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Evaluating Effects of Gene Mutations and Light Intensity on Arabidopsis thaliana Development

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ABSTRACT

Arabidopsis thaliana is a model organism often compared to commercial crops. The completion of sequencing *A. thaliana's* genome has led to the next crucial challenge of determining gene function in these plants. The discovery of gene function within these plants will provide insights on how gene function can affect commercial crop production. This work compared wild-type Columbia (Col-O) *A. thaliana* to single gene mutants VPI/ABI3-like 1 (VAL1*)* and basic region/leucine zipper motif (bZIP*).* These single gene mutations may affect several traits that, in turn, can result in morphological changes and/or time of development in seedlings. Wild-type and mutant plants were grown side by side, and we measured the time of bolt and formation of reproductive parts under both similar light intensities and conditions of varying light intensity. *Val1* and *bZIP67* mutants indicated extended developmental time through a delayed time of bolt as compared to wild-type plants. Under different light intensity ranges measured by photosynthetic photonic flux or PPF, (62-96 PPF, 107-130 PPF, and 117-143 PPF) there was a significant difference in the developmental growth of each plant, mainly in the length of time it took for the plant to bolt. The production of reproductive parts was less within mutant plants (51.45±18.72 reproductive parts for *val1* and 54.31±21.4 reproductive parts for *bZIP67*) as well compared to wild-type (35.05±18.88 reproductive parts), resulting in lower numbers of siliques and flowers after five weeks. This suggests that maturation and overall growth is stunted or prolonged when suppressing genes such as VAL1 and bZIP67 occur in *A. thaliana*. Both types of mutant plants experienced extended lives as compared to the wild type.

INTRODUCTION

Arabidopsis thaliana is a model organism used in many plant biology research studies. The availability of extensive genetic and physical maps of its chromosomes, a short life cycle, and marked growth stages make this plant ideal for these studies. Analyzing and comparing the phenotypic differences between wild type plants and mutants can determine the function of a particular gene. Single gene mutations may affect several traits that in turn can result in morphological changes and/or the timing of development (Boyes et al., 2001). The transcriptional regulator VPI/ABI3-like 1 (VAL1) is one example of a gene that controls developmental processes in *Arabidopsis.* This *val1* gene has been identified to suppress the seed maturation program prior to germination, which involves embryo differentiation in an ungerminated seed (Sharma et al., 2013). In further detail, it has been shown to suppress the accumulation of seed storage compounds known as seed storage proteins, or SSPs, within the seed (Sharma et al., 2013). These SSPs mainly provide a store of amino acids used during germination or seedling growth (Shewry et al., 1995). The fact that SSPs are suppressed is of importance because the pathway that affects accumulation of these proteins could also cause phenotypic differences, especially the timing of development between the WT and *val1*.

Previous studies by Tsukagoshi, Morikami, and Nakamura (2007) analyzed both *val1* and *val2*, which belong to the B3 family of transcription factors. The B3 family is a plant-specific DNA-binding domain that includes all identified *VAL* genes. In *val1* and *val2* double knockout mutants, seed maturation genes are highly expressed during seedling development and lead to shriveled seedlings with reduced seed storage compounds (Suzuki et al., 2007). Suzuki and colleagues (2007) showed phenotypic variations such as no leaves in *Arabidopsis* with *val1 val2*

double-mutant seedlings. The growth and phenotypic variation continued within triple mutants of *val1*, *val2*, and *val3* as growth was slowed and stunted shortly before the emergence of the cotyledons. Studies have also been done involving triple knockouts, but single mutants appear to lack obvious phenotypic differences from wild type. At least one copy of any *VAL* gene is needed for normal seedling development, which requires the repression of seed maturation genes by VAL1 proteins. Normal growth still occurs in single knockouts of *val*, resulting in the hypothesis that VAL1 plays an important role in the seed maturation of *Arabidopsis.* Since normal growth still occurs, the function of the *VAL1* gene can be studied by looking at phenotypic characteristics. It leads to the questioning of what role does this gene plays within *A. thaliana*?

Another family of transcription factors that functions in plants is the basic region/leucine zipper motif (bZIP) transcription factor family. These transcription factors are classified based on a DNA binding domain at the leucine zipper. One such bZIP transcription factor, bZIP67, has also shown the ability to decrease the amount of seed storage proteins in plants, while at the same time there is no difference in total protein content when compared to wild-type (Mendes et al., 2013). Therefore, other phenotypic differences such as time of bolt and the number of reproductive parts produced can also be affected by the knockout of this transcription factor. Furthermore, *bZIP* is known to play key roles in regulating processes including pathogen defense, light and stress signaling, seed maturation, and flower development (Jakoby et al., 2002). Previous studies looked at specific bZIP proteins and have shown their effects on the plants' light-responsive promoters in mediating light control where these promoters are activated under specific wavelengths of light (Chattopadhyay et al, 1998). This suggests that light intensity

may affect *A. thaliana* bZIP67 mutants. Will a change in light intensity have a greater effect on *bZIP67* mutants than wild-type? Does this also hold true for *val1* mutants?

In this experiment, the phenotypes that were screened were involved in the flowering and bolting stages of development. These phenotypic characteristics were compared between the wild type (WT) and the mutant (*val1*) *Arabidopsis* plants to analyze the gene function of *val1*. The experiments were then repeated to compare WT to *bZIP67* mutants. This study also looked at how different light intensities affected WT and the two mutant genotypes. The time of bolting, flowering, and stages of development were observed to test light's effect on these phenotypic characteristics of *val1* and *bZIP67* compared to WT. The reason for looking at these relationships comes of importance in understanding how the plant is allocating its energy and time. *Arabidopsis* is an annual plant and produces seeds at one time before its life ends. Looking at relationships between length of its life cycle and the number of reproductive parts can indicate if these mutants hold any benefit in producing more seeds by having a longer life cycle. Increased seed production can result in more seed oil and therefore the capability to produce more biofuels per plant, which is the main goal of this study. Counting reproductive parts within both mutants (*val1* and *bZIP67*) allowed for the determination if late bolting or possible elongated bolting time had an effect on the number of siliques and flowers the plant produced. Furthermore, by changing the amount of light intensity, we investigated if there are limitations to the amount of light the plant absorbs or if those intensities benefit one genotype over another.

MATERIALS AND METHODS

Bolting Time/Reproductive Parts for *val1* vs. WT

The procedure of this experiment mainly consisted of plant growth and collecting phenotypic documentation. Forty of both seed types (kindly provided by the Collakova lab at Virginia Tech) were grown under the exact same conditions: sown in Germination, Professional Formula soil (Farfard, Sun Gro Horticulture and Technigro, Agawam MA) within 4-inch, plastic, square pots with 4 seeds in each corner. The seeds were stratified for 3 days in a dark, controlled environment with a temperature of 4°C, and then moved into a long-day growth chamber (Norlake Scientific Environmental Chamber, Hudson, WI). The long-day growth chamber was maintained at 23°C and allowed the plants to have 16 h of light and 8 h of dark. The light range was maintained as consistent as possible and ranged from 87-118 PPF when measured from each corner of the growth trays. Approximately every two days, the plants were bottom-watered simply with tap water. When the siliques began to burst and dry, water was halted so that the plants could be bagged to collect seeds.

Two replicates were performed at staggered time intervals. Data were collected around every 2-3 days by marking down the stage (1.08, bolt, 6.0, 8.0), specifically time of bolt, and/or number of flowers present after bolting. Stages 1.08, 6.0, and 8.0 represent the stages when the plant had a rosette of eight leaves, bolted, the first flower opened, and the siliques ripened, respectively (Fig. 1). The number of reproductive parts (siliques + flowers) were counted after 5 and 6 weeks from germination.

Bolting Time/ Reproductive Parts for *bZIP67* vs WT

The growth conditions for the bZIP and WT were the exact same as the experiments done on the mutant *val1*. The only difference was the number of samples: 20 samples of both bZIP and WT were grown side by side. In this experiment, only two seeds of either sample type were sown in either corner of the 4in. square-pot, and the light range varied from 72 PPF-97 PPF as it was maintained at levels as close to previous experiment with *val1* as possible. Stages of life cycle (1.08, bolt, 6.0, 8.0), bolting time, and reproductive parts were collected similarly to the previously detailed experiment using the *val1* mutant. It is important to note that while 20 seeds of each phenotype were planted, each consisted of 4-5 seeds not germinating.

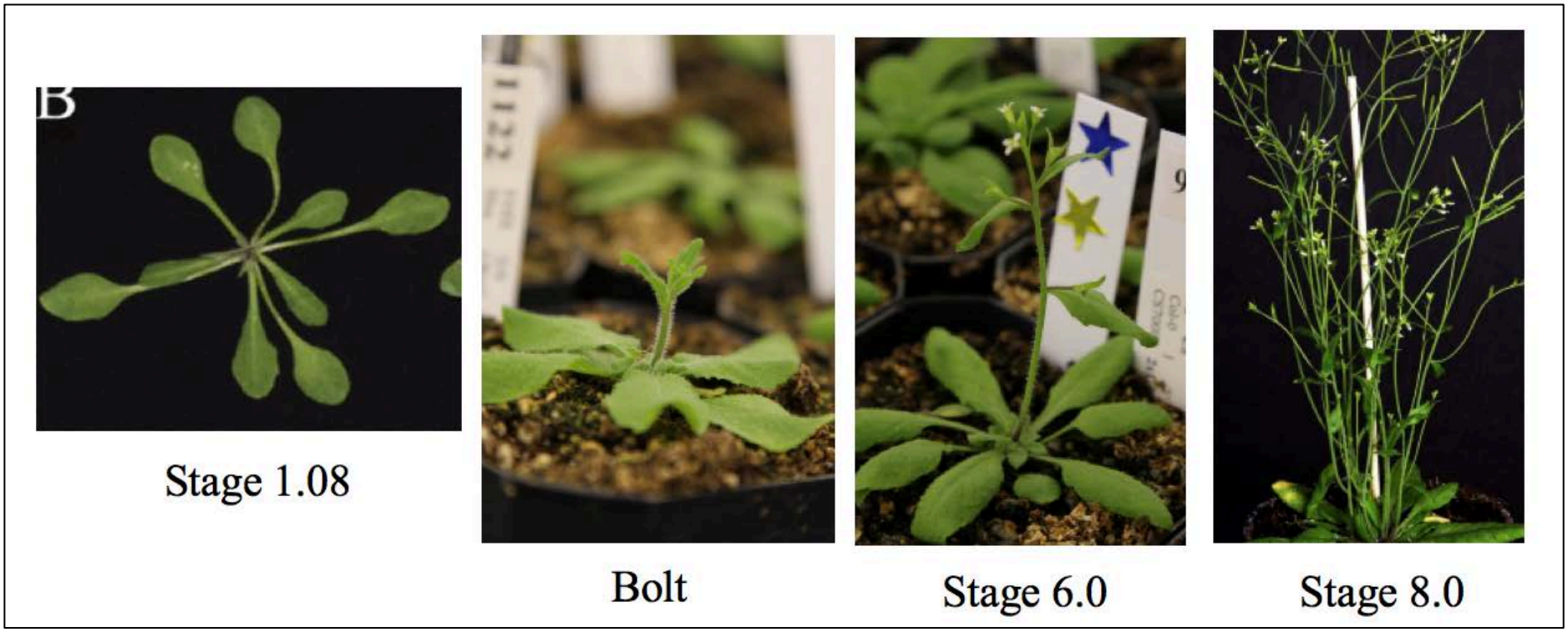

Figure 1: Stages of growth of *A. thaliana* are 8-leaf rosettes (1.08), time of bolt, flower emergence (6.0), and silique ripening. (Bollman et al., 2003) (Silverstone et al., 2007)

Light Intensity of *val1* vs. WT / *bZIP67* vs WT

To test the effects of light intensity, two trays were staggered with WT and val1/bZIP pots consisting of 20 of each strain in each of the trays. The WT was run separately side-by-side for each mutant gene type. The trays were stratified in similar manner as before and placed in different areas of the growth chamber, ranging in light intensity of low (below 90 PPF), medium (approximately 90-110 PPF), and high (above 115 PPF). The medium light in this experiment was consistent to the light intensity performed under normal light conditions detailed in methods above. For this experiment, time of bolting was the main phenotypic characteristic observed. At the same time, other growth stages were observed and marked to compare the overall life cycle of both WT and val1. This measured by recording the date in which each specimen was in these stages. This process was repeated to compare bZIP to WT under varying light conditions as well.

RESULTS

val1 vs. WT

Under similar light conditions (87-118PPF), the overall trend of developmental stages of val1 versus WT indicated that the val1 plants reach the specified growth stage more slowly than the WT plants (Fig. 2). Both genotypes follow a similar initial growth trend and do not show a difference in developmental stage timing until getting to the stage of bolting. From bolting to emergence of flowers, *val1* plants' growth began to slow down when compared to the WT plants' developmental stage.

Focusing on the time of bolting allowed for a more detailed analysis of a specific developmental stage. Figure 3 shows the results of the time of bolting under identical light conditions for each treatment, and the data represent three separate trials of the same experiment that allowed for a total of over 60 samples per genotype. This figure also indicates and paired

statistical tests show that there was a significant difference between gene types. The WT averaged 23±2.9 days to bolt and the val1 averaged 26±2.4, once again showing that the time it takes to get to this developmental stage is longer for val1 individuals.

Figure 3 illustrates the effect of different light intensities. There was significant difference between the genotypes under every treatment (Table 1). Under all three treatments, the number of days to bolt was longer for val1 (Fig. 3). Low light intensity and medium light intensity showed a smaller margin of difference than that of high light intensity. Furthermore, Table 1 shows the results from a two-way ANOVA with replication test. A p-value of 2.79×10^{-52} indicates significant difference between days of bolting of val1 versus wild type under all three treatments since the p-value is below 0.05. The basic trend is similar for both genotypes even when increasing the light intensity and appears to be hypersensitive to high light intensities.

The number of reproductive parts for 20 specimen of val1 and 20 WT were counted after 5 and 6 weeks of plant growth. Figures 4a and 4b show the average number of both flowers and siliques at these time intervals. At week 5 there was a significant difference between WT and val1 in the number of reproductive parts, but after another week there was no significant different between WT and val1 plants. The gap between reproductive parts of these two genotypes decreased after a week’s time.

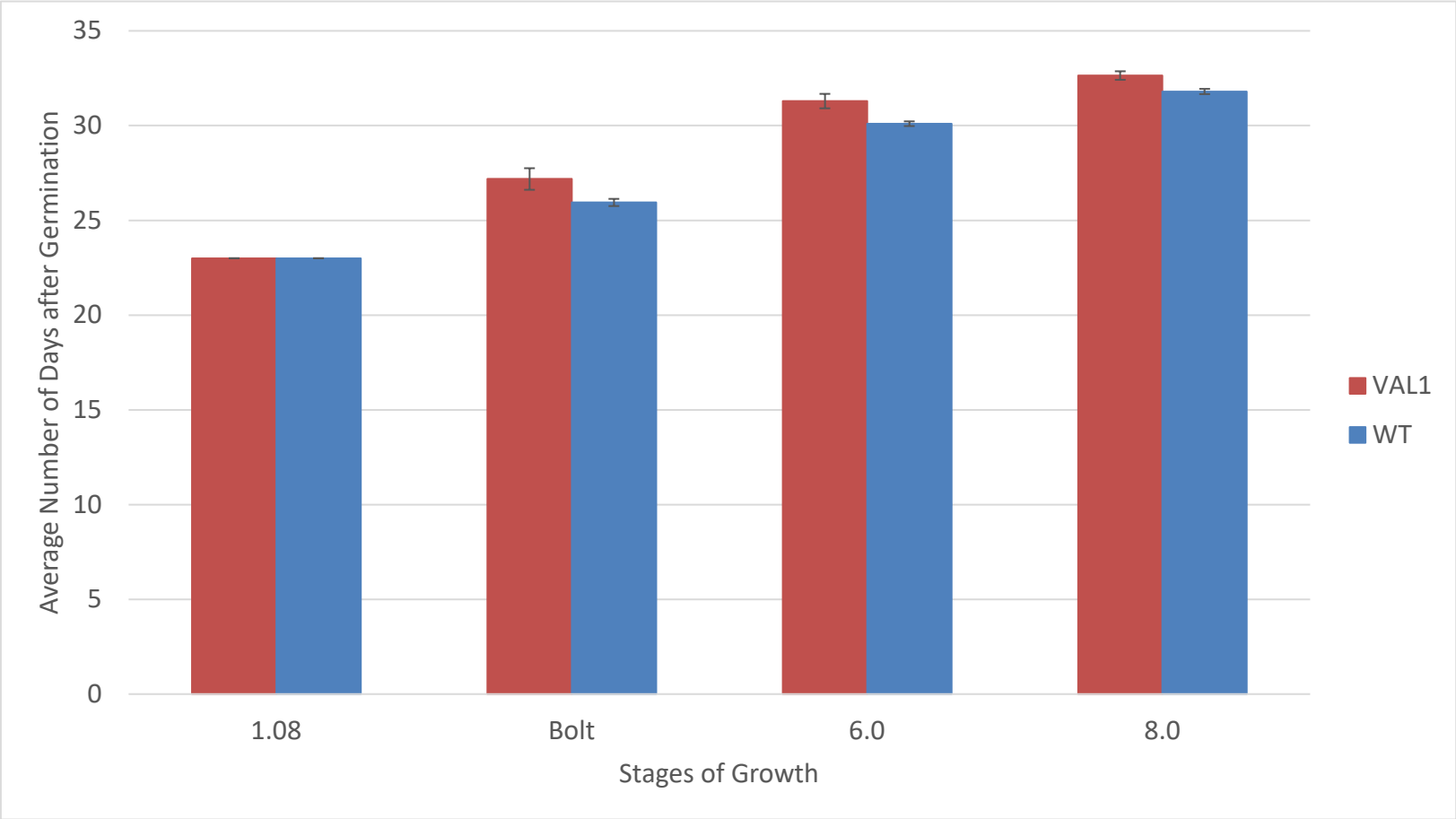

Figure 2: Stages of growth compared between mutant and wild type. This graph looks at stages when there are 8-leaf rosettes (1.08), time of bolt, flower emergence (6.0), and silique ripening.

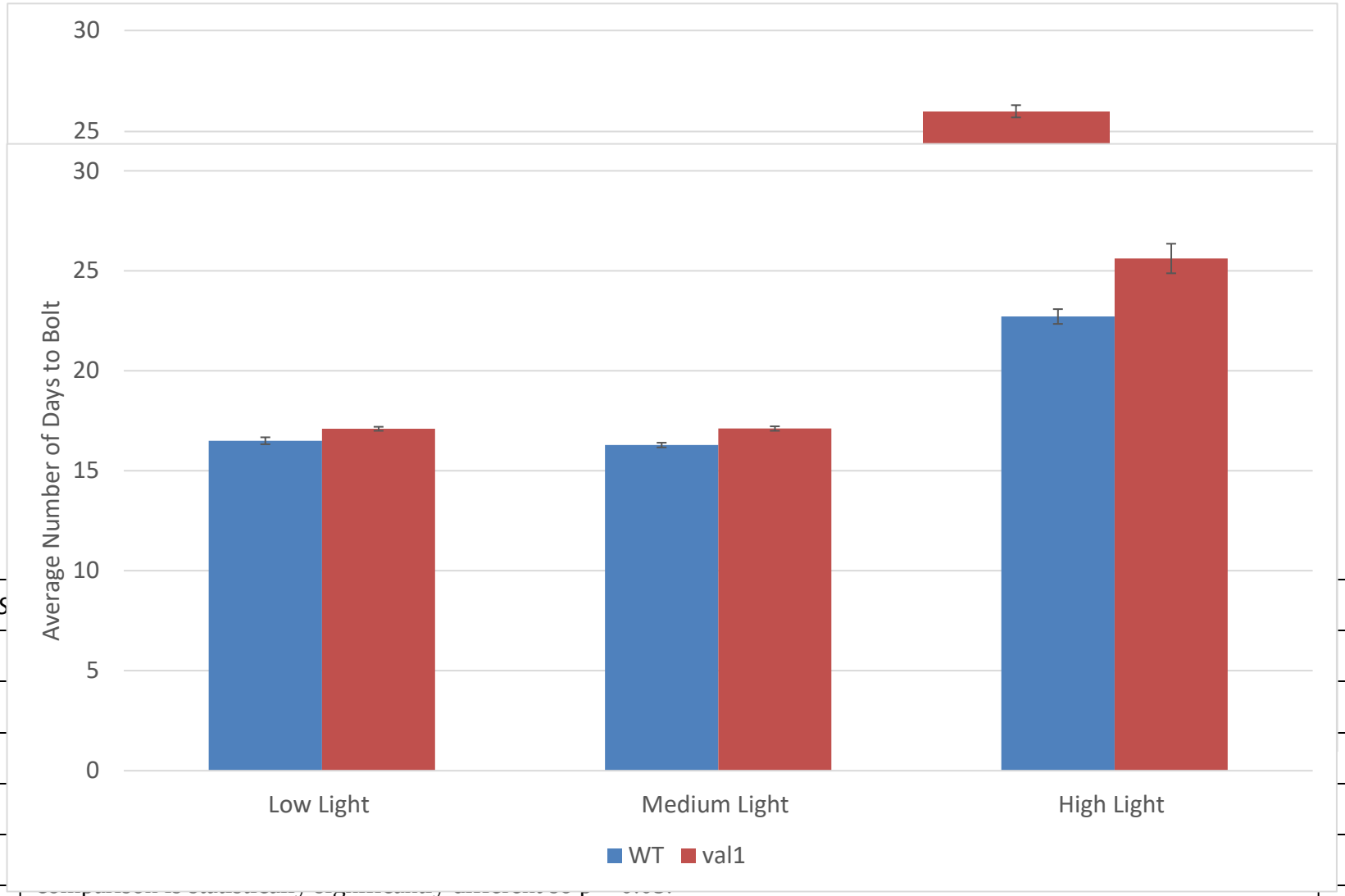

Figure 4: Comparison of the average number of days to bolt for both WT and val1 under low (62-96 PPF), medium light (107-130PPF), and high light (117-143PPF). Error bars signify the standard error of each sample, with 14 seeds averaged per sample type/genotype.

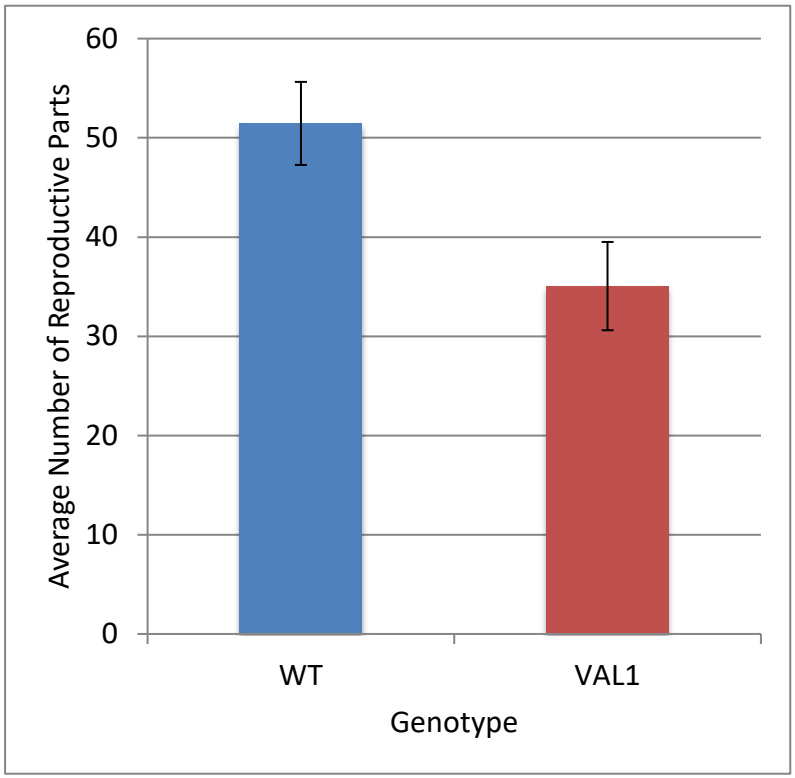

Figure 5a. The average number of reproductive parts (flowers and siliques) under light of 73 PPF after 5 weeks for 20 specimens of val1 and WT. Error bars represent standard error and significant difference under $p<0.05$ based on ANOVA.

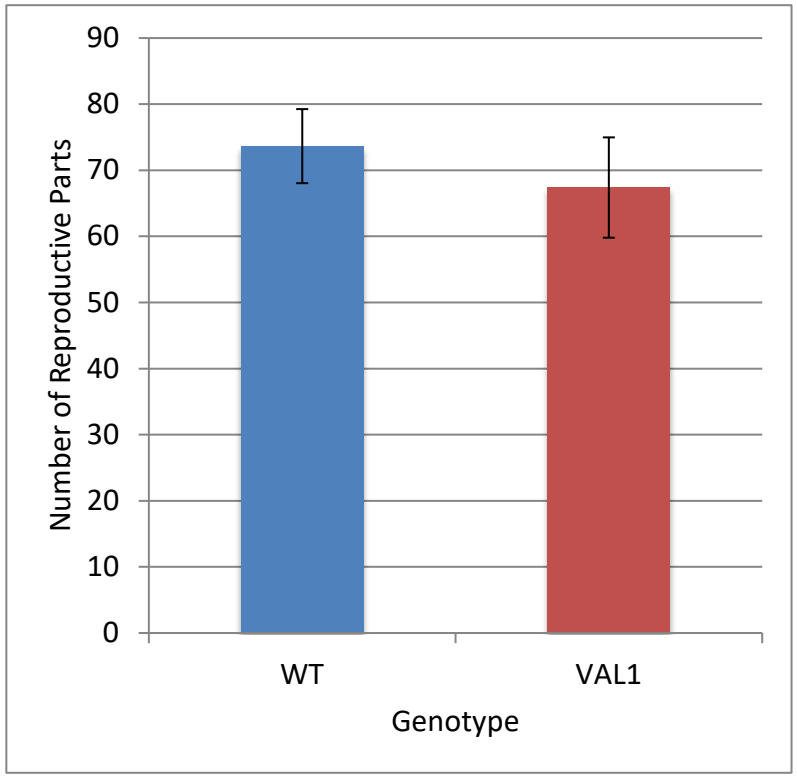

Figure 5b. The average number of reproductive parts (flowers and siliques) under light of 73 PPF after 6 weeks for 20 specimens of val1 and WT. Error bars represent standard error and significant difference under $p<0.05$ based on ANOVA.

bZIP67 vs. WT

Under similar light conditions (average of 72 PPF) *bZIP67* and WT plants were grown side-by-side to measure general life stages, bolting time, and number of reproductive parts. The growth rate of *bZIP*67 appears to be on average three to four days behind the WT up until bolting, and this gap gets smaller as the plants continue growth processes (Fig. 6). Figure 7 more closely shows the comparison of bolting time between the mutant *bZIP67* and WT, as there is a three-day difference at this stage.

The trend for the experiment on altered light intensity was that the number of days to bolt for plants decreased as the light intensity increased (Fig. 8). At the same time, the mutant plants were still showing a slowed growth rate as at every light intensity: *bZIP67* took longer to reach the bolting stage. It appears that *bZIP67* mutants grew faster under low-light conditions than in high-light conditions, as measured by the time to bolt. The two-way ANOVA further shows there was a significant difference (Table 2).

Similarly, to the comparison of reproductive parts in val1 to WT, the *bZIP* mutants showed fewer flowers and siliques at both recorded intervals. There was a significant difference in reproductive parts' production at week 5 of the plants life cycle (Fig. 9a). On the other hand, after another week, this growth gap closed up, and at week 6, there was no significant difference in the numbers of reproductive parts between the genotypes (Fig. 9b).

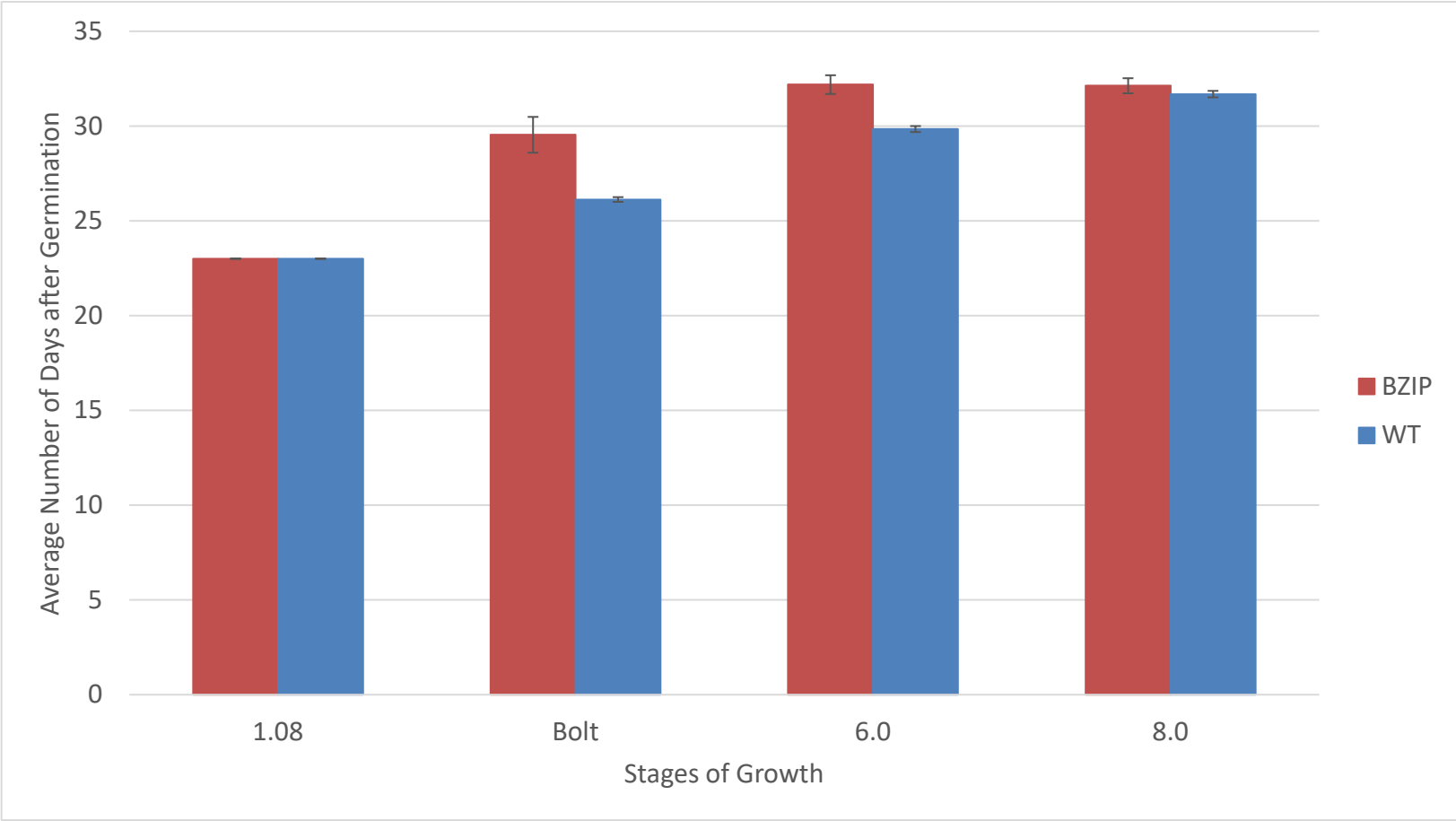

Figure 6: Stages of growth for mutant and wild type plants. This graph looks at stages when there are 8-leaf rosettes (1.08), time of bolt, anthesis (6.0), and silique ripening (8.0) for 20 specimen of each phenotype.

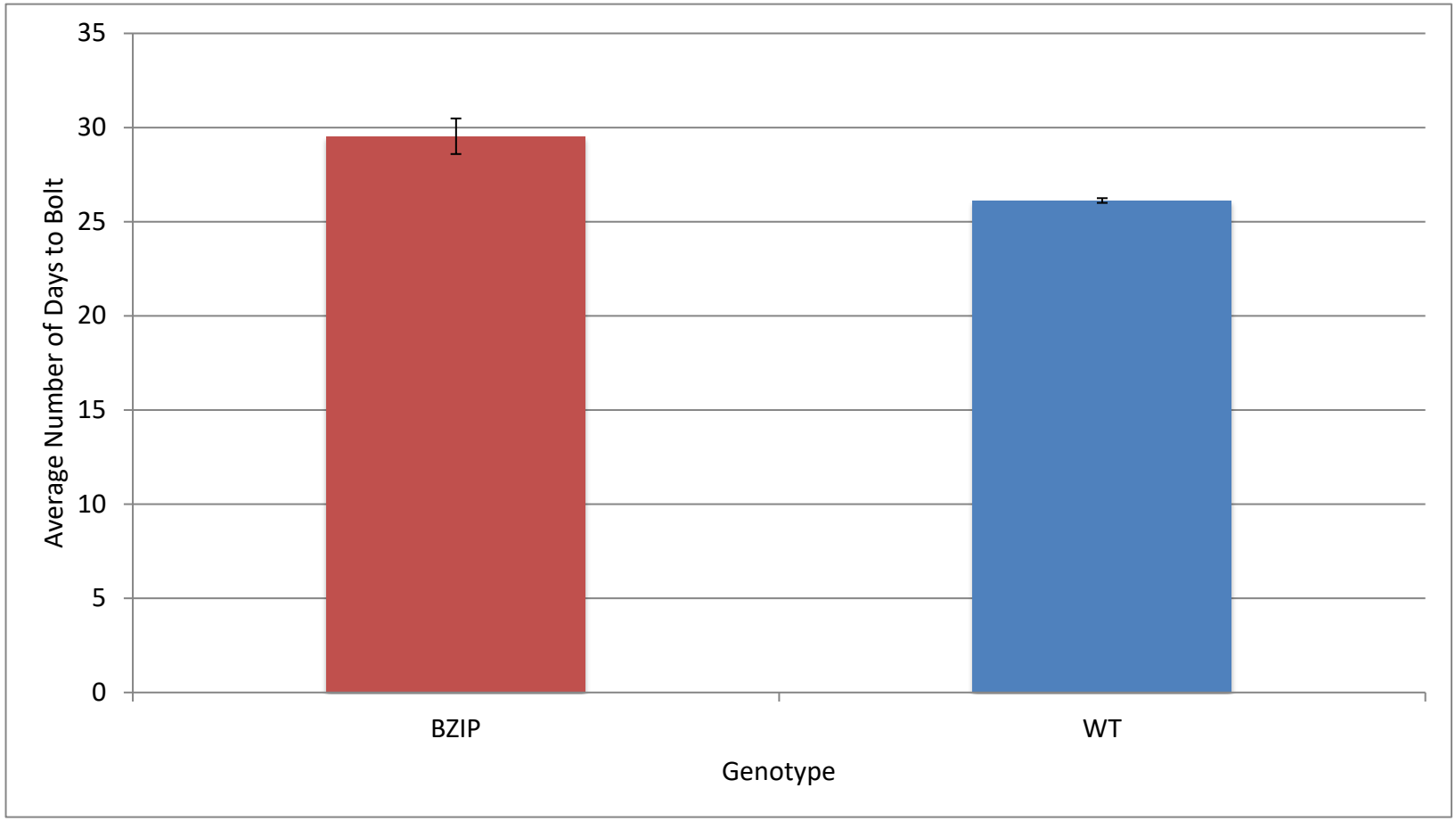

Figure 7: Comparison of the average number of days to bolt for both WT and BZIP. Data was taken from 20 samples per treatment. Error bars signify standard error with significant difference $p<0.05$ as determined by ANOVA.

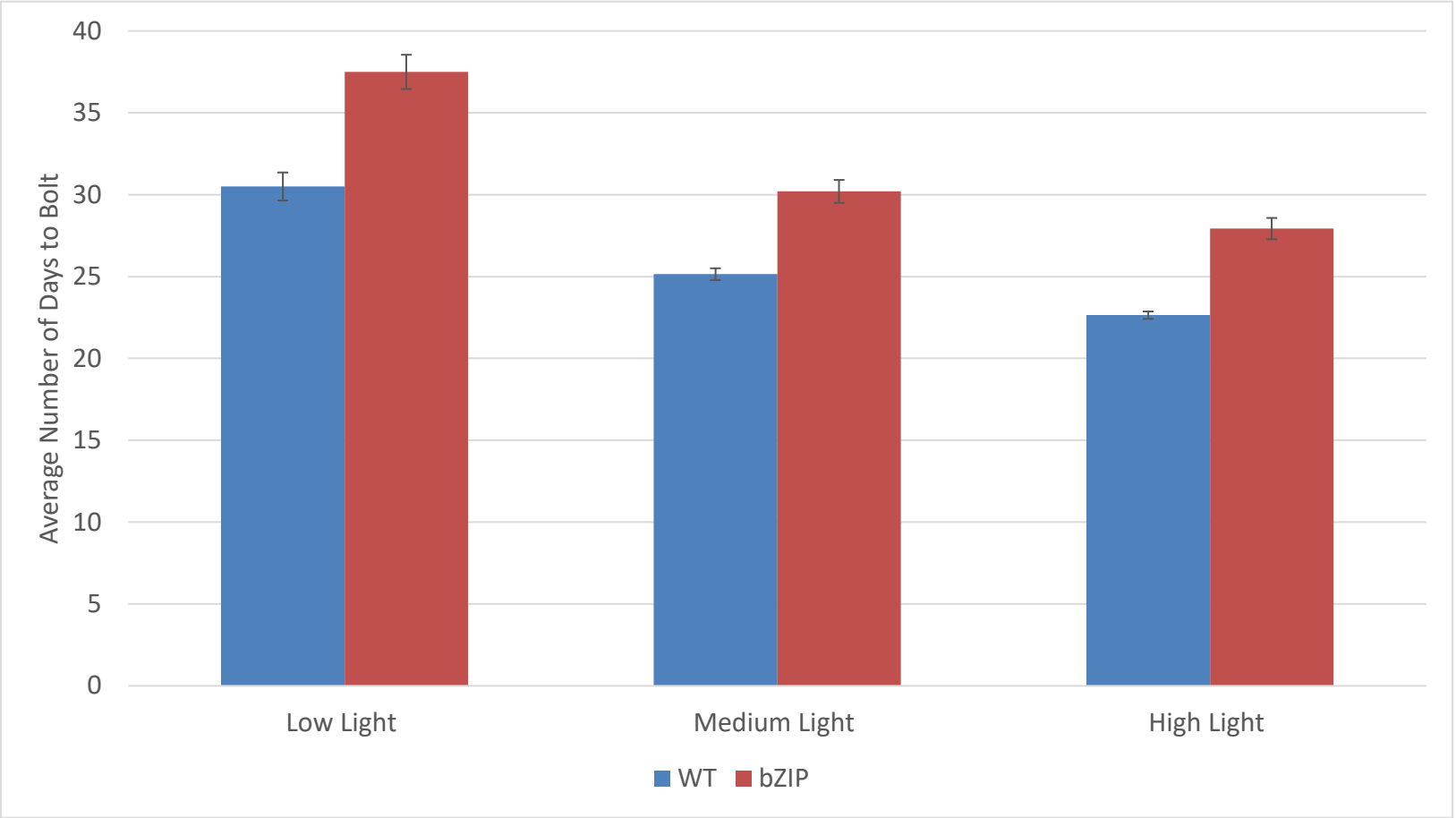

Figure 8: Comparison of the average number of days to bolt for both WT and *bZIP* under low (55-85 PPF), medium light (78-109 PPF), and high light (110-137PPF). Error bars signify the standard error of each sample with 14 plants averaged per sample type/genotype.

Table 2: Two-factor ANOVA replication test results on genotype and light intensity. P-values show significant difference for all treatments.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F critical*
Sample	898.9047619	5	179.780952	34.9698611	7.0681E-24	2.272137291
Columns	31652.59524	1	31652.5952	6156.86391	2.858E-127	3.901760738
Interaction	704.9761905	5	140.995238	27.4255077	7.4577E-20	2.272137291
Within	802	156	5.14102564			
Total	34058.47619	167				

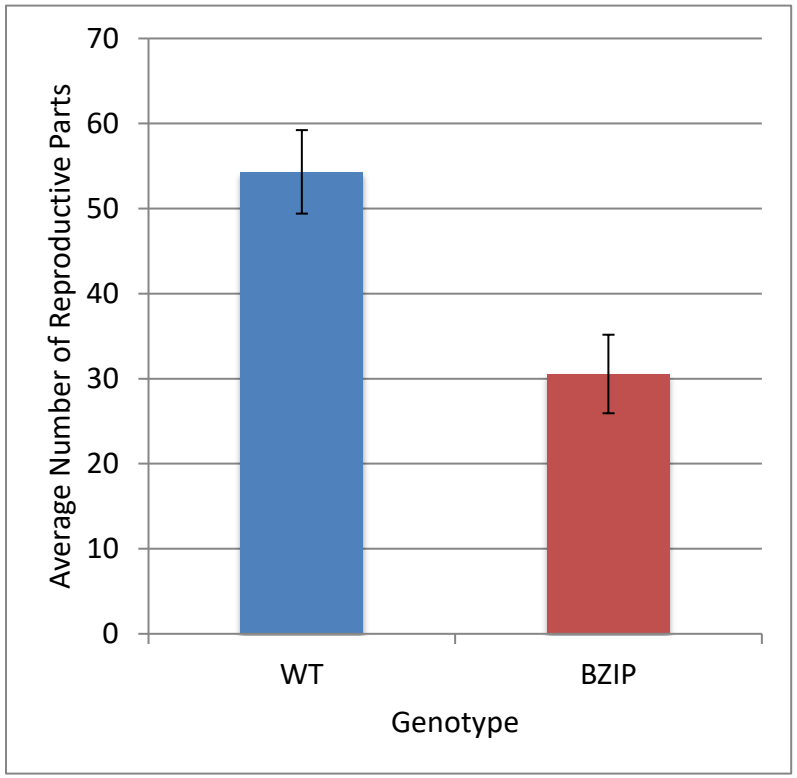

Figure 9a. The average number of reproductive parts (flowers and siliques) under light of 73 PPF after 5 weeks for 20 specimens of *bZIP* and WT. Error bars represent standard error.

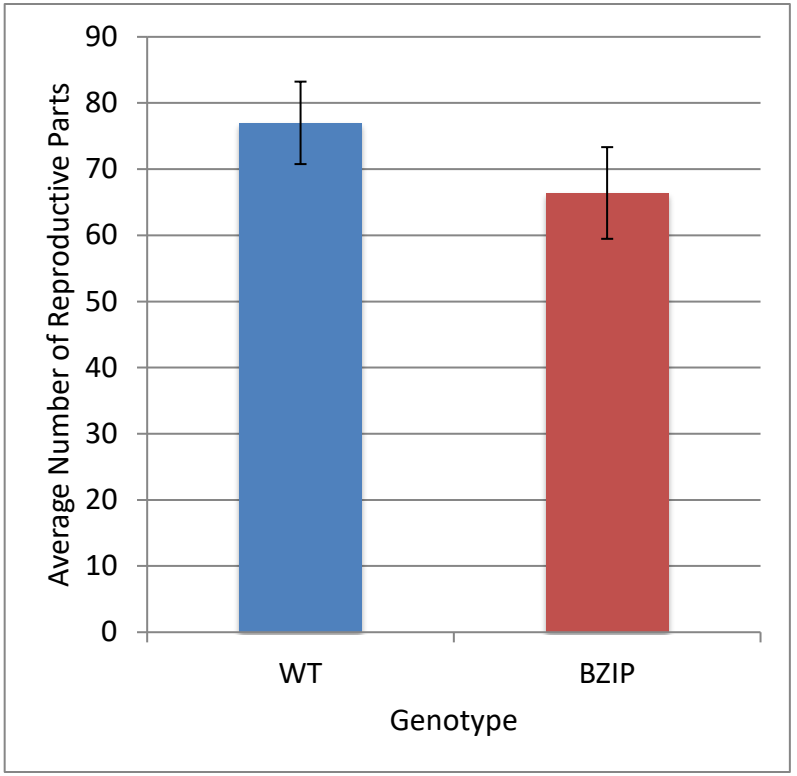

Figure 9b. The average number of reproductive parts (flowers and siliques) under light of 73 PPF after 6 weeks for 20 specimens of *bZIP* and WT. Error bars represent standard error.

DISCUSSION

The two genes, *val1* and *bZIP67,* that were knocked out within seed lines of the model organism, *Arabidopsis thaliana,* have been shown to lead mainly to slower growth. When growing these mutants side-by-side with the WT strain, COL-0, there were obvious phenotypic differences. Plants with mutant *val1* had delays in bolting time that then led to more delays in other stages past bolt. It overall led to more time spent before the plant grew vertically and produce its reproductive parts. This falls in line with previous studies that have indicated a knockout of *val1* led to slower growth (Sharma et al., 2013). This was also seen in the mutant *bZIP67* knockout plants as they were delayed in reaching the bolting and reproductive stages as well. When comparing the two mutants next to each other, there is no significant difference in one mutant taking longer time to bolt than the other, but they did both take longer time to bolt than that of the WT.

Light intensity did play a role in altering the growth of both the mutant and WT plants. The increase in light intensity showed a significant difference in the time it took these plants to bolt. The general trend held true though for both the WT and mutants throughout as the mutants remained slower in time to bolt than the WT. The more pertinent information came from the experiment on counting the number of reproductive parts each genotype produced. More reproductive parts produced correlates with an increase in the number of seeds per plant, provided that all genotypes' siliques remain producing similar numbers of seeds. While this experiment did not count the number of seeds per silique, further research could determine if these numbers were different between the genotypes. It would be viable to see the number of seeds per silique that have embryo in them to show the overall seed production per genotype. However, based on the number of reproductive parts, both *val1* and *bZIP67* had fewer

reproductive structures' counts after weeks 5 and 6 compared to WT. Therefore, the plants with delayed maturation (mutants) are also producing fewer siliques. A longer life cycle produced fewer siliques, which could mean fewer seeds unless the plants are allocating additional energy during the time delay to produce more seeds per silique. This is similar to previous studies by Mendes (2013) and Sharma et al. (2013) that have seen a decrease in seed storage proteins within mutants like *val1* and *bZIP67*. A phenotype producing fewer reproductive parts has the potential to lead to fewer seeds and, therefore, fewer seed storage proteins.

Studying the phenotypic differences between mutant *A. thaliana* plants and the WT is the key to finding which genes may be responsible for effects on the accumulation of seed storage proteins and therefore seed oil. Studying the effects of these gene mutations can shed light on not only the pathways and mechanism of these genes but how we can mutate this plant for the specific need to produce as much seed oil as possible? If we are able to figure out how to create more seed production in *A. thaliana* it can be translated to the plants that really matter. The same changes can be made to plants like rapeseed whose seeds are so commonly used. Arabidopsis are just a model to the bigger picture.

. While, mutations in genes such as *val1* and *bZIP67* have shown an arrangement of effects such as slowed development and less reproductive parts, other genes may provide evidence to differing phenotypic responses. These mutant plants may have longer life cycles because they are allocating their energy toward different processes, such as amount of seeds per silique or amount of oil per seed. Understanding these genes is the key to figuring out how to best fine-tune the expression of metabolic genes to make the most optimal plant to grow seed oil. Looking at this correlation in *Arabidopsis thaliana* can be translated to larger crops such as rapeseeds that are widely used for their seed oil and the production of biofuels.

REFERENCES

Bollman, M., Krista, Aukerman, J., Millo, Park, Mee-Yeon, Hunter, Christine, Berardini, Z., Tanya, Poethig, R., Scott. (2003) HASTY, the *Arabidopsis* ortholog of exportin 5/MSN5, regulates phase change and morphogenesis. *The Company of Biologists.* 130:1493-1504.

Boyes, D., Zayed, A., Ascenzi, R., McCaskill, A.J., Hoffman, N., Davis, K., and Gorlach, J. (2001). Growth stage-based phenotypic analysis of *Arabidopsis*: a model for high throughput functional genomics in plants. *The Plant Cell.* 13, 1499-1500.

Chattopadhyay, S., Ang, L. H., Puente, P., Deng, X. W., & Wei, N. (1998). *Arabidopsis* bZIP protein HY5 directly interacts with light-responsive promoters in mediating light control of gene expression. *The Plant Cell*. 10(5), 673-683.

Jakoby, M., Weisshaar, B., Dröge-Laser, W., Vicente-Carbajosa, J., Tiedemann, J., Kroj, T., & Parcy, F. (2002). bZIP transcription factors in *Arabidopsis*. *Trends in Plant Science*. *7*(3), 106-111.

Mendes, A., Kelly, A.A., van Erp, H., Shaw, E., Powers, S.J., Kurup, S., and Eastmond, P.J. (2013). BZIP67 regulates the omega-3 fatty acid content in *Arabidopsis* seed oil activating fatty acid desaturase. *The Plant Cell.* 25, 3104-3116.

Sharma, N., Bender, Y., Boyle, K., and Robert, P. R. (2013). High-level expression of sugar inducible gene2 (HSI2) is a negative regulator of drought stress tolerance in *Arabidopsis*. *BMC Plant Biology.* 13, 156-170.

Shewry, P.R., Napier, J.A., Tatham, A.S., (1995) Seed storage proteins: structures and biosynthesis. *The Plant Cell.* 7, 945-956.

Silverstone, A., Tseng, T., Swain, S., Dill, A., Jeong, S., Olszewski, N., Sun T. (2007) Functional analysis of SPINDLY in gibberellin signaling in arabidopsis. *Plant Physiol*. 143(2):987.

Suzuki, M., Wang, H.H., and McCarty, D.R. (2007) Repression of the LEAFY COTYLEDON 1/B3 regulatory network in plant embryo development by VP1/ABSCISIC ACID INTENSITIVE 3-LIKE B3 genes. *Plant Physiology.* 143, 902-911.

Tsukagoshi, H., Morikami, A., and Nakamura, K. (2007). Two B3 domain transcriptional repressors prevent sugar-inducible expression of seed maturation genes in *Arabidopsis* seedlings. *Proceedings of the National Academy of Sciences of the United States of America.* 104, 2543-2547.