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Characterizing Phenotypic and Genetic Responses to Light Stress in Ferns

by

Alexa C. Nolan

An Honors Capstone

submitted in partial fulfillment of the requirements

for the Honors Diploma

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Dedication

I dedicate this thesis to Dr. Paul Wolf, whose remarkable guidance, support, and patience have been integral to the successful completion of my research. His unwavering commitment to excellence and exceptional mentorship has inspired me to strive for nothing less than my best. I also sincerely thank Dr. Themistoklis Chronis for his expert assistance with spectrometry measurements and calibration. Moreover, I would like to acknowledge Dr. Fay-Wei Li for his knowledge and expertise as I have begun to investigate neochrome. His insights and advice have been crucial to my understanding of this topic. Lastly, I thank my parents for their unwavering and ever-loving support throughout my collegiate years. Their encouragement and sacrifices have been instrumental in shaping my academic journey.

Abstract

Neochrome is a chimeric photoreceptor gene found in various fern species. While the evolutionary history of a horizontal gene transfer of the neochrome genes has begun to be investigated, the changes in the expression of neochrome during fern development are relatively uninvestigated in current literature. This study aims to bridge that gap in the literature by testing for the expression of neochrome under various light conditions and across a selection of species in both the gametophyte and sporophyte developmental stages. The design of this experiment calls for the fern species *Ceratopteris richardii*, commonly known as C-fern, to be grown from spores in red light, far-red light, blue light, and white light conditions. Observations regarding gametophyte and sporophyte development were then recorded, providing a benchmark for further experimentation regarding neochrome expression with RNA and real-time PCR sequencing.

Introduction

Photoreceptors and Development:

Light plays a crucial role in plant growth and development. As photosynthesis is a plant's primary way of creating energy, a plant must have the necessary biological framework to maximize photosynthetic machinery. Thus, all plants have developed receptors specialized in light reception at different ends of the visible light spectrum to capture signatures from approximately 320 nm blue light to 730 nm far-red light (Foreman et al., 2010).

The red light receptor found in all plants is known as phytochrome and is often reactive at red (650nm) and far-red (730nm) wavelengths (Kawai et al., 2003; Taiz et al., 2018). The phytochrome receptors present in plants play a crucial role in the generation of fruit and floral budding as well as the elongation of the stem in response to changing light signatures (Taiz et al., 2018). Phytochrome also helps regulate photoperiodism and flowering effects in plant signaling, often by switching between the active and inactive forms of the protein (Kawai et al., 2003; Franklin & Quail, 2009; Taiz et al., 2018). Phytochrome itself is a reversible photochromic billiprotein that switches between the biologically inactive red light form and the active far-red light form to rapidly respond to light changes in a plant's environment (Miller & Miller, 1964; Kawai et al., 2003; Franklin & Quail, 2009). Phytochrome has a unique ability to reallocate phototropic resources in order to gain an advantage over competitor species due to its ability to react to different lighting conditions. However, overexposure to red and far-red light may lead to increased stem growth and apical dominance, likely due to an imbalance of growth hormones such as auxins (Taiz et al., 2018). The increased expression of growth hormones like auxin may lead to reduced leaf development and branching as the plant funnels all of its resources into stem elongation (Liu et al., 2011). A common syndrome for plants exposed to too much far red light is known as "red-light syndrome," which is characterized by severe photosynthetic impairment in the leaves (Trouwborst et al., 2016). While a plant can recover from the severe photosynthetic damage done to the chloroplasts with red-light syndrome, constant exposure to red light can yield permanent damage and cause long-lasting effects (Trouwborst et al., 2016). In ferns, phytochrome's ability to react to far-red light, which is often one of the lights most likely to reach the forest floor where the ferns reside, provides a critical evolutionary advantage for survival along the forest floor (Li et al., 2014; Kawai et al., 2003). Some studies indicate that the specific developmental pattern of ferns under red light stresses varies greatly from species to species, and therefore more studies must be conducted before any significant generalizations regarding phytochrome machinery in ferns can be drawn (Miller & Miller, 1964).

Another form of light receptor in plants is the blue light receptor. The two major blue light receptors are known as cytochromes and phototropins (Taiz et al., 2018). Cryptochrome is known to play roles in maintaining the circadian clock and stress responses (Wang et al., 2014; Taiz et al., 2018). Cryptochrome responses can range from regulating stem growth and flowering time to controlling factors related to the stomatal opening (Wang et al., 2014; Taiz et al., 2018). In addition to the circadian rhythm responses, cryptochrome can play a role in plant stress responses such as seed germination and leaf senescence (Wang et al., 2014; Taiz et al., 2018).

Phototropins, on the other hand, are the main receptors for blue light. The phototropins mediate phototropism, induce leaf area development, and help reduce photodamage (Takemiya et al., 2005; Taiz et al., 2018). Changes in light intensities can indicate to a plant that a rearrangement of chloroplasts may be necessary to minimize the photosynthetic damage from light exposure (Taiz et al., 2018). Because of the high impact of blue light receptors on the maintenance of a plant's stomatal regulation and circadian rhythms, overexposure of the plant to blue light may lead to increased stresses and, thus, stunted growth. In ferns, phototropins are the most highly investigated blue light receptor. However, because of the low light levels on the forest floor, many low-lying plants, like ferns, also use red-light-mediated responses to control chloroplast movement and minimize damage (Sanchez-Puerta et al., 2011; Kawai et al., 2003).

Neochrome and Fern Development:

Ferns go through two major life cycle stages: gametophyte and sporophyte (Hickok et al., 1998). As primarily a ground plant, ferns grow upward and rarely increase in diameter (Hickok et al., 1998). Due to high competition with angiosperms that compose the shade-inducing canopy of the forests where most ferns evolved, the life cycle of ferns has developed to help confer an evolutionary advantage in these shaded conditions (Hickok et al., 1998; Kawai et al., 2003). Model ferns like *Ceratopteris richardii* and *Adiantum capillus-veneris* are homosporous, meaning they develop into a gametophyte with both male and female organs (Hickok et al., 1998; Alongi et al., 2009). In comparison, most plants are heterosporous, meaning they develop into separate male and female gametophytes. In the first stage of development, a spore is released from the fern, and it grows and develops into a young homosporous gametophyte. Then, the male reproductive antheridium and female reproductive archegonium develop independently at different times based on the concentration of hormones in the environment to help prevent self-fertilization and allow for genetic diversity (Hickok et al., 1998; Miller & Miller, 1964). After fertilization of the fern, the gametophyte transitions into a sporophyte, a diploid organism capable of producing spores. After mature sporophyte development and spore release, the life cycle begins again. A major physiological change is marked by the transition from the haploid gametophyte to the diploid sporophyte (Hickok et al., 1998; Alongi et al., 2009). The development of an upwards shoot is one of the major signs that a fern has transitioned to a sporophyte. However, relatively little research has been done regarding genetic expression changes in response to this major transition, especially in stressed lighting conditions. Few studies have investigated fern development under pure light stress conditions. Understanding how gene expression and resource allocation change under light stress conditions is critical to understanding the usage of unique photoreceptor genes like neochrome.

Phototropins, phytochrome genes, and protein expression all play major roles in the growth and development of plants. Several genes found in a plant often control these receptors, and while feedback loops control expression systems, the blue light and red light receptors remain separate systems in most organisms (Kawai et al., 2003; Trouwborst et al., 2016). However, in recent years, several chimeric photoreceptors that combine phototropin and

phytochrome protein components have been identified in low-lying shrub plants like ferns (Li et al., 2014; Kawai et al., 2003). In fact, for ferns to adapt and survive in low-light environments of the forest floor, more efficient ways to use the little light received were a necessity to adapt and compete against the larger angiosperms that compose forest canopies (Li et al., 2014; Kawai et al., 2003).

Originating independently in both hornworts and algae, neochrome provided a necessary solution to these organisms' challenges on the heavily shaded forest floor (Li et al., 2014). Generally, neochrome consists of a red-sensing phytochrome N-terminus component, blue-sensing phototropic LOV (light, oxygen, voltage) domain, and kinase domain; neochrome incorporates the red and blue light phototropic responses into one gene (Suetsugu et al., 2005; Kawai et al., 2003). An extensive study of the fern *Adiantum capillis-veneris* helped identify the phylogenetic origins of ferns and investigate their function (Suetsugu et al., 2005; Li et al., 2014). There were two neochrome genes in *Adiantum capillis-veneris* are referred to as NEOCHROME1 (NEO1) and NEOCHROME2 (NEO2) (initially identified as PHY3 chimeric light receptor) (Kawai et al., 2003). The discovery of these genes helped demonstrate the importance of neochrome receptors in chloroplast resource reallocation as well as gametophyte and sporophyte development in ferns such as *Adiantum capillis-veneris* (Suetsugu et al., 2005). However, few studies have been conducted on fern neochrome expression and development in extreme light conditions. Even fewer have been reported and conducted on ferns that are not *Adiantum capillis-veneris*. The lack of developmental studies related to neochrome expression in various fern species comes from the need for phylogenetic resolution of ferns in neochrome. Recent studies suggest that modern ferns were able to obtain the unique neochrome gene via a horizontal genome transfer (HGT) from hornworts to ferns (Suetsugu et al., 2005; Li et al., 2014). The genetic presence and expression of neochrome is a relatively recent diversification from traditional angiosperm light receptors (Suetsugu et al., 2005; Li et al., 2014). This diversion of the modern fern (~165 million years ago) from the angiosperm indicated that the conferred advantages of better and quicker red/far-red reversibility in responses to changes in environmental stimuli are a relatively new advantage ferns have gained over competitors (Li et al., 2014; Kawai et al., 2003). However, it should be noted that the phylogeny of the HGT to ferns has not been resolved at length, as current studies suggest that the distribution of neochrome does not follow the current published phylogeny of ferns (Li et al., 2014). Some scholars suggest that there may have been several incongruent HGT events from fern-to-fern that may have contributed to the current distribution of neochrome in ferns (Li et al., 2014). Internal fern-to-fern genome transfers are not unheard of after an initial HGT between hornwort and ferns (Li et al., 2014; Sanchez-Puerta et al., 2011). For example, the mitochondrial intron *cox1* has had at least 80 instances of internal fern-to-fern transfer after the initial HGT between fern and fungi (Li et al., 2014; Sanchez-Puerta et al., 2011).

This study aims to create a baseline of developmental differences between ferns of the same species under lighting stress conditions. First, observing model fern *Ceratopteris richardii* (C-fern) under light stress conditions helps establish control and baseline data sets for fern

development. Secondly, by running a PCR on both C-fern and other fern species to confirm the presence or absence of neochrome and further phylogenetically resolve the neochrome gene, and lastly, running real-time PCR and potentially RNA sequencing on both C-fern and other fern species under light stress conditions to give a comprehensive understanding of expression rates of neochrome. The first objective has been achieved as described in this paper.

Methods

Chamber Setup and Growth Conditions:

Four chambers were set up on shelving units 40 cm tall by 60 cm wide. They were wrapped in greenhouse grade sunlight blocking tarp, creating an isolated chamber for growth. Each chamber contained a different light source; white light, red light, far-red light, and blue light. All light sources' wavelengths and intensity were verified using spectrometry. Each light source was 33 cm from the bottom of the tray. A cup of deionized water was placed in each chamber to help regulate the humidity. Ferns were irradiated for 14 hours a day as set by a timer and were watered on an as-needed basis at least twice a week for a four-month period which is still ongoing.

Spectrometry Calibration and Light Setup:

The far-red and blue lights were 30-watt commercial-grade LED supplemental UV boosters from FGI. The red light was a 36-watt HIGROW LED bulb. The white light was a 10-watt full spectrum LED Barrina grow light. To cover the surface area of the chamber, the far-red and blue lights were strung across the top. Two white lights were strung across the top to create the same effect. The red bulb was attached to a lamp mounted on the top of the chamber.

Spectrometry measurements were taken using the PASCO Wireless Spectrometer. The fiberoptic sensor was held approximately 33 cm from the light source to give accurate measurements at the growth level. Each light source was measured in 25 separate trials to ensure accuracy. The readings are included in Table 1 below. Additional readings were conducted on light intensity using the PASCO Spectrometer. The intensity readings at the peak in lumens per meter, or lux, are indicated in Table 2 below.

| Light Source | Wavelength in nm |
|---------------------|-------------------------------|
| White Light | No peaks, full spectrum range |
| Red Light | 650 peak, 630-670 range |
| Blue Light | 380 peak, 370-400 range |
| Far-red Light | 705 peak, 680-750 range |

Table 1: Light source measurements from the PASCO Spectrometer in nm

| Light Source | Intensity at Peak Wavelength in lux |
|---------------|-------------------------------------|
| White Light | 1.8 |
| Red Light | 11.5 |
| Blue Light | 13 |
| Far-red Light | 11 |

Table 2: Light Intensity measurements from the PASCO Spectrometer in lux

Fern Preparation:

Media for C-fern was made following the protocol as detailed by Carolina Biological Supply, product number #156728 (2003). The *Ceratopteris richardii* spores were also acquired from Carolina Biological Supply. To plate the spores, one loopful of spores was collected and suspended in 1.5 mL of distilled water. The suspension was mixed thoroughly, and 30uL of the suspension solution was added to each media plate. The plates were labeled and covered, with four replicates placed in each light condition. Growth data was collected at regular intervals, and significant growth changes were recorded at the two-week and two-month marks. The plates were watered twice weekly on an as needed basis, and on March 6th, after two months of growth, the grown ferns were transplanted into growth cups and covered to maintain humidity.

Results

Summary:

Ceratopteris richardii growth was highly monitored under the four lighting conditions. Significant comparisons were performed and marked as benchmarks for growth, the 19-day (two-week), 59-day (two-month), and 91-day (three-month) sporophyte development. Observations relating to root development, leaf development, size, and chlorophyll intensity were all monitored for each condition.

White Light Results:

The *Ceratopteris richardii* grown under the white light control conditions exhibited normal growth. After two weeks, the four plates under each condition began to develop into gametophytes in the petri plates. Visible under the microscope, these spores developed slowly from the hermaphroditic gametophytes to young sporophytes after hitting the 59-day, or two-month, growth benchmark. An extensive root system began to develop at this two-month mark, with each plate containing 3-4 young sporophytes. Each young gametophyte had approximately 2-3 major root systems present.

The leaves were bright green, indicating a healthy chlorophyll level, with short shoots measuring 5 mm in length. Each gametophyte also had approximately 4-5 leaves of an average bright green coloration. After two months of growth in the plates, all four plates were transferred to growth cups with more C-fern medium and grown in the chambers. During this time, they grew to a 5-6 cm height and began transitioning to the mature sporophyte phase. All four white light plates appeared to develop at relatively the same rate, with approximately the same number of ferns developing in each replicate. Additionally, all four within the replicate have similar coloration and leaf architecture, consistent with teaching guides on traditional *Ceratopteris* growth. The images are depicted in Figure 1 below.

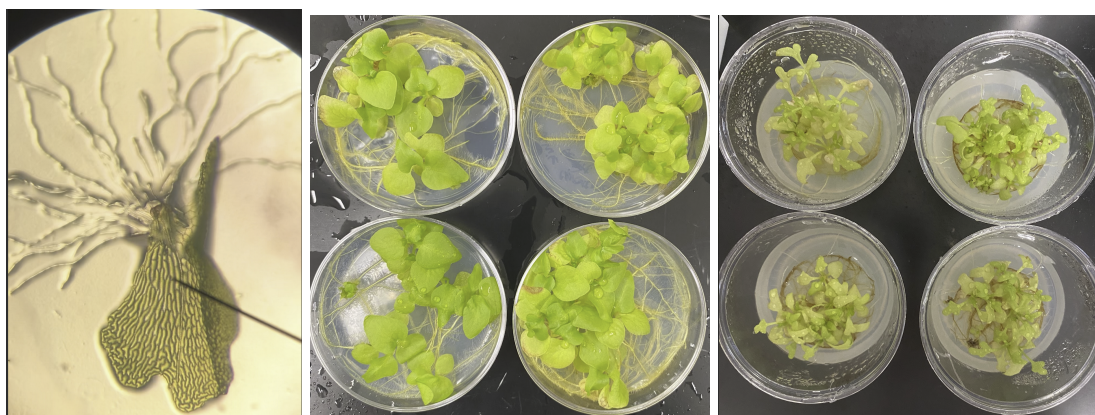


Figure 1: *C*-fern development under white light conditions. Far left image taken after 19 days of growth, middle image taken after 59 days, and far right image taken after 91 days of growth.

Red Light Results:

The *Ceratopteris richardii* grown under the red light conditions exhibited normal growth in the early development stage. Similar to the control conditions, all four plates began to develop into gametophytes after 19 days in the petri plates. The roots of these gametophytes appeared long and spindly, with little development of the leaves. Additionally, after sporophyte emergence at 59 days, an extensive root system began to develop, with each plate containing 3-4 mature young gametophytes. After 96 days or three months of growth and development, each young sporophyte had approximately 2-3 major root offshoots comparable to the white light control conditions.

The leaves were lighter green, indicating a lack of chlorophyll content, with short shoots at 5 mm in length. Each gametophyte also had approximately 4-5 leaves and appeared to have a healthy level of chlorophyll compared to the white light. These leaves appeared to have curled edges and were less rigid. After two months, they were transferred to growth cups with more C-fern medium and grown in the chambers. They grew to 5-6 cm during this time and began transitioning to the mature sporophyte phase. They also began to develop new foliage though the color remained a lighter green in the leaves, and the curled leaves began to gain some rigidity. Throughout the growth process, all four replicates displayed similar root development patterns, with little leaf area growth in the early gametophyte to sporophyte (19 to 51 day) period. All four grew at approximately the same rate, with the same concentration of plantlets. The images are depicted in Figure 2 below.

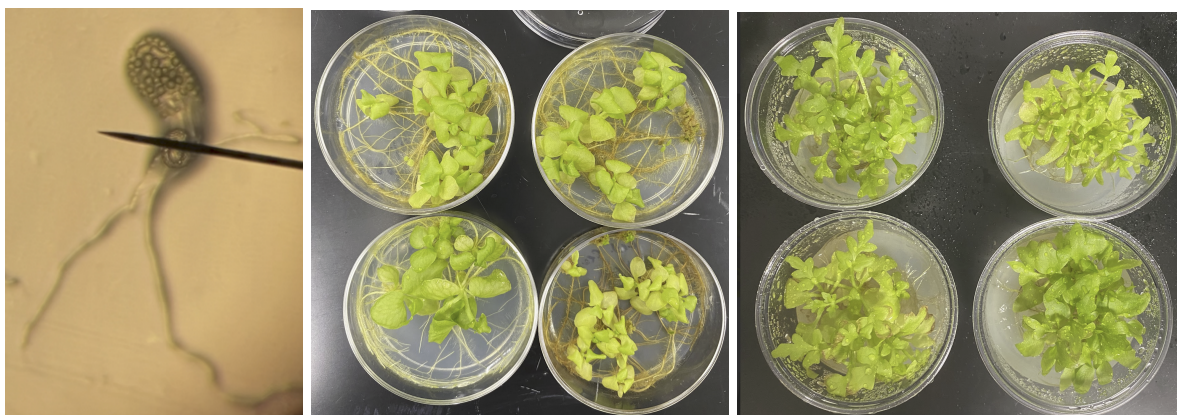


Figure 2: *C-fern* development under red light conditions. Far left image taken after 19 days of growth, middle image taken after 59 days, and far right image taken after 91 days of growth.

Far Red Light Results:

Under the experimental far-red light conditions, the *Ceratopteris richardii* exhibited stunted growth, with 2-10 small plantlets beginning to develop from spores. At 19 days, each plate contained 3-4 mature young gametophytes with elongated leaf cells and little root development. Each plate contained at least two spores that stalled in development compared to expected growth. After 51 days and development into the young sporophyte, most of the apparent growth is focused on the upward shoot of the fern, with very little leaf development. The shoot development measured at 3-4 mm in length. Only one dominant root was established, extending 2-4 mm into the media.

After 91 days, three months of growth, each young gametophyte had approximately 2-3 root offshoots, with the roots extending only a short way into the media at approximately 4-5mm. The gametophytes had several leaves, though they were poorly developed, measuring only several millimeters wide. The stems have increased in number and length, measuring 6-7mm tall with more than double the number of stems in each dish. At all points, the coloration of the leaves was a light green, indicating a chlorophyll deficiency. All replicates developed plants with a focused development in the shoot system and very little leaf development. Additionally, the roots of all four replicates were thin and spindly. Figure 3 below shows images of the growth process.

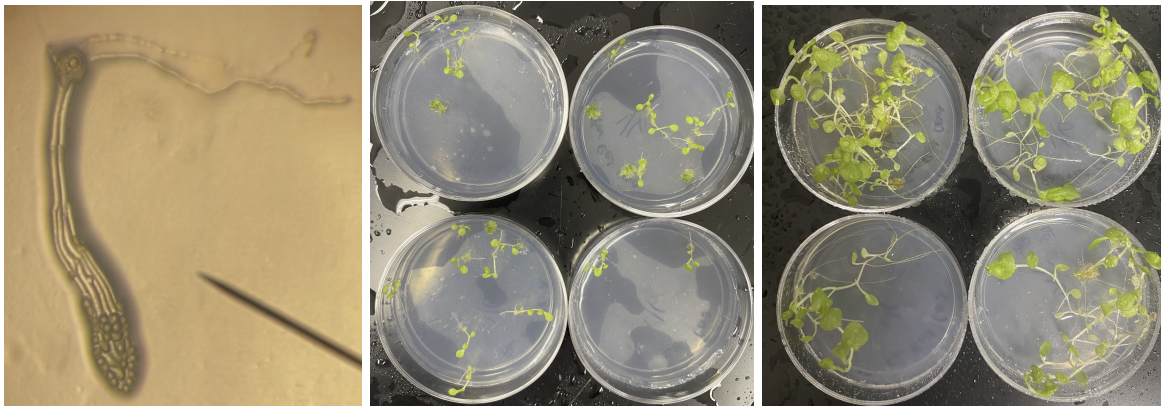


Figure 3: *C-fern* development under far-red light conditions. Far left image taken after 19 days of growth, middle image taken after 59 days, and far right image taken after 91 days of growth.

Blue Light Conditions:

Ceratopteris richardii demonstrated stunted growth under blue light conditions. Of the four growth plates, only one exhibited growth, with only one plantlet present. There were no young gametophytes present at the 19 day mark visible to the microscope. The emergence of the young gametophyte occurred at the 51 day, or two-month mark, and quickly developed into a sporophyte, possessing 3-4 leaves in the early stage. These leaves were very rigid and of a light green color, indicating a slight chlorophyll deficiency. There was very little upward growth of the fern, but there was extensive root system development after 91 days of growth, with the sporophyte having seven long root offshoots.

After 51 days of development, the fern has produced several leaves with a high surface area and are several millimeters long. There was also an increase in the intensity of green coloration in the leaves but a decrease in leaf rigidity from earlier developmental stages. Only one of the four replicates produced any gametophytes or sporophytes, leading to the development of one sporophyte total. The growth is demonstrated in Figure 4 below.

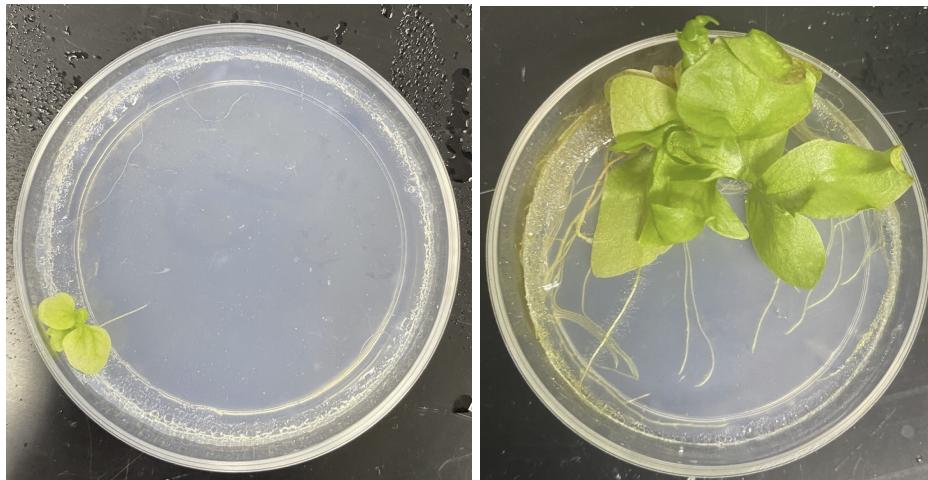


Figure 4: C-fern development under blue light conditions. Far left image taken after 59 days, and far right image taken after 91 days of growth.

Discussion

Shoot and Root Development:

Comparing the shoot growth of each sample set, it is apparent that the far-red lighting conditions induced shoot growth at the highest capacity. The ability of the far-red conditions to induce the extreme development seen in the results follows the same general trends proposed by researchers (Miller & Miller, 1964). The phenotypic results that we see here likely indicate that the phytochrome expression changes are causing a drastic increase in stem elongation (Miller & Miller, 1964). The red light growth chamber effects further support these conclusions. However, because the red light growth seems to more closely mimic that of the control plants, the neochrome receptor may be playing a role in regulating the more extreme physiological changes that would more often be seen with plants under such conditions (Miller & Miller, 1964; Taiz et al., 2018). Additionally, the plants grown under blue light conditions have stem shoot lengths similar to that of red light plants. These results further indicate that there may be some relationship between neochrome and the regulation of stem growth in both the red and blue light receptors.

Research suggests that roots formed under intense blue light conditions would more often than not form a denser network of roots due to the increased promotion of lateral root growth (Kumari & Panigrahi, 2019). While the results from the blue light ferns do indicate that there is a promotion of lateral root growth, there is not a large and dense root network. This is more than likely due to the stage of development at these experimental results were taken, and more than likely, the change in root growth can be attributed to blue light receptor expression. Under red light conditions there is an increase in the expression of the hormone auxin, which helps regulate plant growth (Datta et al., 2005). These root systems, while not necessarily more dense than the blue light growth, are often thinner and longer under red light stress (Datta et al., 2005). The results are consistent with these expectations. Therefore, it is likely that root development is primarily controlled by traditional red and blue light receptors, as opposed to neochrome. However, neochrome may impact plant hormone regulation, similar to the results here, indicating that no significant conclusions can be made regarding neochrome.

Leaf development:

Studies have shown that blue light stress can cause an increase in leaf area development (Wang et al., 2014). That hypothesis was supported by the large leaf area sizes of the blue light fern throughout all stages of development present in the experimental results. In addition, the blue light leaves demonstrate an elongated shape compared to the white light control and the other red light experimental ferns. This finding is also consistent with literature on blue light control, where the blue light-controlled hormones cytokinin and gibberellin may have played a role signaling increased leaf area development (Wang et al., 2014). On the other hand, the red ferns experienced normal leaf area development compared to white light ferns. The similar appearance of the red light fern leaf developed compared to the white is consistent with typical *Ceratopteris richardii* growth conditions, as there would not be as much stress on the cytokinin

and gibberellin expression as the blue light (Wang et al., 2014; Brown et al., 1995). However, the growth of the leaf area is still visibly stunted, which is likely partly due to the lack of blue light promotion for healthy leaf expansion and growth. In contrast, the fear-red ferns hardly had any development of the leaves at all, with a significant emphasis on the promotion of stem growth. Therefore, the discrepancies in leaf area development can likely be attributed to expressions of other light-receiving elements in the cell other than neochrome.

The ability of a plant to senesce its leaves is another critical factor in leaf lifecycle development (Fan et al., 2013). However, there was no significant development of fern senescence throughout the experiment, but it should be noted that there are phytochrome-controlled factors found in *Arabidopsis thaliana* that may contribute to negative regulation of age-induced light-mediated senescence, effectively counterbalancing the photoinduced senescence we planned to observe in the red light conditions (Tian et al., 2020). These observations to senescence conditions may be made as the plant is transplanted to soil and develops into a mature sporophyte.

The coloration of the leaves was as expected in each experimental condition. All experimental replicates displayed some photosynthetic impact, largely visible in the reduction of chlorophyll in the leaves. While studies propose a variety of factors could be attributing to the lack of chlorophyll, it is not possible to determine if neochrome expression would play a role in the chlorophyll content (Tian et al., 2020; Huang et al., 2011). However, studies done on neochrome expression in *Adiantum capillis-veneris* have demonstrated that there seems to be a photosynthetic impact related to neochrome expression (Suetsugu et al., 2005).

Future Experimentation

After the phenotypic characterization of the *Ceratopteris richardii*, as presented in this report, genotypic characterization of neochrome expression in C-fern will be conducted. Based on results from a BLAST search, no neochrome should be present in the C-fern genome. PCR will be performed to confirm the lack of neochrome in the C-fern control samples. Additional PCR will be performed on a sample selection of ferns that prior studies have identified as possessing neochrome and grown under normal lighting conditions, including *Adiantum capillis-veneris* (Li et al., 2014). Confirming these positives will provide a baseline of neochrome expression, which can be identified in further experiments. Additionally, the confirmation of the neochrome positives and negatives will further bolster the current developed phylogeny of neochrome in fern species by reinforcing conclusions from prior studies (Li et al., 2014).

After the initial conformation of neochrome in *Adiantum capillus-veneris* and lack of it in *Ceratopteris richardii*, additional experiments will be conducted to identify the presence of neochrome in *Woodwardia areolata*, *Dryopteris ludoviciana*, and *Christella denata*. Each of these ferns has only partial genomes assembled, so it is impossible to determine if neochrome is present from a BLAST search, as was previously done with the C-fern. A large part of the trouble that scientists face when assembling complete phylogeny of neochrome comes from the lack of published genomes for many species of ferns. Using our PCR methods adapted from prior studies (Li et al., 2014) and used for initial confirmation of C-fern and *Adiantum*, more information regarding the genetic distribution of neochrome among ferns can be established.

After establishing the presence of neochrome in *Woodwardia areolata*, *Dryopteris ludoviciana*, and *Christella denata*, they, alongside *Adiantum capillis-veneris*, will be grown in the same conditions as the C-fern above. Resulting phenotypic differences will be recorded at different stages in the life cycle. Additionally, real-time PCR experiments will be performed on the samples at an intermediate period to determine the expression levels of neochrome in response to the light stress conditions. These experiments will help isolate the effects and determine the function of neochrome as it relates to conferring advantages in shaded and differing light conditions.

Conclusions

From the phenotypic responses of *Ceratopteris richardii* in light stress conditions, a baseline of generic stress responses of ferns could be created. Although C-fern does not have a presence of neochrome, recognizing the phenotypic changes in response to different light conditions helps generate a baseline of stress responses in ferns that will be useful in monitoring future development of ferns that do possess neochrome. This data will help researchers determine if neochrome has a visible phenotypic expression effect or if the effect is specific to internal plant factors (Li et al., 2014).

Additionally, these results and further experimentations will help determine the phylogeny of neochrome, which at this point has relatively little research in the scientific literature (Li et al., 2014). Neochrome is a relatively new finding that has changed the scientific understanding of how light receptors may provide evolutionary advantages to low-lying plants such as ferns. Further elucidation of the neochrome gene and its corresponding expression and factor may play a critical role in understanding the function and development of light receptors, thus providing critical scientific gains in the ever-important process of photosynthesis.

References

- Alongi, D. A., Hill, J. P., & Germino, M. J. (2009). Opportunistic heterotrophy in gametophytes of the homosporous fern *Ceratopteris richardii*. *Botany*, 87(8), 799–806.
<https://doi.org/10.1139/b09-039>
- Brown, C. S., Schuerger, A. C., & Sager, J. C. (1995). Growth and photomorphogenesis of pepper plants under red light-emitting diodes with supplemental blue or far-red lighting. *Journal of the American Society for Horticultural Science*, 120(5), 808–813.
<https://doi.org/10.21273/jashs.120.5.808>
- Carolina Biological Supply. (2003). *C-fern Spores Instructions*. Burlington, NC; Carolina Biological Supply Company
- Datta, S., Hettiarachchi, G. H. C. M., Deng, X.-W., & Holm, M. (2005). Arabidopsis constans-LIKE3 is a positive regulator of red light signaling and Root Growth. *The Plant Cell*, 18(1), 70–84. <https://doi.org/10.1105/tpc.105.038182>
- Fan, X.-X., Xu, Z.-G., Liu, X.-Y., Tang, C.-M., Wang, L.-W., & Han, X.-lin. (2013). Effects of light intensity on the growth and leaf development of young tomato plants grown under a combination of red and Blue Light. *Scientia Horticulturae*, 153, 50–55.
<https://doi.org/10.1016/j.scienta.2013.01.017>
- Foreman, J., Johansson, H., Hornitschek, P., Josse, E.-M., Fankhauser, C., & Halliday, K. J. (2010). Light receptor action is critical for maintaining plant biomass at warm ambient temperatures. *The Plant Journal*, 65(3), 441–452.
<https://doi.org/10.1111/j.1365-313x.2010.04434.x>
- Franklin, K. A., & Quail, P. H. (2009). Phytochrome functions in Arabidopsis Development. *Journal of Experimental Botany*, 61(1), 11–24. <https://doi.org/10.1093/jxb/erp304>
- Hickok, L. G., Warne, T. R., Baxter, S. L., & Melear, C. T. (1998). Sex and the C-fern: Not just another life cycle. *BioScience*, 48(12), 1031–1037. <https://doi.org/10.2307/1313459>
- Huang, D., Wu, L., Chen, J. R., & Dong, L. (2011). Morphological plasticity, photosynthesis and chlorophyll fluorescence of *Athyrium Pachyphlebium* at different shade levels. *Photosynthetica*, 49(4), 611–618. <https://doi.org/10.1007/s11099-011-0076-1>
- Kawai, H., Kanegae, T., Christensen, S., Kiyosue, T., Sato, Y., Imaizumi, T., Kadota, A., & Wada, M. (2003). Responses of ferns to red light are mediated by an unconventional photoreceptor. *Nature*, 421(6920), 287–290. <https://doi.org/10.1038/nature01310>
- Kumari, S., & Panigrahi, K. C. (2019). Light and auxin signaling cross-talk programme root development in plants. *Journal of Biosciences*, 44(1).
<https://doi.org/10.1007/s12038-018-9838-2>
- Li, F.-W., Villarreal, J. C., Kelly, S., Rothfels, C. J., Melkonian, M., Frangedakis, E., Ruhsam, M., Sigel, E. M., Der, J. P., Pittermann, J., Burge, D. O., Pokorny, L., Larsson, A., Chen, T., Weststrand, S., Thomas, P., Carpenter, E., Zhang, Y., Tian, Z., ... Pryer, K. M. (2014). Horizontal transfer of an adaptive chimeric photoreceptor from bryophytes to Ferns. *Proceedings of the National Academy of Sciences*, 111(18), 6672–6677.
<https://doi.org/10.1073/pnas.1319929111>

- Liu, X., Cohen, J. D., & Gardner, G. (2011). Low-fluence red light increases the transport and biosynthesis of auxin. *Plant Physiology*, 157(2), 891–904. <https://doi.org/10.1104/pp.111.181388>
- Miller, J. H., & Miller, P. M. (1964). Blue Light in the development of fern gametophytes and its interaction with far-red and Red Light. *American Journal of Botany*, 51(3), 329–334. <https://doi.org/10.1002/j.1537-2197.1964.tb06639.x>
- Sanchez-Puerta, M. V., Abbona, C. C., Zhuo, S., Tepe, E. J., Bohs, L., Olmstead, R. G., & Palmer, J. D. (2011). Multiple recent horizontal transfers of the COX1INTRON in Solanaceae and extended co-conversion of flanking exons. *BMC Evolutionary Biology*, 11(277). <https://doi.org/10.1186/1471-2148-11-277>
- Suetsugu, N., Mittmann, F., Wagner, G., Hughes, J., & Wada, M. (2005). A chimeric photoreceptor gene, neochrome, has arisen twice during Plant Evolution. *Proceedings of the National Academy of Sciences*, 102(38), 13705–13709. <https://doi.org/10.1073/pnas.0504734102>
- Taiz, L., Zeiger, E., Møller, I. M., & Murphy, A. S. (2018). *Fundamentals of Plant Physiology*. Oxford University Press.
- Takemiya, A., Inoue, S.-ichiro, Doi, M., Kinoshita, T., & Shimazaki, K.-ichiro. (2005). Phototropins promote plant growth in response to blue light in low light environments. *The Plant Cell*, 17(4), 1120–1127. <https://doi.org/10.1105/tpc.104.030049>
- Tian, T., Ma, L., Liu, Y., Xu, D., Chen, Q., & Li, G. (2020). Arabidopsis far-red elongated HYPOCOTYL3 integrates age and light signals to negatively regulate leaf senescence. *The Plant Cell*, 32(5), 1574–1588. <https://doi.org/10.1105/tpc.20.00021>
- Trouwborst, G., Hogewoning, S. W., van Kooten, O., Harbinson, J., & van Ieperen, W. (2016). Plasticity of photosynthesis after the ‘red light syndrome’ in cucumber. *Environmental and Experimental Botany*, 121, 75–82. <https://doi.org/10.1016/j.envexpbot.2015.05.002>
- Wang, Q., Zuo, Z., Wang, X., Liu, Q., Gu, L., Oka, Y., & Lin, C. (2014). Beyond the photocycle — how cryptochromes regulate photoresponses in plants? *Current Opinion in Plant Biology*, 45, 120–126. <https://doi.org/10.1016/j.pbi.2018.05.014>
- Wang, X. Y., Xu, X. M., & Cui, J. (2014). The importance of blue light for leaf area expansion, development of photosynthetic apparatus, and chloroplast ultrastructure of *Cucumis sativus* grown under weak light. *Photosynthetica*, 53(2), 213–222. <https://doi.org/10.1007/s11099-015-0083-8>