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Environmental stress response genes crosstalk with the floral developmental program in Arabidopsis thaliana

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Environmental stress response genes crosstalk with the floral developmental program in *Arabidopsis thaliana*

by

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Thesis

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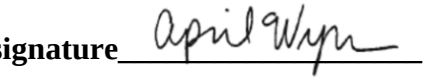
4/28/2020	**signature** 
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Abstract

As climate change continues to destabilize precipitation and temperature regimes for economically significant crops that we consume daily, the global supply of agricultural products may become limited. This purpose of this study was to determine if climate change may affect developmental and stress response regulatory gene networks. By examining the crosstalk between the floral developmental program and the environmental stress response program in *Arabidopsis thaliana*, we were able to determine key genes that integrate the inputs of these two pathways to affect plant reproductive development. Gene expression was examined in four genotypes of plants (Col-0, c*or413*, *erd10*, and *seu)* grown in three conditions (optimal, cold and drought). I expected to see increases in the expression of all three genes compared to the wild type control under both stressful conditions. qRT-PCR analysis was used to compare gene expression of two environmental stress response genes, *COR413* and *ERD10*, as well as the developmental gene *SEU* between the genotypes and growth conditions. Fertilized ovule counts were also analyzed. I expected to see increased ovule numbers in all of the mutants under stressful conditions. The results show that growth in cold conditions raises the levels of all three genes significantly. This indicates that the drought response gene *ERD10* is responsive to temperature stress and that genes for ovule development respond to environmental stress. Fertilized ovule counts also revealed a significant difference in the number of mutant ovules compared to the wild type control, which further indicates that the interaction between the environmental stress pathway and the developmental pathway result in alterations in plant development and reproductive ability.

Table of Contents

Introduction

Climate change will continue to threaten the supply of agricultural products worldwide. Global warming is currently increasing temperatures and humidity levels around the world, thus changing timing and lengths of seasons (Brown et. al 2015). As climate change worsens, where plants can survive will shift, but more importantly we will see the impact of higher temperatures and changes in precipitation on economically significant crops. Additionally, recent studies have shown that global warming has already caused a shift in the polar vortex, meaning cooler than normal temperatures are moving south due to increased ozone depletion, sea-ice loss, and changes in arctic oscillation (Zhang et. al 2016). With the significant impact of climate change in mind, I utilized tools developed with the model organism *Arabidopsis thaliana* to extend basic understanding of plant processes that can be later translated into crops. Utilizing Arabidopsis gave us the ability to gain insight into the interaction between the environmental stress program and the growth/developmental program for flowering and seed production.

Wynn et. al (2011) showed that in *Arabidopsis thaliana*, the two environmental stress response genes of interest, *COLD REGULATED 413 (COR413)* and *EARLY RESPONSIVE TO DEHYDRATION 10 (ERD10)*, are located downstream of *SEUSS (SEU)* and *AINTEGUMENTA (ANT)*, which are the two important transcription factors that promote organ initiation and growth in *Arabidopsis thaliana*. Research also shows that in *seu ant* double mutants, the genes *COR413* and *ERD10* are upregulated (Wynn et. al 2011). This means that when *SEU* and *ANT* are present, there are reduced the expression of *COR413* and *ERD10*, indicating that there is communication between the environmental response pathway (represented by *COR413* and *ERD10)* and the floral/ovule development pathway (represented

by *SEU* and *ANT*). The intersection of the action of *SEU* and *ANT* and response to environmental stressors has not been examined to determine how they mutually affect flower and seed development in *Arabidopsis.* Additionally, no research has been conducted on how the expression of *COR413* and *ERD10* changes in wild type and *SEU* mutants under stressful conditions.

Developmental genes, stress response genes and the floral developmental pathway

To understand how abiotic stress factors impact floral and ovule development, it is important to first understand the developmental pathway of the flower. Research completed by Azhakanandam et. al. (2008) showed that *AINTEGUMENTA (ANT)* is a gene that encodes a DNA-binding protein functioning in early organ development to initiate cellular division. *SEUSS (SEU)* is a gene that encodes a transcriptional coregulator that acts in a protein regulatory complex with *LUG* that represses *AGAMOUS* (*AG)* - class C gene in perianth organs (Azhakanandam et. al 2008). These genes work together to direct the development of the floral reproductive organs. Results from Azhakanandam et. al (2008) showed that *seu ant* double mutants did not develop any ovules while *ant-1* single mutants developed only half the number of ovules compared to wild type plants. In *seu-3* single mutants the number of ovules did not vary statistically from the wild type number but there was some reduction in number. Therefore, their data indicated that *SEU* and *ANT* share partially redundant functions in patterning ovules in *Arabidopsis thaliana* (Azhakanandam et. al. 2008).

Cold stress (temperatures between <0°C and <20°C) negatively impacts the growth of plants and constrains agricultural productivity. Cold acclimation consists of remodeling the cell and tissue structures while reprogramming metabolism and gene expression. In

Arabidopsis, the *COR* family of genes (of which *COR413* is a member) make up approximately 4% to 20% of the genome (Chinnusamy et al. 2007). Members of the *COR* family experience upregulated gene expression in response to low temperatures as well as drought conditions (Su et. al. 2018). Kim and Nam (2010) also found that in *erd10* mutants there is reduced tolerance to low temperatures.

Genes that are members of the *ERD* family are known to be rapidly induced by dehydration. *ERD10* specifically has been shown to have chaperone-activity when it comes to protecting proteins within the plant under stressful conditions (Kim and Nam 2010). Kim and Nam (2010) also determined that the overexpression of *ERD10* in response to drought stress could be lethal in the postembryonic stage. In *erd10* mutants, there is reduced tolerance to drought when compared to wild type plants, and when ERD10 is not present there is reduced seed maturation and germination percentage compared to the wild type (Kim and Nam 2010).

As *ERD10* and *COR413* are located downstream of *SEU* and *ANT* (Figure 1), and upregulated in the *seu ant* double mutants, one of the functions of SEU and ANT is to (directly or indirectly) downregulate *ERD10* and *COR413.* This provides an indication that that the ovule developmental program genes crosstalk with the environmental stimuli genes during the growth and development of the flower.

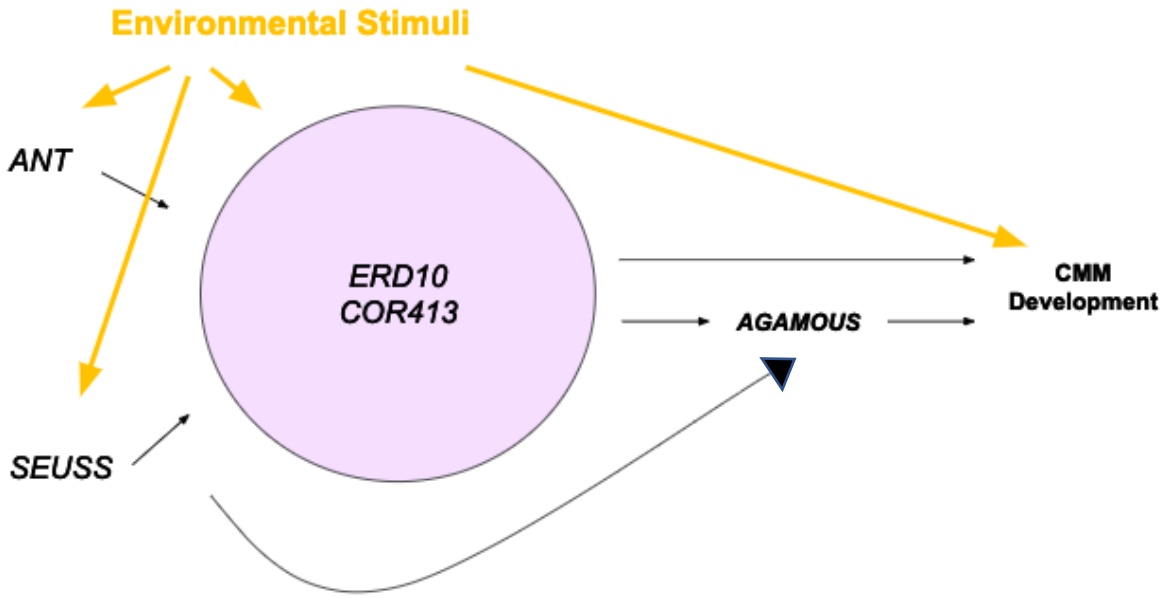

Figure 1. Diagram showing the genetic path from *SEU* and *ANT* to the development of the carpel margin meristem where plant ovules develop. *ERD10* and *COR413* are located downstream of *SEU* and *ANT* (Wynn 2011).

Predictions

I predicted that environmental stress (cold and drought) will increase the expression of *COR413*, *ERD10*, and *SEU* in wild type *Arabidopsis thaliana* to protect the plant from degradation. In *seu* mutants under stressful conditions, I expected to see an increase in *COR413* and *ERD10* expression because the presence of *SEU* typically downregulates *COR413* and *ERD10* (Wynn et al. 2011). In *erd10* mutants I expected an increase of COR413 expression in both cold and drought conditions as previous research has shown COR413 to be responsive to drought as well (Su et. al. 2018). In *cor413* mutants, I expected the expression of *ERD10* to increase in response to drought conditions but not cold conditions as no previous research has shown *ERD10* to be responsive to cold. Finally, I expected ovule counts to increase in all three mutants compared to the wild type under

stressful conditions because some plants are likely to set seed quickly when dying in hopes of increasing their fitness.

Materials and Methods

Plant growth

All of the samples were grown in Percival growth chambers, where the humidity was held constant at 55% and light/dark cycles were set at 16 hours of light and 8 hours of no light. Plants were planted in two-part grow-mix and one-part vermiculite. The four genotypes grown were *seu*, Col-0, *erd10*, and *cor413*. Three treatments were conducted: cold (18°C), drought (watered to 50% field capacity), and optimal temperatures of 23°C. Four replicates (apical inflorescences) were collected of each genotype for each treatment, giving a total of 48 samples. All plants were initially treated with optimal watering and temperature until they were just about to bolt, or the 10-12 leaf growth stage (Harb et. al 2010).

Cold and optimal plants received the optimal amount of DI water (with Miracle Grow fertilizer supplement once per week), where they were given 1400mL of water for two hours and then excess water was removed from the tray. Drought plants were given 50% of field capacity (based on the amount of water absorbed by the control plants on each watering date) (Harb et al. 2010). 50% field capacity was determined by measuring the water remaining in the control plant tray was subtracted from 1400mL, then dividing that by two. This ensured that as the plants were getting bigger (requiring more water) that the drought plants were still receiving 50% of the water the control plants were taking up.

qRT-PCR for gene expression analysis

Apical inflorescences were collected after the plant produced approximately 10-15 siliques. RNA was extracted from apical inflorescences of each treatment/control using the

Thermo Scientific™ GeneJET Plant RNA Purification Kit, and then synthesized into cDNA using the Thermo ScientificTM Maxima First Strand cDNA Synthesis Kit with dsDNAse using gene specific primers (Table 1). A QuantStudio™ 3 Real Time PCR System was used to run qRT-PCR using a SYBR green reporter and the ΔΔCT method was used to determine gene expression levels. Expression levels were normalized to the housekeeping gene *ADENOSINE PHOSPHORIBOSYL TRANSFERASE* 1 (*APT1*) (Azhakanandam et. al. 2008).

Table 1. qRT-PCR primer sequences

Gene	FW	RV
seu	CTCCGGTGATTTGACTGATGTC	GACCAGGCAGTGCAGATGAA
cor413	AGCTACACACGCCTTCACACTCA	ACACCAACAAGTATATGGCGGCGA
erd10	CACCACCATGCCAGCACCACA	GCTGTGGCCTGGAAGCTTCTCC
APT1	GTTGCAGGTGTTGAAGCTAGAGGT	TGGCACCAATAGCCAACGCAATAG

Statistical analysis of fertilized ovules and Ct values

To examine the number of fertilized ovules produced, three replicates were collected for each genotype and treatment. Each replicate consisted of a silique from the plant collected around the 15-silique growth stage. The siliques were gently opened by hand over a piece of white paper and the seeds (fertilized ovules) were counted. The data was recorded in Excel and analyzed via chi-squared with the wild-type control group used as the expected value. To examine changes in gene expression, four replicates were collected for each genotype and treatment and analyzed using qRT-PCR. Average normalized Ct values were analyzed via t-test in Excel.

Results

Changes in COR413, ERD10, and SEU expression in cold, control, and drought Col-0 plants

The average normalized Ct values for the amount of *COR413* expression in Col-0 cold, Col-0 control, and Col-0 drought plants were 1.01, 2.25, and 2.09 respectively. There was a significant difference in *COR413* gene expression between the Col-0 cold treatment group and the Col-0 control ($t = -3.64$, $df = 6$, $p = 0.01$, $n = 4$) (Figure 2). There was no significant difference in *COR413* gene expression between the control and drought Col-0 treatments ($t = -0.49$, $df = 6$, $p = 0.64$, $n = 4$). The average normalized Ct values for the amount of *ERD10* expression in Col-0 cold, Col-0 control, and Col-0 drought plants were 17.90, 29.89, and 18.85 respectively. There was no significant difference between the cold treatment or the drought treatment group in the amount of *ERD10* gene expression compared to the control ($t = -3.76$, $df = 2$, $p = 0.06$, $n = 2$; $t = -1.59$, $df = 2$, $p = 0.25$, $n = 2$) (Figure 3). The average normalized Ct values for the amount of SEU expression in Col-0 cold, Col-0 control, and Col-0 drought plants were .09, 2.71, and 0.16 respectively. Figure 4 shows a significant difference in *SEU* gene expression in Col-0 cold and Col-0 drought plants when compared to the Col-0 control separately ($t = -9.27$, $df = 6$, $p < 0.0001$, $n = 4$; $t = -9.51$, $df = 6$, $p < 0.0001$, $n = 4$).

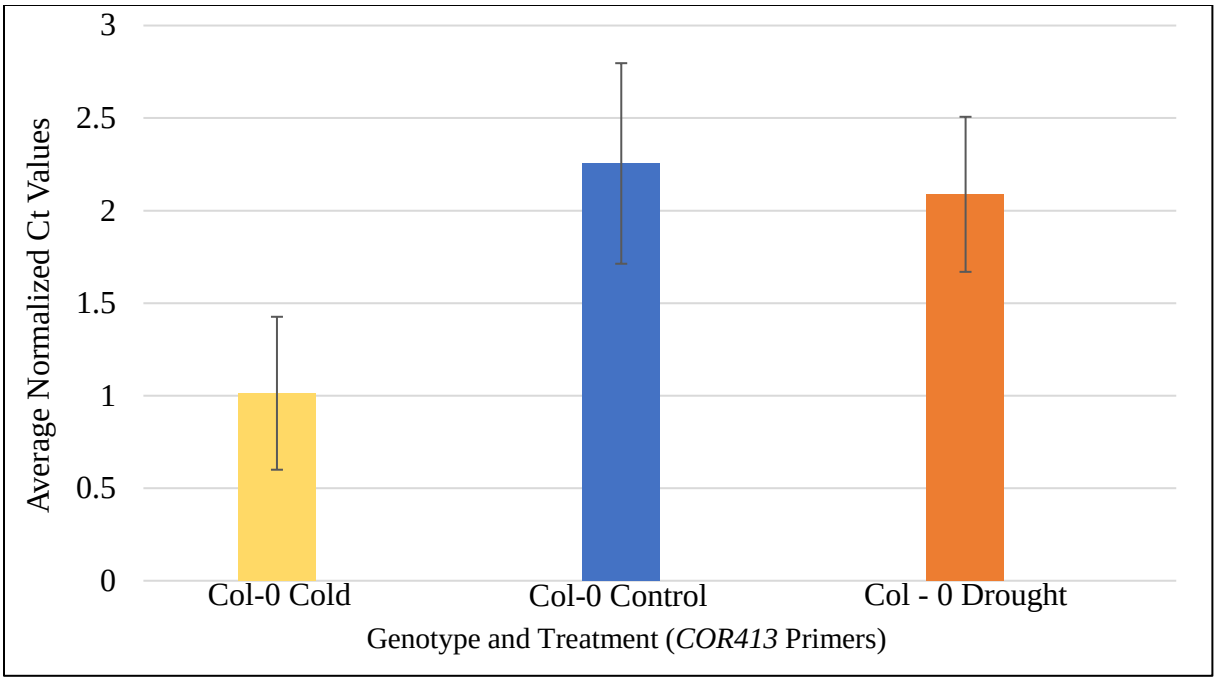

Figure 2. *COR413* expression in wild type plants. There was a significant difference in *COR413* gene expression between the Col-0 Cold treatment group and the Col-0 Control (t = -3.64, df = 6, p = 0.01, n = 4). There was no significant difference in *COR413* expression between the control and drought Col-0 treatments (t = -0.49, df = 6, p = 0.64, n = 4).

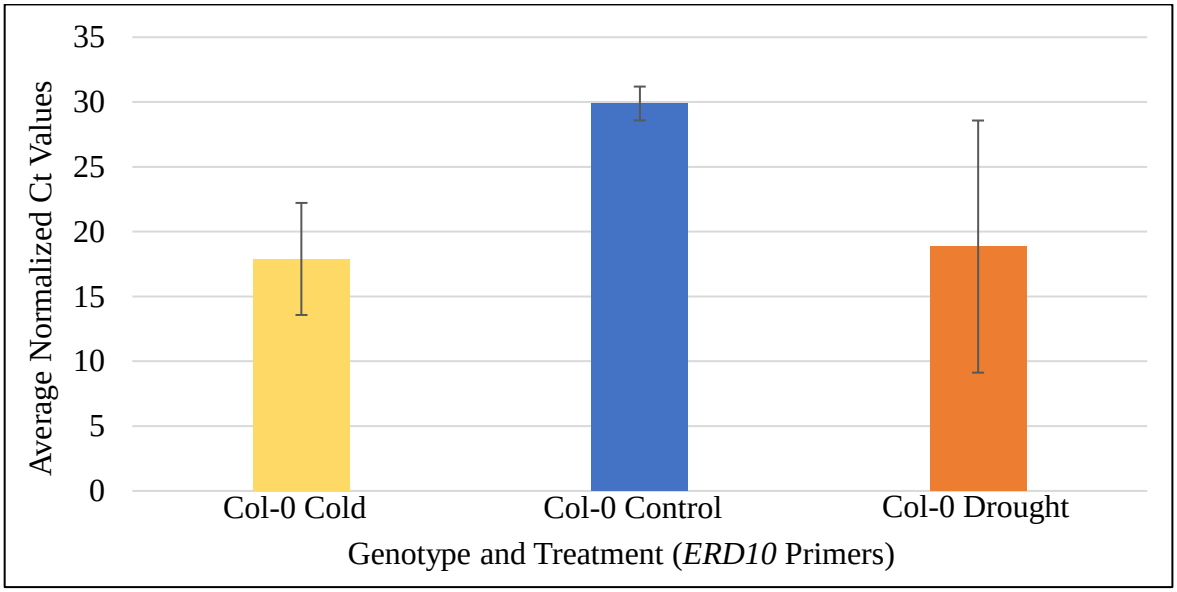

Figure 3. *ERD10* expression in wild type plants**.** There was no significant difference between the cold treatment or the drought treatment group in the amount of *ERD10* gene expression compared to the control (t = -3.76, df = 2, p = 0.06, n = 2; t = -1.59, df = 2, p = 0.25, n = 2)

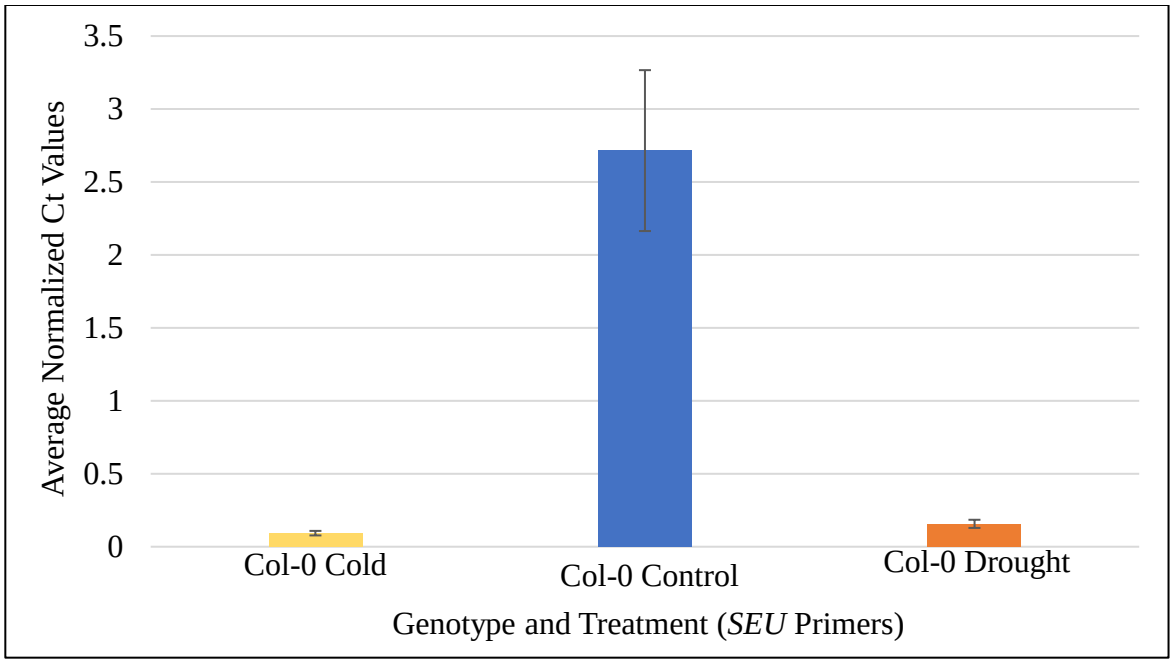

Figure 4. *SEU* expression in wild type plants. There was a significant difference between both the cold and drought treatments and the wild type control plants in the amount of *SEU* gene expression ($t = -9.27$, $df = 6$, $p < 0.0001$, $n = 4$; $t = -9.51$, $df = 6$, $p < 0.0001$, $n = 4$).

Changes in COR413 expression in erd10 mutants under cold, control, and drought treatments

The average normalized Ct values for COR413 expression in erd10 mutants under cold, control, and drought treatments were 0.88, 2.20, and 1.34 respectively. Figure 5 shows that there was no significant difference in C*OR413* expression between *erd10* drought and *erd10* control treatments, but there was a significant difference in *COR413* gene expression between the *erd10* cold and *erd10* control treatments ($t = -2.74$, $df = 6$, $p = 0.03$, $n = 4$).

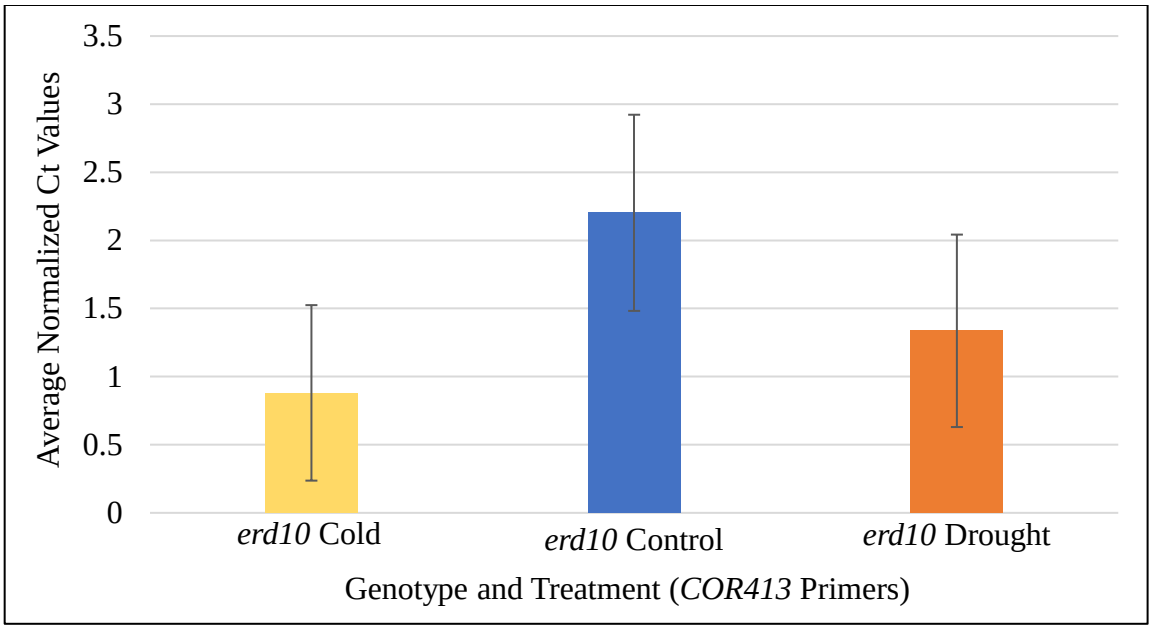

Figure 5. *COR413* expression in *erd10* mutants. There was a significant difference in *COR413* gene expression between *erd10* cold and *erd10* control treatments (t = -2.74, df = 6, p = 0.03, n = 4), but no significant difference between *erd10* drought and *erd10* control treatments (t = -1.72, df = 6, p = 0.14, n = 4)

Changes in ERD10 expression in cor413 mutants under cold, control, and drought treatments

The average normalized Ct values for ERD10 expression in cor413 mutants under cold, control, and drought treatments were 8.30, 22.15, and 26.30 respectively. There was a significant difference in *ERD10* gene expression between *cor413* cold and *cor413* control treatments (t = -2.86, df = 6, p = 0.03, n = 4) (Figure 6). Figure 6 also shows that between the *cor413* drought and *cor413* control treatments, there was no significant difference in *ERD10* gene expression.

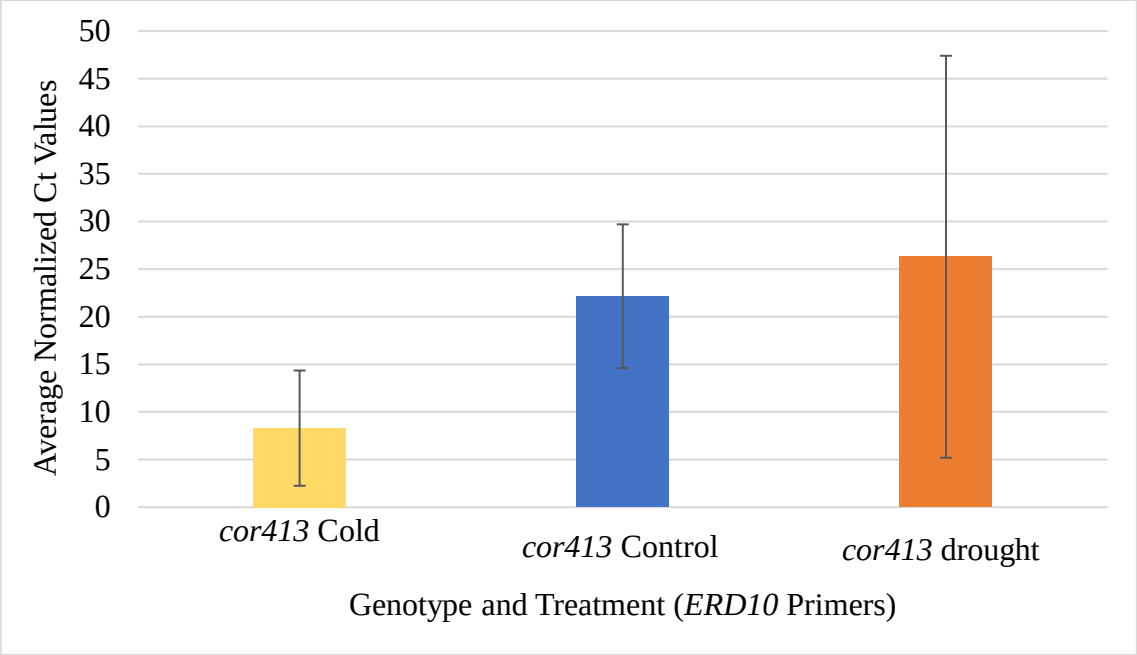

Figure 6. *ERD10* expression in *cor413* mutants. There was a significant difference in *ERD10* gene expression in *cor413* cold and *cor413* control treatments (t = -2.86, df = 6, p = 0.03, n =4). There was no significant difference between *cor413* control and *cor413* drought treatments in relation to *ERD10* gene expression (t = 0.37, df = 6, p = 0.72, n = 4).

Chi-squared analysis of fertilized ovule counts between genotypes and treatments

Most of the genotypes were similar in seed averages except for the *seu* mutants which had a reduced seed count no matter the treatment as seen in previous research (Azhakanandam et. al. 2008) (Table 2). Table 3 shows the p-values given from the chi-squared analysis for each genotype and treatment compared to Col-0 in optimal conditions. All of the mutants and treatments except for *cor413* under cold conditions showed a significant difference in the number of fertilized ovules compared to the wild type control (Table 3).

Table 2. Fertilized ovule counts and averages for each genotype and treatment.				
	Replicate 1	**Replicate 2**	**Replicate 3**	**Average**
seu				
Cold	28	36	21	28.33
Drought	29	34	31	31.33
Control	29	33	32	31.33
Col-0				
Cold	49	51	53	51.00
Drought	53	52	50	51.67
Control	51	44	44	46.33
erd10				
Cold	61	53	54	56.00
Drought	43	63	52	52.67
Control	48	53	54	51.67
cor413				
Cold	45	50	44	46.33
Drought	61	53	59	57.67
Control	54	49	41	48.00

Table 3. Fertilized ovule count chi-squared test (compared to Col-0 control).	
Category	**P-value**
***seu* cold**	8.3773E-06
***seu* drought**	0.0006
***erd10* cold**	0.0321
***erd10* drought**	0.0313
***cor413* cold**	0.8001
***cor413* drought**	0.0107

Discussion

How does environmental stress (cold and drought) impact the expression of COR413, ERD10 and SEU in wild type Arabidopsis thaliana?

In the wild type plants, there were a few significant differences in gene expression based on changes in environmental conditions. There was a significant increase in *COR413* expression in the cold Col-0 plants compared to the control. This is likely because the gene is responsive to cold temperatures (Su et. al 2018). Unlike the research completed by Su et. al., there was no significant difference in *COR413* gene expression in the Col-0 drought treatment compared to the control which the researchers originally mentioned being a possibility (2008) (Figure 2).

ERD10 expression data showed that there were no significant differences between the wild type cold, control, or drought conditions (Figure 3). Previous research would predict increased *ERD10* gene expression in the Col-0 drought plants compared to the controls, as the gene functions as a response to dehydration (Kim and Nam 2010). This inconsistency may have been due to differences in experimental methods, where Kim and Nam (2010) grew the seedlings on ½ MS plates for 10 days, implemented the cold stress for only 24 hours at 4°C, and then extracted tissue for RNA extraction immediately.

Finally, there was a significant increase in *SEU* expression in both the cold and drought Col-0 plants compared to the wild type (Figure 4). This leads to an indication that there is an interaction between the environmental stress response program and the developmental program because we know that *COR413* and *ERD10* are located downstream of *SEU* (Wynn et al. 2011). These results would indicate that there may be a feedback loop between the environmental pathway and the developmental pathway. It would be interesting

to determine if increased or decreased expression of *COR413* and/or *ERD10* caused by environmental stress changes the expression of *SEU* in the future.

In seu mutants how does the expression of COR413 and ERD10 change under stressful conditions?

I was able to extract the RNA and synthesize the cDNA for most of these samples, but time constraints limited my ability to complete the plates. The next step to answer this question would be to run qRT-PCR on cold, drought, and control *seu* cDNA using *COR413* and *ERD10* primers. Research previously completed by Wynn et al. (2011) showed that in *seu ant* double mutants *COR413* and *ERD10* were downregulated, but they did not test this idea using *seu* mutants facing drought or cold stress. Without this data, I was unable to determine how the *seu* mutation could impact *COR413* and *ERD10* expression under stressful environmental conditions, which may have further validated the idea that there is crosstalk between the developmental and environmental pathways in Arabidopsis.

In erd10 mutants how does the expression of COR413 change?

Results indicate a significant increase in *COR413* expression in *erd10* mutants in cold conditions compared to the control, but no significant changes in *COR413* expression in drought conditions compared to the control (Figure 5). This trend is supported by Su et. al (2018) where under cold conditions there is an expected increase in *COR413* expression. The results also indicate that it is unlikely that the presence of *ERD10* changes the function of *COR413*.

In cor413 mutants how does the expression of ERD10 change?

The data showed a significant increase in *ERD10* expression in *cor413* cold plants compared to *cor413* control plants, while there was no significant difference in *ERD10*

expression between the cor413 drought and control plants (Figure 6). The standard deviation for the c*or413* drought treatment plants was very high so it is difficult to say whether those data are valid. However, the increase in *ERD10* expression in the *cor413* cold plants could be indicative of some sort of interaction between *COR413* and *ERD10*. When *COR413* is not present, as in this case, *ERD10* may act as a non-specific stress response gene to help buffer the plant to the effects of the cold. This could allow the plant can either set seeds quickly or remain small in cold weather until more permissive temperature are reached.

How do changes in COR413 and ERD10 expression impact floral and seed development in Arabidopsis thaliana?

Results found many significant differences in fertilized ovule counts when compared the stressed mutant plants to the wild type control, indicating that there is likely some interaction between the environmental stress response pathway and the floral developmental pathway (Table 2, Table 3). In *seu* mutants, there was a significant decrease in fertilized ovules in both the cold and drought plants. According to Azhakanandam et. al (2008), *seu* mutants typically show a reduction in the number of ovules they produce, but the same research did note that the difference was not by half, which is what these results show. In *erd10* mutants, there was a significant increase in ovule number in both cold and drought treatments compared to the wild type control. This could support the conjecture that *erd10* mutants are more likely allocate resources towards setting seed quickly under stressful conditions, while the *seu* plants are more likely to save their energy and wait to switch to a reproductive program until environmental conditions improve. In *cor413* mutants, there was no significant difference in ovule counts between the cold treatment and the wild type control. However, there was a significant increase in the number of fertilized ovules present in the *cor413* drought plants. The fact that there was no difference in ovule count between

the *cor413* cold and control plants likely means that the cold stress did not negatively impact the reproductive pathway in this case. However, the drought *cor413* plants seem to have set seed more quickly in order to increase their overall reproductive success.

Conclusion and future directions

Due to time limitations and COVID-19, it is difficult to confidently say that there is a definite interaction between the floral developmental program and the environmental stress response pathway. The results indicate there is crosstalk between the two pathways, but further testing would need to be done to come to a definite conclusion. In the future, it would be important to complete the *in-situ* hybridization analysis to determine where the genes are expressed within the flower and ovule. If *COR413* and *ERD10* are expressed in the reproductive organs, those findings can be used as further evidence to prove the interaction between the two pathways. Additionally, it is important to run the remaining *SEU* plates to examine how the presence of *SEU* might change the expression of *COR413* and *ERD10* under stressful conditions. I was able to cross *cor413* and *seu*, and *erd10* and *seu* and collect F1 seeds, which could be planted, genotyped, and analyzed using the same methods to determine if losing two of the three genes affect flower or ovule development more severely than single mutants.

It is extremely important that we understand how the environmental response program and the developmental program work together to protect the plant, especially as we see climate change across the globe. We will need crops that are able to survive stressful drought situations in areas that are not normally impacted by lack of precipitation, and we additionally will need crops that can survive and set seed in areas where harsh cold winters were not the norm, but will be sooner than we think.

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