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Changes in Amaranth Microgreen Growth in Response to Differing Light Conditions

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ABSTRACT

Microgreens are small, fast-growing greens that are gaining popularity for their high levels of flavor and nutritional content. Climate change has pushed food production indoors. With the implementation of controlled environment agriculture, determining the proper daily light integral (DLI), photoperiod, and environmental conditions is imperative for energy efficiency and sustainable production. We tested for changes in Amaranth (*Amaranthus cruentus* 'Copper-head') microgreen height, leaf surface area, and biomass in response to differing amounts of light. We aimed to test two replicates of eight light conditions with differing DLI, photoperiods, and photosynthetic photon flux density (PPFD). After germination, we took weekly pictures to determine leaf surface area and height. We then took measurements of these pictures using ImageJ. After three weeks of growth, we harvested the plants. After we finished, we analyzed the data for a correlation between DLI and plant size.

Introduction

As climate change progresses, so do the issues that it inevitably worsens. The United States and the world, we must start thinking about the future of our food and agriculture, so food production may have to be pushed indoors where there would be less variables by implementing controlled environment agriculture [5]. However, we must find the conditions with which the growth would be most efficient. When talking about the most efficient conditions for each type of plant, many components come into play. Each type of plant may have different needs when it comes to humidity, soil, temperature, nutrients, and lighting. Even within those areas, there are more specific conditions that would need to be looked at for maximum efficiency. In an effort to focus the scope of this experiment, we concentrated on three conditions within lighting. The three conditions were applied through light emitting diodes (LEDS) and are as follows:

Photoperiod	The total time during which a plant is subjected to light in the span of 24 hours.
Photosynthetic Photon Flux Density (PPFD)	The intensity of the light by the second.
Daily Light Integral (DLI)	The total daily light that a plant receives. It is a function of PPFD with the unit of day.

We also decided to use microgreens for this experiment. Microgreens have gotten recent attention due to their fast-growing tendencies as well as their high nutritional content. [3] They are classified as germinated seedlings that have grown to about 5 centimeters and include cotyledons and at least one or two true leaves. [4] Furthermore, many microgreens can be grown in a relatively small area since 1-10 seeds can be planted per cm2 (this depends on

the species of the microgreen). [4] If humans were to have to decide which crops to grow in controlled environments, there might be an emphasis on high nutrition and low volume crops. Since microgreens are known to be small, they seem like a great choice.

Many microgreens can be grown without soil by using hydroponic systems. [3] These systems do not use soil and can instead use standardized substrates such as rockwool. Rockwool is made of thin fibers creating high water retention and a great environment for some microgreens, such as Amaranth. Amaranth is also known to have a distinguishing structure that contains a high concentration of important amino acids even when compared to barley, maize, wheat, and legume species [2]. To avoid too many variables, we used the same substrate (Rockwool) and seed batch ('Copperhead') throughout this experiment.

Methods and Procedure

Methods

The experiment took place in a lab setting. The humidity and temperatures were kept the same throughout all the experiments. There were three sections in the lab where the overhead lights were blocked off using curtains for easy access to the microgreens. In each section, there was one overhead LED light system. These LEDs could change colors but we decided to keep them close to a natural, sun-like, color using a lot of yellow, some green, and a little bit of red and blue. Overall, the light the LEDs gave off was close to white. When deciding what conditions we were going to apply, we created combinations of photoperiod, PPFD, and DLI. By the end, we had eight different conditions (Figure 2).

Table 1: Experimental light conditions used.

ID	Treatment (DLI / photoperiod)	Photoperiod (h)	PPFD (umol/m^2/s)	DLI (mol/m^2/d)
1	12/8	8	400	11.5
2	12/12	12	280	12.1
3	12/16	16	200	11.5
4	12/24	24	140	12.1
5	10/8	8	330	9.5
6	10/12	12	230	9.9
7	10/16	16	170	9.8
8	10/24	24	110	9.5

Each of the eight conditions was replicated two to three times.

Procedure

When preparing the microgreens for each condition, we used two containers with rockwool and water placed inside of each. We measured one gram of Copperhead Amaranth seeds and spread them evenly on the rockwool surface. Finally, we sprayed the seeds with diluted hydroponic solution to help with germination. We then repeated the same treatment for the other container. We left the containers in the dark for two to three days for the seeds to germinate, after which we put both containers under their designated lighting condition. We repeated this two more times to have three different light conditions happening at the same time.

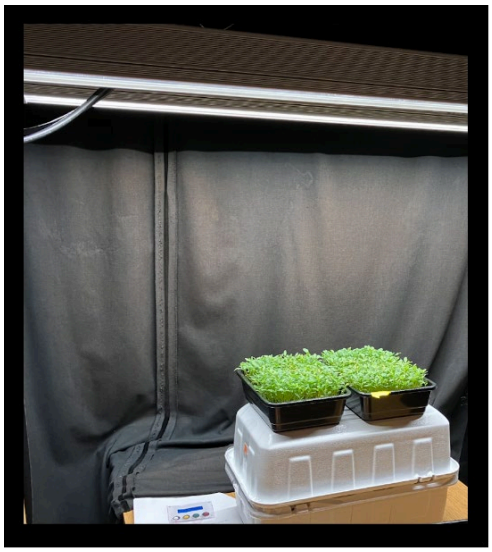

Figure 1: Inside a section of the lab with a lighting condition. Note: this shows Week 3 plants.

A week later, we took 30 plants out of one container (which will now be known as the Stem Group) to measure stem length. With the other container in the same condition (which will now be known as the Area Group), we took an overhead picture to measure the larger canopy leaf areas. To acquire these measurements, each picture included a ruler to scaling reference. Using ImageJ software, we translated pixel lengths into centimeters and pixel areas into centimeters squared.

Figure 2: Example of plants taken from Stem Group.

This process was repeated in the following weeks as well, until the third week which was also harvest week.

Harvest Week

On harvest week, we still took 30 plants out of the Stem Group and took an overhead picture of the Area Group. We then harvested all the microgreens from the largely undisturbed Area Groups and weighed the plants on a scale for biomass. Finally, we placed as many of the microgreens as possible in labeled tubes to freeze them using liquid nitrogen. They were then placed in a freezer if needed for further studies.

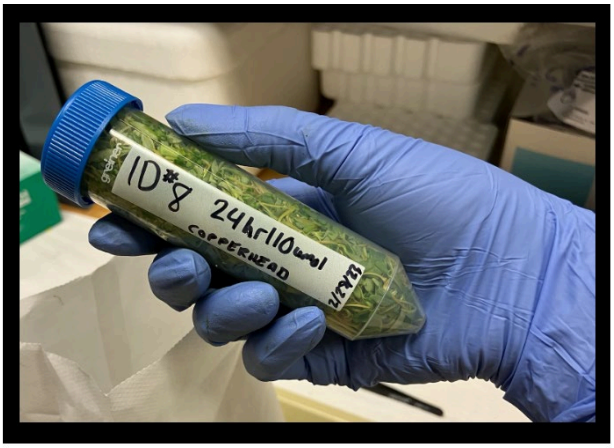

Figure 3: Harvested microgreens before being frozen

Results

Overall Results

The measurements gathered by using ImageJ were placed in a large Google Sheet where the data were annotated with the corresponding condition, week number, and replicate number (since each condition was replicated two to three times). Once in the Google Sheets, each condition's stem length and area was averaged for each week. When looking at the results, we mostly focused on the final week to figure out which condition resulted in the tallest or biggest microgreens. We also compared biomass between light conditions.

The biomass tended to vary a lot and seemed to lack a pattern. However, by looking at the data displayed in Table 2, we can focus on maximums. By looking at the maximum values, ID 2 and ID 6 were the heaviest batches of microgreens. When looking at their similarities, they both have similar PPFDs with ID 2 having a PPFD of 280 umol/m^2/s and ID 6 having a PPFD of 230 umol/m^2/s. They also both have a photoperiod of 12 hours. However, ID 4 also had a high biomass despite it having a PPFD of 140 umol/m^2/s and a photoperiod of 24 hours.

When analyzing leaf areas, it was also difficult to see a concrete pattern. However, IDs 5-8 areas were overall larger than IDs 1-4. The main difference between these two groups was the DLI. While IDs 1-4 had a DLI closer to 12 mol/m^2/d, IDs 5-8 had DLI values closer to 10 mol/m^2/d. However, no DLI was exactly those values with IDs 1-4 ranging from 11.5-12.1 mol/m^2/d and IDs 5-8 ranging from 9.5-9.9 mol/m^2/d (Table 1).

Table 2: Final results to experiment.

Condition Description	ID #	Biomass (g)	Final Area on Week of Harvest (cm^2)	Final Stem Length on Week of Harvest (cm)
8hr400	ID 1	35.63	1.0001	4.7668
12hr280	ID 2	38.41	1.3207	3.9372
16hr200	ID 3	30.48	1.4354	4.0654
24hr140	ID 4	38.12	1.9529	2.9106
8hr330	ID 5	35.46	2.0743	5.6897
12hr230	ID 6	38.19	2.4254	3.6430
16hr170	ID 7	34.00	2.7830	3.7633
24hr110	ID 8	34.73	1.8531	3.5352

Note: All these values are the maximum average from across all the replicates for each condition on the final week.
Note: The largest value in each category is highlighted in gray.

Stem Results

When analyzing the stem lengths, there might not be any patterns by looking at the maximum values. However, by plotting the stem lengths on a bar graph, we start to see a pattern emerge (Figure 4). As the photoperiod gets longer, the plants get shorter stem lengths. With a DLI of 12 $mol/m^2/d$, a gradual decrease is seen as the plants are subjected to their respective lighting condition for a longer time. With a DLI of 10 $mol/m^2/d$, there is a sharper slope initially, but it then becomes a very slight change throughout IDs 6-8.

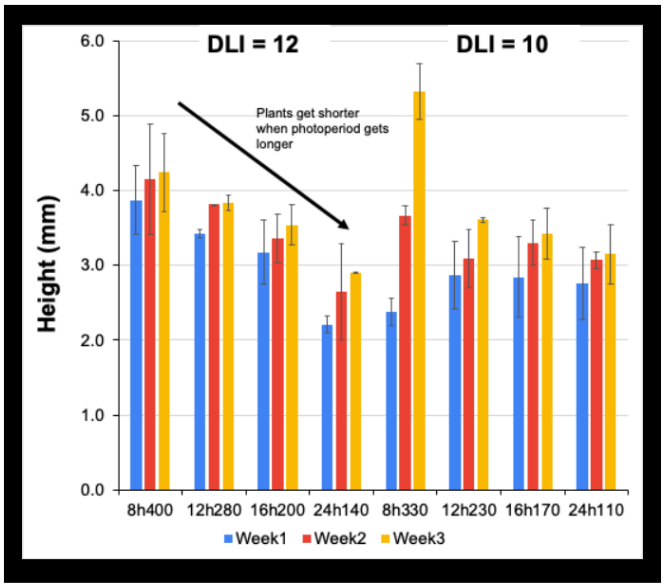

Figure 4: Stem height results

Discussion

Stem Significance

Overall, our result that longer photoperiods lead to shorter stem lengths corroborates previous studies. When a plant receives too much light, it can inhibit cell elongation and there are visible changes. On the leaves, for example, burned or pale spots start to appear. By looking at this study and Figure 4, it's reasonable to conclude that longer photoperiods also negatively affect stem lengths by making the microgreen stems shorter. However, the photoperiod response depends on the DLI, so when regarding the data, the decrease in growth is more visible when DLI is 12 mol/m^2/d rather than 10 mol/m^2/d.

A possible reason for this is that, since there is so much light all the time, microgreens don't have to grow taller to absorb more light when it's available to them. Light being available 24 hours a day could make growing closer to the lights a waste of the plant's resources. Furthermore, though we did observe pale spots in some microgreen containers that had more extreme conditions (longer photoperiods or higher PPFD), longer photoperiods didn't seem to affect the biomass nor the leaf areas by a significant quantity when compared to conditions with shorter photoperiods (Table 2).

A Note on Amaranth

Amaranth has a C4-type photosynthesis [1]. This type of photosynthesis is common among plants which are used to grow under high intensity light, such as corn. In Figure 4, it can be seen that the conditions with higher intensities lead to a better microgreen yield. These conditions are also the ones that have a shorter photoperiod, so the better microgreen growth could be a result of photoperiodism. Photoperiodism is a control over the plant by a circadian clock, and if the process is circadian and rhythmic, it means that its regulation has been optimized to its environmental condition. If the stem growth is rhythmic, it normally means that the growth happens at the end of the night. So, the conditions that led to the most growth make sense since amaranth comes from a hot and harsh environment with high light intensity.

Data Limitations

Sometimes the stem lengths could be unpredictable and odd since the lengths from week one could be bigger than week two, and sometimes the lengths from week two could be bigger than those in week three. However, we realized since we always took the top 30 canopy microgreens from the Stem Group, the following week the plants that had been growing best were gone. Furthermore, sometimes not all the measurements could be perfect. It's possible some containers had slightly more rockwool, water, or hydroponic solution than others. However, it shouldn't have affected the results in an extreme way since realistic patterns still emerged in our data.

Future Studies

Though this experiment and study is a good step in the effort of creating efficient controlled environment agriculture, there is still much to be done. We barely scratched the surface when discussing all the possible lighting combinations. Furthermore, we didn't use all the different colors possible with the LEDs, and there is a possibility that a different color light could stimulate more growth from the Amaranth microgreens.

Even more generally, there are many experiments and studies that could be done by looking at all the other components of agriculture we kept constant in this experiment such as soil, humidity, and temperature. To further this

study specifically, we could try to see if there are any differences or patterns in the nutritional content of the microgreens we harvested and stored. It is possible there is a difference between microgreen size and microgreen nutrient concentration.

Finally, there is a big spike during the condition with a photoperiod of 8 hours, PPFD of 330 umol/m^2/s, and a DLI of 10 mol/m^2/d. Since it doesn't follow as much of a pattern as the plants from DLI 12 mol/m^2/d, more could be explored with this condition. Especially since, though the regulation by photoperiod is interesting from a basic science standpoint, we showed that the best microgreen yield was from DLI=10 mol/m^2/d, which is the most sustainable condition from a production standpoint.

Conclusions

With successful agriculture slowly becoming more of an issue, we need to look at less common sources of food and nutrition. In the US, many western states have become subject to hurricanes, floods, droughts, and huge storms. This creates an issue since those states produce most national agricultural products. As natural conditions worsen and resources must be used more carefully, microgreens could help feed our population due to their small size and nutritional content. However, to mass produce them for our large and growing population, we need to be able to do it in the most efficient way possible. Using studies such as this, we can start steering people in the right direction.

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