Examining the role of the *ARI11* gene in plant growth and development, and resilience.

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INTRODUCTION

Ubiquitin Proteasome System (UPS): The UPS is an important mechanism involved in the degradation of eukaryotic proteins. The UPS controls protein abundance by attaching ubiquitin, a highly conserved protein, to selected substrates, which are then targeted by the 26S proteasome for degradation (Figure 1). Within the UPS system, E3 ligases play a critical role as the final step in attaching the ubiquitin molecule to the target protein, thereby defining the specificity of the system. One of the important roles of the UPS is its involvement in the stress response pathway, which allows plants to adapt to various stressors such as drought, salinity, high temperatures, pathogens, etc.

This project investigates the role and impact of the *ARIADNE11* (*ARI11)* gene, which encodes for an E3 ligase, on plant growth, development, and stress tolerance. While there is limited knowledge about its function, *ARI11* seems to be central to plant development and tolerance as indicated by previous lab studies that has observed significant differences in mutant survivability and morphology. To further understand *ARI11's* function, a mutant analysis was conducted using *Arabidopsis thaliana* (Arabidopsis) with normal and decreased expression of this gene to assess morphology, and drought survivability rates. Growth parameters, such as height, number of siliques (seed pods), rosette shape and size, seed shape and size and biomass, were analyzed to compare wild-type plants with normal *ARI11* expression and mutants with decreased *ARI11* expression. Likewise, a drought stress assay was used to determine the survivability rates. By comparing the post-drought recovery rates of plants with normal expression of *ARI11* and decreased expression of *ARI11*, the gene's impact on stress tolerance was also evaluated.

This project aims to enhance our understanding of plant stress responses. This knowledge may translate into helping us develop effective strategies for mitigating the impact of climate change on crop production and yield.

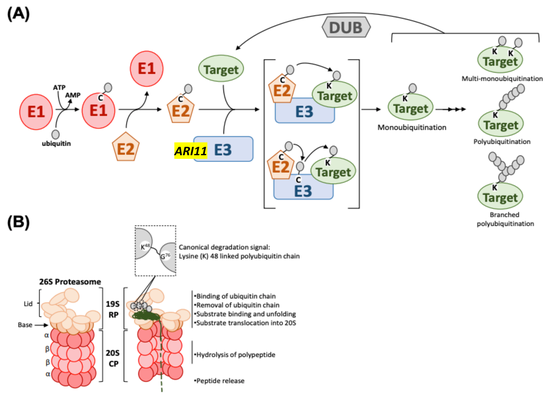

Figure 1: **(A)** The Ubiquitin Proteasome System (UPS) and involved enzymes E1, E2 and E3. *ARI11* as an E3, marked in the UPS pathway. **(B)** The subunits and overall structure of the 26S proteasome, the structure that degrades target proteins (Stone 2019)

RESEARCH QUESTIONS

1. Does the expression of *ARI11* impact Arabidopsis growth parameters in terms of leaf shape and size, seed production, height, biomass, etc.?
2. Does *ARI11* expression levels (normal vs. knockdown) impact the rate of survival after drought ?

METHODS

Genotyping Reverse Transcription Polymerase Chain Reaction (RT-PCR) were used to confirm homozygosity and measure expression levels of *ARI11* in Arabidopsis mutant lines *ari11*-1, *ari11*-2, and *ari11-3*, respectively with WT and housekeeping genes (Ubiquitin) as control. Homozygous wild-type (WT) and mutant (*ari11*-1, *ari11*-2, and *ari11*-3) lines were then utilized in a mutant analysis assay to compare differences in leaf shape and size, silique (seed pod) production, and seed size.

A drought stress assay was conducted to compare the tolerance and survivability of wild-type (WT) and homozygous mutant (*ari11-1*) lines. The drought assay involved a control treatment with a fixed, continuous watering schedule, compared to a two-week drought period in the treatment group (Figure 2). To examine the involvement of *ARI11* in stress response, RT-qPCR was used to assess levels of *ARI11* expression in WT plants throughout the progression of drought.

Significance of values was determined using a one-way ANOVA test.

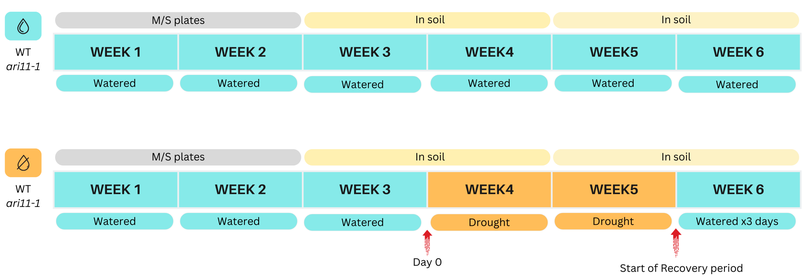

Figure 2: Experimental setup, watering schedule and timeline for drought stress assay with wild-type (WT) and *ari11-1* mutants. The drought treatment period of 14 days marked, starting from Day 0 and ending with the start of the recovery phase (after 2 weeks). Plant growth conditions (plates and soil) specified.

RESULTS
Mutant Analysis

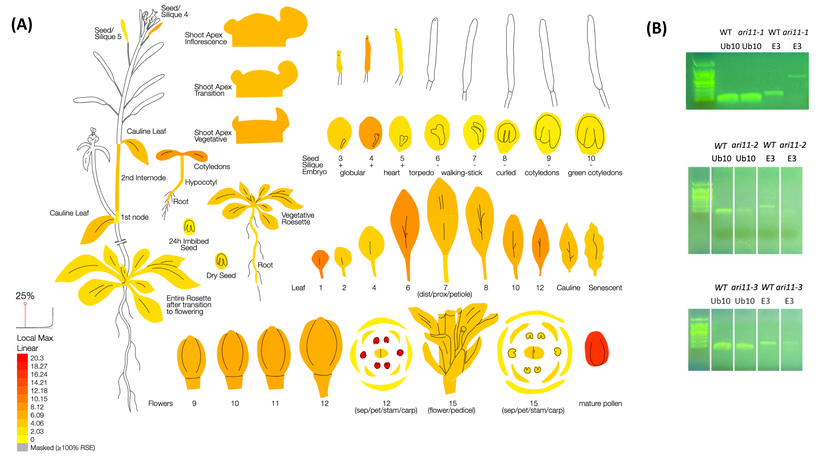

Figure 3: **(A)** Expression map of *ARI11* in different organs of *Arabidopsis thaliana* at specific developmental times (Schmid et al. 2005). **(B)** RT-qPCR results for *ARI11* expression levels in WT and *ari11-1* mutants (Mackinnon Unpublished), Ub10 for ubiquitin (housekeeping gene) and *ARI11* expression, RT-qPCR results for *ARI11* expression levels in WT and *ari11-2* and *ari11-3* mutants, Ub10 for ubiquitin (housekeeping gene) and *ARI11* expression.

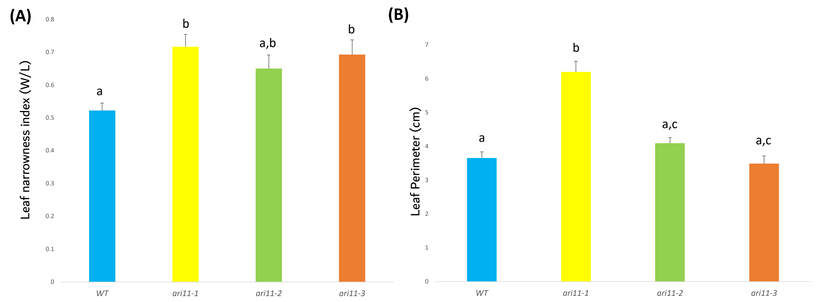

Figure 4: **(A)** Leaf Narrowness Index (width/length) for WT, *ari11-1*, *ari11-2*, and *ari11-3* Arabidopsis at ~5-6 weeks old. Error bars represent +/- SE. Significance was determined using one-way ANOVA tests. Shared letters indicate no significant difference. **(B)** Leaf perimeter for WT, *ari11-1*, *ari11-2*, and *ari11-3* Arabidopsis at ~5-6 weeks old, measured in cm. Error bars represent +/- SE. Significance was determined using one-way ANOVA tests. Shared letters indicate no significant difference. **(C)** WT and *ari11-1* rosettes (~4 weeks old) on the left and, WT, *ari11-1*, *ari11-2*, and *ari11-3* Arabidopsis largest leaf in an individual at ~5-6 weeks old on the right.

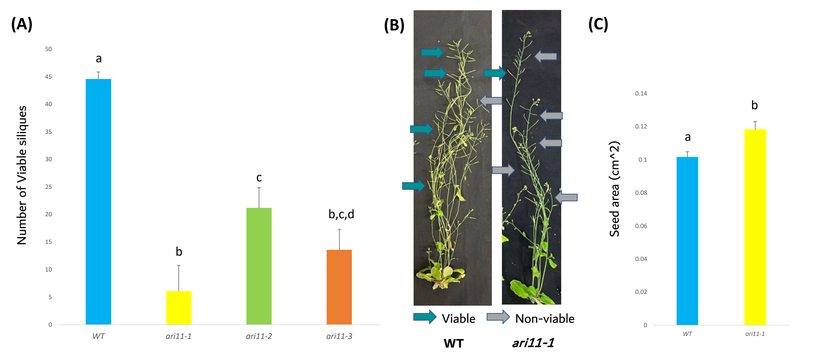

Figure 5: **(A)** Number of viable siliques (siliques with seed pods) for WT, *ari11-1*, *ari11-2*, and *ari11-3* Arabidopsis at ~5-6 weeks old. Error bars represent +/- SE. Significance was determined using one-way ANOVA tests. Shared letters indicate no significant difference. **(B)** Viable versus non-viable siliques on WT and *ari11-1* Arabidopsis bolt. **(C)** Average seed area in cm^2 of individual Arabidopsis seeds for WT and *ari11-1* lines. Error bars represent +/- SE. Significance was determined using one-way ANOVA tests. Shared letters indicate no significant difference.

RESULTS
Drought Stress Assay

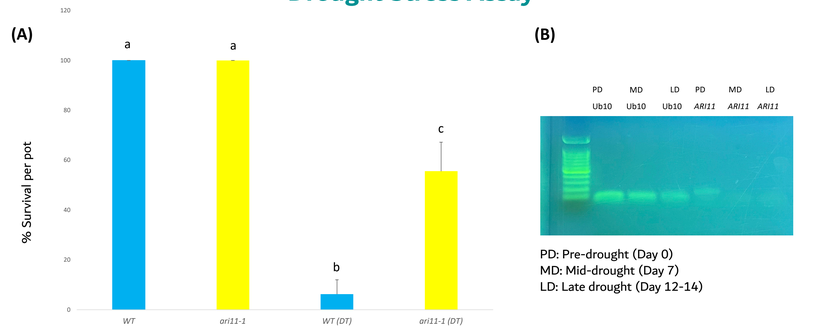

Figure 5: **(A)** Average **p**ercent survival per replicate (pot) for WT and *ari11-1* Arabidopsis post drought (12-14 days drought period) after 3-day recovery period. Watered and drought treatment (DT) for both lines included. Error bars represent +/- SE. Significance was determined using one-way ANOVA tests. Shared letters indicate no significant difference. **(B)** *ARI11* expression in wild-type Arabidopsis at different stages of drought treatment: pre, mid and late drought with housekeeping gene expression (Ub10) as control.

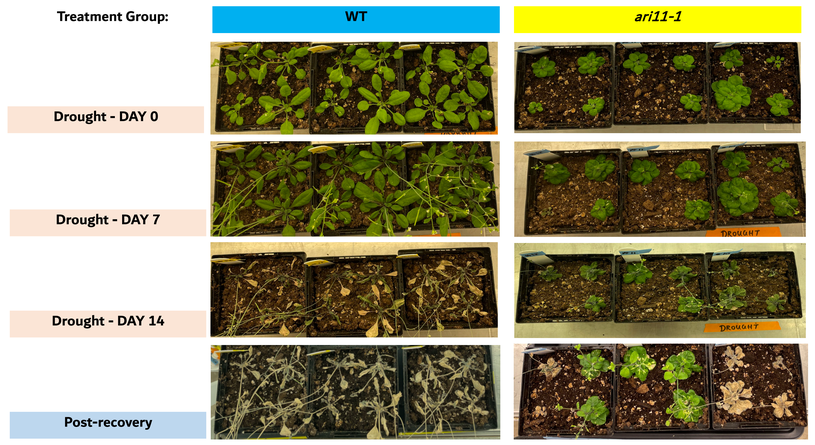

Figure 6: WT (Left column) and *ari11-1* (right column) Arabidopsis during pre-drought (Day 0), mid- drought (Day 7), late drought (Day 12-14), and post-recovery (after 3 days of watering).

CONCLUSIONS

ARI11 is highly expressed in early stages of leaf, silique, seed and pollen development and is involved in determining leaf shape and size, as well as in proper silique and seed production. Mutant analysis of WT, *ari11-1*, *ari11-2* and *ari11-3* lines with differing levels of *ARI11* expression revealed the following differences:

- **Leaf morphology:** *ari11-1* with the lowest *ARI11* expression had significantly larger, more rounded leaves (large narrowness index and perimeter values). *ari11-2* and *ari11*–3 leaves were similar in size and shape to WT.
- **Yield**: *ari11-1* had a significantly lower number of viable siliques, along with a larger number of non-viable siliques, compared to WT. *ari11-2* and *ari11-3* had lower numbers of viable siliques compared to WT (though higher than *ari11-1*), with a larger decrease in siliques observed in *ari11-3* compared to *ari11-2*. Conversely, *ari11-1* had larger individual seeds (larger seed area values) compared to WT.

ARI11 also functions as a negative regulator for drought stress tolerance (expression in WT decreases as drought progresses). *ari11-1* with reduced *ARI11* expression, showed better survivability and tolerance under mild drought conditions. This suggests that *ARI11* is a negative regulator for drought stress tolerance, decreasing survivability in wild-type (WT) with normal *ARI11* expression compared to *ari11-1* with reduced *ARI11* expression.

REFERENCES AND ACKNOWLEDGEMENTS

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