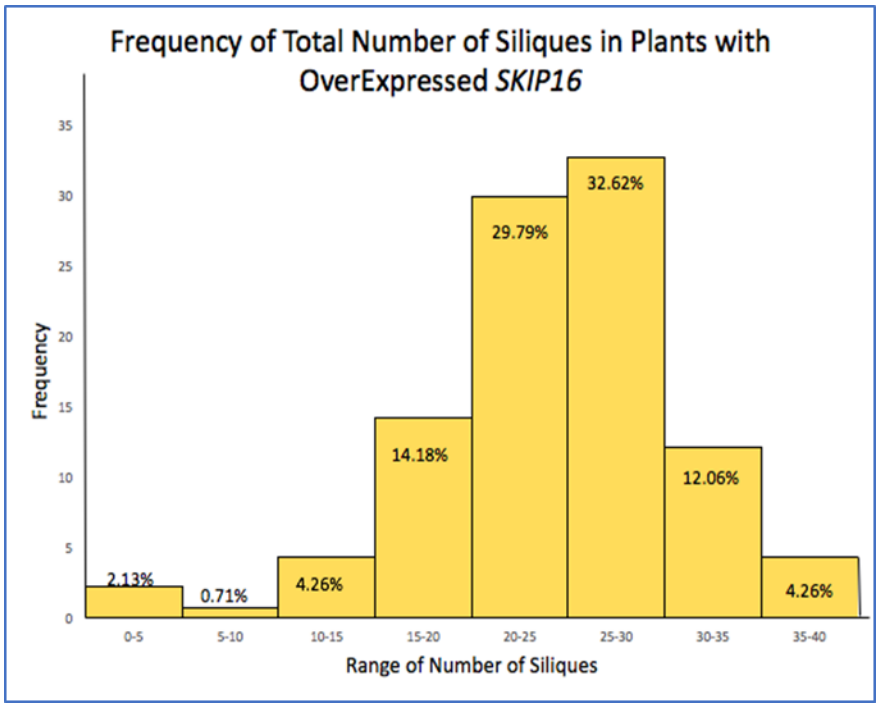

Figure 2 - Frequency of number of total siliques in transgenic plants. The average for total siliques in the wild-type plants we observed was 28.0

Results

At the end of each T_1 plant's reproductive growth stage, the siliques were counted and identified as either fertile or infertile. Figure 2 and Figure 3 show the distribution of the numbers of total siliques and infertile siliques per transgenic plant. We compared these numbers, as well as the plants' morphology, with 3 wild-type plants to see how the overexpression of *SKIP16* affected the plants' appearances and ability to reproduce. Compared to the wild-type plants, 62.41% of transgenic plants were within the range of total silique number observed in wild-type plants and 64.09% of transgenic plants were within the range of the number of infertile siliques as the wild-type plants. The rest fell outside the range observed in the wild-type plants. Phenotypically we observed many different responses to the insertion of the promoter *ASK1*, ranging from heavily affected (Figure 4), to moderately affected (Figure 5), to mildly affected (Figure 6). The cause of this range of response to the overexpression is most likely because of the location of the insertion of the mutated gene. Floral dip transformations randomly insert genes into the plants' genomes, and different locations could cause the gene to be expressed at different levels. In total, there were 5 heavily affected plants observed, 3 that produced completely infertile siliques and 2 that produced only a terminal silique on each inflorescence stem.

Discussion

After observing the phenotypes of the hemizygous transgenic plants, we determined there was indeed a negative effect on reproductive development compared to wild type plants. This was shown by plants such as the ones in Figures 4, 7, and 8 which were heavily affected by the overexpression, and produced almost all infertile siliques or only a terminal silique on each inflorescence stem. Although the frequency of total number of siliques and infertile siliques showed that most plants fell into a normal range compared to wild-type plants, 37.59% of transgenic plants had an abnormal range of total siliques and 35.91% of transgenic plants had a higher number of infertile siliques than the range shown by the wild-type plants. This shows that the overexpression of *SKIP16* can lead to a significant number of plants displaying abnormal reproductive development in *Arabidopsis thaliana*. The implications of

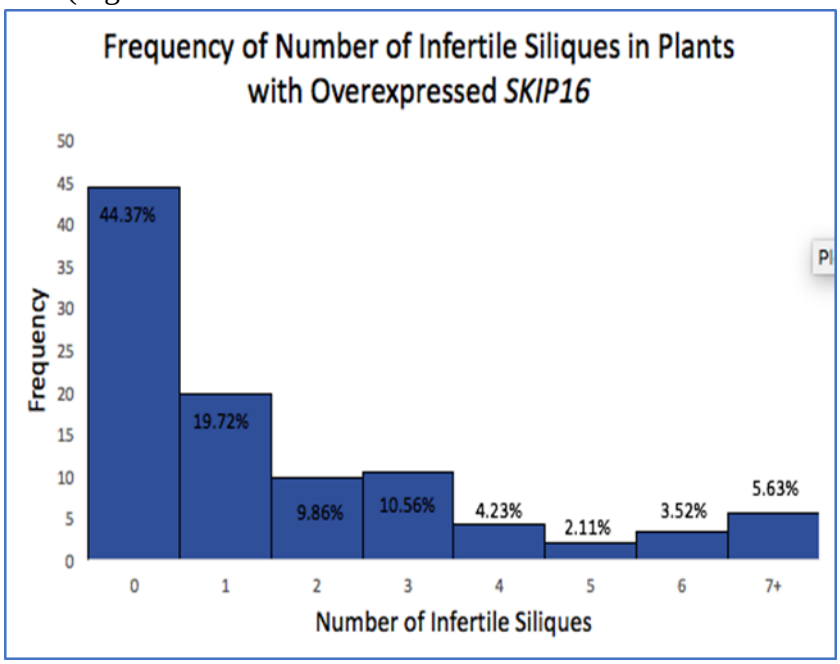

Figure 3 - Frequency of number of infertile siliques in transgenic plants. The average for infertile siliques in the wild-type plants we observed was 0.667

Figure 4 - Heavily Affected infertile or no siliques Wild-Type on far left for comparison

Figure 6 - Mildly Affected, almost all fertile siliques (0-1 infertile) Wild-Type on far left for comparison

Figure 5 - Moderately Affected infertile and fertile siliques present wild-Type on far left for comparison

Figure 7 - Heavily affected plants with no fertile siliques present

this are that it could affect the reproductive capability of agriculturally significant plants such as corn or wheat, which also contain SCF complexes. The seeds from this T_1 generation have been harvested and will be used in further research to study the impact *SKIP16* on reproductive development.

Literature Cited

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Figure 8 - Heavily affected plants with no fertile siliques present

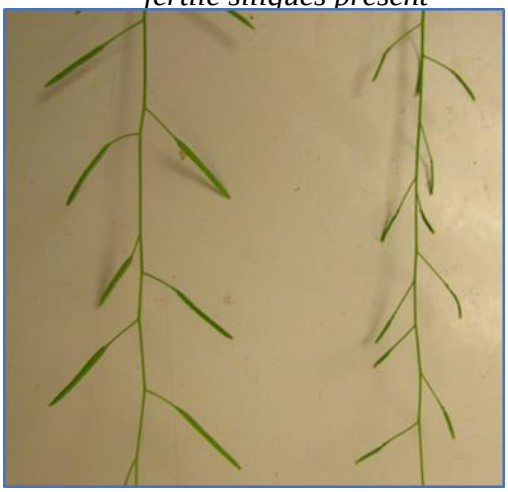

Figure 9 - Comparison of Fertile Siliques from Wild-Type plant (left) to Infertile Siliques from transformed plant (right)

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