Investigating the Effect of Light Intensity on Programmed Cell Death in Lace Plant (*Aponogeton madagascariensis*) Leaves

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BACKGROUND

The Lace Plant & PCD

- An aquatic monocot that is native to Madagascar. [1]
- The mature leaves have a lattice-like perforated morphology that occurs via developmental programmed cell death (PCD). [2]
- PCD is a genetically encoded, controlled process by which cells undergo death to support developmental processes. [2]
- Its characteristics make it an excellent model for studying PCD [3]:
  - Spatial and temporal predictability of perforations
  - Established sterile culture system for plant propagation
  - Thin, semi-transparent leaves are ideal for live cell imaging
- Young leaves are pink due to anthocyanin pigments that are masked by chlorophyll as the leaves mature. [2]
- The effect of environmental factors such as light, pH, temperature, and nutrient availability on lace plant PCD is currently unknown.

Figure 1 The lace plant (*Aponogeton madagarcariensis).* Left to right: Plant in a sterile culture jar and lay out showing the developmental stages of leaves (right). Scale bar = 2 cm

Phenolic Compounds

- Anthocyanins are phenolic compounds that belong to the flavonoid group. They are responsible for the red, blue, and purple colours of plant tissues. [4]
- Play a role in programmed cell death in lace plant leaves, though the exact function is unknown. [6]
- Anthocyanin vacuolar inclusions (AVIs) are condensed bodies of pigment located in the cell vacuole. [7]
- Upregulated in tissues by environmental factors such as light. [4,5]
- The effect of light on phenolic compounds during lace plant PCD is unknown.

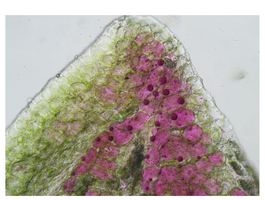


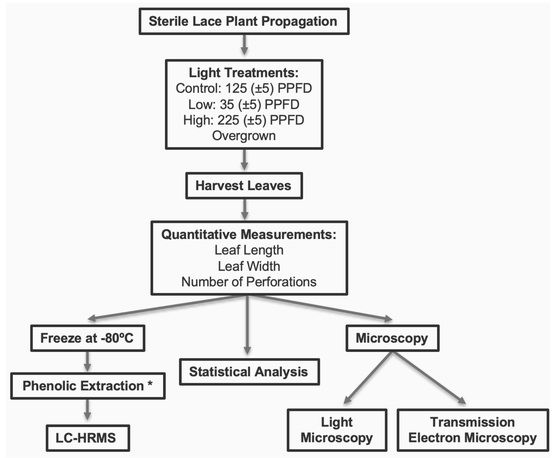
Figure 2 Apex of pre-perforation stage leaf showing anthocyanin vacuolar inclusions and anthocyanin pigment

Objectives

1. To determine the effects of light intensity on programmed cell death during leaf development in the lace plant.
   a. Compare phenolic compound profiles in leaves grown under different light intensities using LC-HRMS.
   b. Examine the quantity and localization of AVIs in window leaves at different light intensities.

METHODS & RESULTS

Overview



1) Leaf Morphology

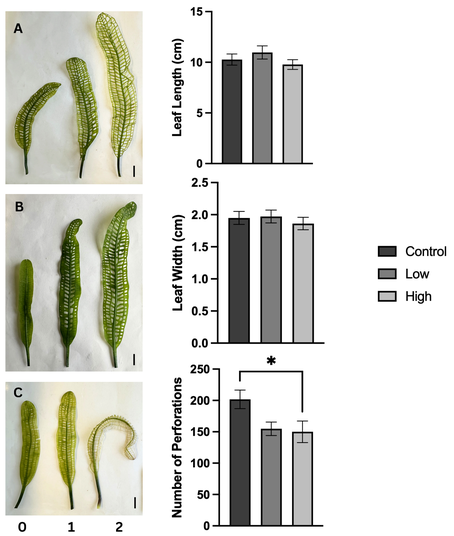


Figure 3 Mature stage leaf layouts from A) control, B) low, and C) high light treatments. From left to right: before treatment, the first leaf, and the second leaf formed during treatment. Bar graphs from top to bottom show average leaf length, leaf width, and number of perforations between the treatments. Means were analyzed using a one-way ANOVA and Tukey Test. Control (n=27), low (n=22), high (n=21). * P < 0.05. Scale bars = 1 cm

2) LC-HRMS

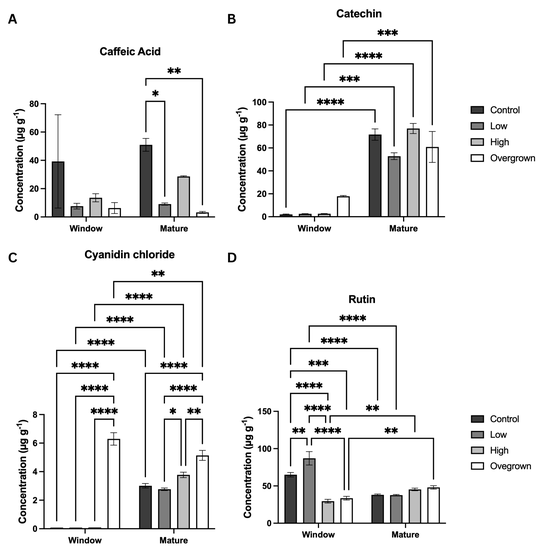


Figure 4 Phenolic compound concentrations between mature and window stage leaves in control, low, high, and overgrown treatments. Means were analyzed using 2-way ANOVA and Tukey test. Window (n=2), mature (n=3). * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001

3) AVIs

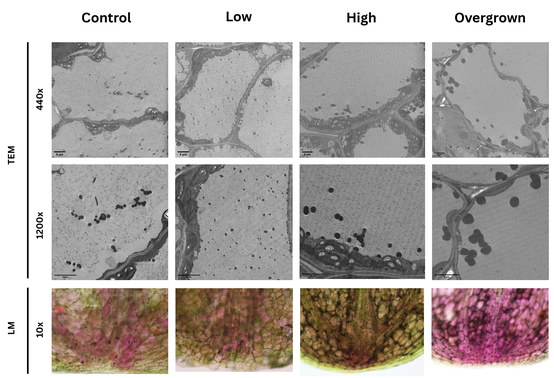


Figure 5 Transmission electron and light microscopy micrographs of window stage leaves between treatments. The purple (LM) and black (TEM) spherical bodies are anthocyanin vacuolar inclusions. The samples observed were approximately 0.5 cm of the leaf apices. Rows from top to bottom: TEM 440x, TEM 1200x (Scale bars = 5 µm), and LM 10x.

DISCUSSION

1) Leaf Morphology

- There is no significant effect of the experimental light intensities on leaf length and width. However, light intensity showed a decreasing trend from low to control to high light.
- There was a significant (P < 0.05) decrease in the number of perforations between the control and high light intensity treatment.

2) LC-HRMS

- Caffeic acid (Figure 4A) only showed significant (P < 0.05; 0.001) differences between treatments in mature stage. Other light intensities showed lower concentrations than the control for both stages.
- Catechin (Figure 4B) showed significant (P < 0.0001) differences between window and mature stages, but not within treatments.
- Cyanidin chloride (Figure 4C) showed significant (P < 0.0001) differences between mature and window stage leaves, and between the treatments in each stage.
- Rutin (Figure 4D), the most abundant compound, showed significant differences (P<0.0001) between all treatments in the window stage.

3) AVIs

- AVIs were discovered in the window stage leaves of all treatments.
- Preliminary data suggest that AVIs are higher in number in overgrown and high-light treatments.
- More replicates are needed to determine if there are any major differences between the groups.

CONCLUSION

- Differing light intensity does have *some* effect on overall leaf morphology in terms of leaf length, width, and number of perforations.
- Light intensity has a varying effect on the concentration of phenolic compounds present within the window and mature leaves.
- Rutin was observed to be the most abundant phenolic compound, while cyanidin chloride was the most abundant anthocyanin.
- Anthocyanin vacuolar inclusions were observed in all treatments, but more replicates need to be completed to determine any definitive differences.

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