IMPACT OF FAR-RED LIGHTING IN TISSUE CULTURE MICROPROPAGATION OF HEMP

A Project Paper

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By

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ABSTRACT

With the rapidly changing landscape of Hemp and Cannabis legislative policy in the United States and many countries around the world, its social and economic impact as an agricultural crop is rapidly expanding. With this expansion there are many discoveries. Some of these discoveries come in the form of new pests, viruses, and pathogens for which hemp and cannabis are susceptible.

The tissue culture micropropagation of hemp and cannabis can be used to eradicate these viruses, pests and pathogens from the plant. Once sterile and virus free explants can be multiplied exponentially. The stage ii multiplication and stage iii rooting of these propagules is the focus of this research.

It is widely accepted that hormone treatments within the tissue culture media signal different types of growth within the explants. It is also accepted within the science community that light quality and quantity signal different types of growth within plants. This project aims to gain some insights on the interactions between far-red light and the hormones BA (6-Benzylaminopurine) and IBA (Indole-3-butyric acid) in tissue culture micropropagation of hemp in three different hemp cultivars.

The results of this experiment show that the far-red lighting treatment produced some of the tallest explants, and longest roots. There was great variability across cultivars. Some cultivars produced more nodes with IBA than BA. The genetic diversity in *Cannabis s.* justifies the need for more research in tissue culture micropropagation with many more cultivars.

BIOGRAPHICAL SKETCH

Franklin Henderson was born and raised in Houston, Texas. Upon graduating from the High School for the Performing and Visual Arts he attended the Maryland Institute College of Art. He received a bachelor's degree in Fine Art majoring in photography. While in school Franklin noticed that photographic darkroom processes required some understanding of physics and chemistry. Simultaneously technology was disrupting the world of photography with digital cameras. Franklin had always been tech savvy and the dawning of the digital era opened up a world of new possibilities.

Post-graduation Franklin moved to Brooklyn, New York where he formed an independent record label with some of his friends from high school and college. As he dove into the deep end of digital audio production and engineering, he began to see the underlying themes of science, art and technology synergizing again. Franklin and his friends enjoyed some success along the way, while building an expansive network and lifelong relationships throughout the industry.

Franklin's strengths in creativity, and entrepreneurship have allowed him to reinvent himself as the social, economic, and political world around him evolved. As the green wave of cannabis legalization swept through the west coast of the United States, he began working in a licensed cannabis cultivation company in Mendocino, California. Mendocino county is part of what is known as the 'Emerald Triangle' the mecca of cannabis cultivation in North America. During the three years he spent in California he immersed himself in learning cultivation and post-harvest science.

Shortly after the birth of his son, Franklin accepted an offer from Cornell University Cooperative Extension in New York City. Here he was able to apply his agricultural and cultivation knowledge while engaging in science communication in New York City communities. In 2021 almost a year into the Covid pandemic Franklin was hospitalized with a pulmonary embolism. He submitted his application to the Cornell MPS degree program from that hospital bed.

During his academic studies at Cornell, he pursued his interests in hydroponics, biotechnology, hemp breeding, and business. At the time of this writing Franklin is completing his master's in professional studies under the advisement of Dr. Neil Mattson specializing in Controlled Environment Agriculture.

ACKNOWLEDGEMENTS

I'd like to begin with an acknowledgement of Mitochondrial Eve through whom all living humans are connected. We are all one species on mother earth. Race, religion, gender, and many other social constructs have been used to divide us. With this acknowledgement I reaffirm our oneness.

It's imperative that I acknowledge the indigenous lands on which I have been blessed to have lived, learned, grown, and connected through. I thank the Karankawa, Coahuiltecan, Atakapa-Ishak, Sana Nations in what is now called Houston, Texas. I thank the Lenape and Cayuga Nations in what is now called Brooklyn and Ithaca where Cornell University is located. I thank the Pomo and Yuki Nations in what is now called Mendocino County, California which has been an epic part of my journey.

I would like to thank my Mother Therese A. Wyatt, who cultivated a love of writing and learning that has nourished and developed me and continues to do so. I would like to thank my Father James J. Henderson who challenged me and encouraged my creativity and independence. I cannot thank my wife enough for her unwavering love and support that ensures I shine through as my authentic self. To my son Elias, thank you for so much medicinal and healing laughter it keeps me motivated. To all my closest friends, family and kin, my energetic family, you are a part of my success and I appreciate all of the love and support you have invested in me.

David Sheard, Eileen Montgomery, Diane Marks, Rick Jennings and Dr. Leslie King-Hammond, thank you for being amazing educators and mentors for me in both high school and college. The ways in which I learned from you has enriched my life.

Dr. Jennifer Sarah Tiffany, Dr. Neil Mattson, Nick Kaczmar, Dr. Carlyn Buckler, Dr. Heather Grab, Dr. Mark Bridgen, Georgia Neighly, and Victor Zayas this Cornell MPS Degree would not have been possible without your support, care, and attention. It would not have been nearly as enjoyable without your unique talents, gifts, personalities, and connection. I thoroughly enjoy the times I've spent with each of you, I'm humbled by your brilliance, and cannot thank you enough.

Finally, I am so grateful to be doing academic research with Hemp (*Cannabis sativa).* Growing up during the height of the 'War on Drugs' and mass incarceration I never dreamed I'd be where I am today. I would like to thank the cultivators, artisans, advocates, and martyrs, who have lost their freedom and in some cases their lives to ensure this plant, with which humans have had prolific experiences, is accessible to future generations.

LIST OF ABBREVIATIONS

B	Blue
BA	6-Benzylaminopurine
FR	Far-Red
IBA	Indole-3-Butyric Acid
MS	Murashige and Skoog
PAR	Photosynthetically Active Radiation
pH	Power of Hydrogen
R	Red
TC	Tissue Culture
THC	Tetrahydrocannabinol
W	White

INTRODUCTION

Tissue culture, its associated tools and techniques had its inception in the theory that living cells can survive and function outside of a living organism. In artificial environments animal tissues have survived in different mediums as early as 1907(Britannica, 2020; Del Pozo et al., 2005).

Since that time, it has been a beneficial tool to scientists across many disciplines and its requisite features have expanded to the demands of those disciplines (Parker, 1950). In plant tissue culture micropropagation we are concerned with the multiplication of entire plants, identical in genetic makeup to the original parent stock (George et al., 2007). Depending on the species of plant these explants may be cuttings from organized growing points like shoots, roots, stems, leaves, nodes, embryos, pollen, or anther (Adhikary et al., 2021).

In totipotency we find the key feature central to the theory behind plant tissue culture micropropagation. Totipotency is the ability for somatic cells to reproduce and differentiate into all the necessary cell types to produce an entire plant (Fehér, 2019).

Small cuttings from parent plants placed on a medium containing the necessary carbohydrates, hormones, nutrients, and minerals signal to the explant which type of differentiated cells to multiply and grow (Del Pozo et al., 2005). The other factor at play in this process are photoreceptors including but not limited to cryptochromes and phytochromes that regulate cell elongation and other actions based on the quality and quantity of light (Reed et al., 1993).

In Vitro Micropropagation of plants is typically preceded by growing and maintaining Parent stock plants that carry the desired genotypic and phenotypic traits. Micropropagation in tissue culture consists of 4 stages (Loyola-Vargas & Vázquez-Flota, 2006; Van Huylenbroeck, 2018).

Stage I: Initiation of the excised plant tissue begins with sterilization to remove any pests, microbes, bacteria and or fungi that would thrive, proliferate and harm the explant within the tissue culture vessel (Van Huylenbroeck, 2018).

Stage II: Multiplication of the plant tissue from small cuttings into many shoots occurs during Stage II. It is common to signal this type of growth with the presence of cytokinin in the medium on which the explant is placed (Van Huylenbroeck, 2018).

Stage III: Rooting explants in vitro occurs during Stage III. It is common to signal this type of growth with the presence of auxins in the medium on which the explant is placed (Van Huylenbroeck, 2018).

Stage IV: Acclimatization occurs during Stage IV. The explant is removed from its vessel and acclimated with great care to its new in vivo environment (Van Huylenbroeck, 2018).

The term tissue culture is often used in the context of micropropagation but has many other applications. Those applications are still tissue culture and should be differentiated using terms that specify the application (Van Huylenbroeck, 2018). Some of the different applications for plant tissue culture include but are not limited to, genetic

transformation, meristem culture, somatic embryogenesis, synthetic seed, microspore culture, cryopreservation, and secondary metabolite culture (Adhikary et al., 2021).

In addition to germplasm maintenance tissue culture in its many forms is a valuable tool for advancing breeding techniques (Stack et al., 2021). Considering global warming and water scarcity, recent advances in biotechnology and genomics may play a pivotal role in the conservation of medicinal, agricultural, and endangered plant species (Wochok, 1981).

Hemp

Hemp (*Cannabis sativa* L.) is an annual, mostly diecious plant native to central Asia that has been cultivated across the planet over the last 5000 years or more. (Adhikary et al., 2021; Cranshaw et al., 2019). In the United States Hemp is *Cannabis sativa* L. that does not contain more than 0.3% Delta9 THC concentration by dry weight. (*7 CFR 990.1 -- Meaning of Terms.*, n.d.) The diecious nature of this plant, meaning it forms pistillate and staminate inflorescence on separate male and female plants, lends itself to great genetic diversity (Dayanandan & Kaufman, 1976). As such it has developed many varied uses including but not limited to nutritional, medicinal and industrial uses (Adhikary et al., 2021, p. 1). Because this crop is so diverse, I'd like to propose three lenses through which we can address the science of its cultivation.

1. **Agricultural Crop**

Hemp can be grown as an agricultural crop on a large scale for fiber, seed, and biomass from which secondary metabolites can be extracted. This style of cultivation adopts some of the machinery and practices found in other large field crops like corn and wheat (Cherney & Small, 2016).

2. **Horticultural and Botanical Crop**

Both Cannabis and Hemp can be grown in greenhouses or controlled environments like horticultural crops with attention given to plant propagation and cultivation to improve plant growth, yields, quality, nutritional value, and resistance to insects, diseases. Because of the medicinal qualities of the secondary metabolites found in the inflorescence of Hemp (*Cannabis sativa* L.) aspects of its cultivation can be considered botanical. (Adhikary et al., 2021, p. 1; Chandana et al., 2018)

3. **Floricultural Crop:**

Some segments of the Hemp and Cannabis market are strictly concerned with the cut and dried flowers for aesthetic beauty and high concentrations of metabolites. Areas of study in this science include special aspects of floriculture, such as spacing, training and pruning plants for optimal flower harvest; and post-harvest treatment such as cold treatments, storage, preservation, and packaging (Van Huylenbroeck, 2018).

Hemp and Cannabis contain more than 500 phytochemicals, more than 100 of them are Phyto cannabinoids (ElSohly et al., 2017; Liktor-Busa et al., 2021). Because of the psychoactive nature of one Phyto cannabinoid, Delta9 THC legal restrictions have

prevented the research necessary to fully potentiate this crop in the ways that are common for many other agricultural crops globally. The history around cannabis policy in the U.S. is rooted in racism (Hudak, 2020). Recent changes to legalization in Canada, Europe, Israel and in the U.S. at state levels and the 2018 Farm Bill have allowed new growth in the scientific literature (Abuhasira et al., 2018). This crop is at the center of a multibillion-dollar global market that would greatly benefit from an expanded knowledge base integrated across multiple disciplines.

Tissue Culture and the Hemp/Cannabis Industry

With the adoption of medicinal and adult use cannabis laws in many states within the U.S. along with the 2018 Farm Bill, C. sativa is being cultivated at a much larger scale than any time in recent history (Decorte & Potter, 2015). With this rapid growth, pests, and vectors for disease are presenting newly discovered problems (Cranshaw et al., 2019).

Meristematic techniques in tissue culture micropropagation have proven to be effective in eliminating virus, and viroid from previously infected plant stock (Holmes et al., 2021). Because unique and diverse genotypes create high value within the cannabis marketplace, maintaining the integrity of these cultivars is vital to the success of cultivators. As of 2021 the global cannabis market was worth $340 B2 (Adhikary et al., 2021). Using the available data, it is estimated that the global expected market size for tissue culture clones/manual clones could be predicted around $5B (Adhikary et al., 2021). Because every gram of hemp or cannabis that gets consumed on earth annually

begins with either a seed, in vivo clone, or in vitro tissue culture. Propagators serve a vital role in the supply chain. It is important that we maintain the diversity and integrity of germplasms across market classes (Chandana et al., 2018; Stack et al., 2021). The potential for tissue culture to provide clean plantlets, free of pest, viral, viroid, bacterial, and fungal infections at scale could be vital to advancing this crop to its greatest potential (Holmes et al., 2021).

Hemp Tissue Culture Research

I had the timely fortune to begin this capstone project just as Conor Stephen was graduating. Although our paths did not cross, I found his love of the art and science of hemp micropropagation, evident in his thesis resonated with me. I also had the great fortune to learn from Dr. Mark Bridgen and Victor Zayas who shared invaluable insights with me generously. *The Micropropagation of Hemp Thesis* defined optimal conditions for the micropropagation of TJ's Hemp cultivar across several factors (Stephen, 2021).

Tissue culture micropropagation is a valuable tool because of its scalability. Minimizing waste and maximizing efficiency in the tissue culture micropropagation of hemp has crucial economic implications for an industry rife with volatility and changing market forces. There are several factors that are required for the tissue culture of hemp to be successful. Sucrose concentrations, Basal Media, Gelling Agent, pH, Explant type, temperature, hormone treatment depending on which stage the explant is intended for, each have multiple options (Stephen, 2021). Stephen's research does a good job at distilling those options into recommendations across one cultivar TJ's CBD.

In this project the focus was the results from Stage II - Multiplication and Stage III In vitro Rooting. By focusing on the formulations that gave the most optimal results. In the subsequent research found in this paper we would replicate these recommendations across multiple cultivars to add to the available data, and base of knowledge available in this burgeoning area of plant science in Hemp.

Sucrose

In tissue culture sucrose is a widely used carbohydrate to support osmotic potential and serve as an energy source for the plant's biological processes in vitro (Yaseen et al., 2013).

One of the significant determining factors for explant growth and quality was sucrose. 1.5% sucrose concentration in the medium was optimal. These explants exhibited greater fresh weight, shoot length, and quality rating (Stephen, 2021). Sucrose concentrations of 1.5% and 3% did not yield significantly different results from one another. 1.5% sucrose concentration would present a 50% reduction in sucrose cost in media preparation (Stephen, 2021).

Basal Media

In plant tissue culture the basal media serves as the substrate for explant growth. Basal tissue culture media typically contains a combination of the following components: macronutrients, micronutrients, vitamins, amino acids or nitrogen supplements,

source(s) of carbon, undefined organic supplements, growth regulators and solidifying agents (Saad & Elshahed, 2012).

Two commercially available basal media acceptable for hemp tissue culture micropropagation include MS (Murashige and Skoog) and LS (Linsmaier and Skoog). The basal media used reveal no significant difference between MS and LS. LS produced slightly higher average shoot weight, shoot length, and number of shoots (Stephen, 2021).

Gelling Agent

Gelling agent is used in the formulation of tissue culture media to provide the support necessary for the explant to maintain an upright position and proper polarity. Stephens' report indicates that the type of gelling agent in the media, agar, agargellan, and gellan gum produced no statistical differences between treatments (Stephen, 2021).

pH

It is commonly accepted that pH of a substrate or medium directly correlates a plants ability to uptake nutrients and minerals, as well as overall implications for plant health. Precise control of pH in tissue culture media is desirable. This thesis suggests a standard pH of 5.8 yields explants of the best quality (Stephen, 2021).

Explant Type

Apical shoot tips are ideal organized growing points but limited in number depending on the maintenance of the parent stock. Single node explants taken from nodal points along the stem or branch are typically more abundant. Shoot tip and single node explants both produce prolific growth under ideal conditions in stage ii multiplication. Shoot tips will be preferred when performing meristem culture (Stephen, 2021).

Temperature

Typical temperatures for growth chambers in plant tissue culture vary from 15°C-30°C. Hemp performs best at a temperature of 28°C/82°F producing higher quality, faster growing plants with more nodes and greater root formation. It falls slightly on the warmer end of the temperature range. (Stephen, 2021)

Hormone Treatment

Cytokinin is the plant growth regulating hormone associated with the proliferation of new shoots and nodes. For stage ii the results indicate that a concentration of cytokinin BA (6-Benzylaminopurine) at 1.0 µM produced taller explants with more shoots and nodes consistently (Stephen, 2021).

Auxins are the plant growth regulating hormone associated with the proliferation in the development of the root zone. For stage iii the results indicate that a

concentration in the realm of 2.5 µM IBA (Indole-3-butyric acid) is optimum for in vitro root induction (Stephen, 2021). IBA has been the preferred auxin for other in vitro rooting protocols as well (Lata et al., 2009).

In the fall of 2021, I discovered a paper published by Pepe et al. addressing optimization for tissue culture micropropagation of *Cannabis sativa.* The algorithms and machine learning aspects of the study were interesting. Artificial Intelligence, machine learning, automation and cooperative robotics are exciting new frontiers that deserve more exploration in tissue culture and controlled environment agriculture (Pepe et al., 2021).

I found the results around light quality and quantity to be an interesting variable that I would like to experiment with myself. Through advising and consulting with Dr. Neil Mattson I was able to use the findings from this study to refine and identify what spectrums of interest I would use in this project, particularly far-red.

Quantifying the economic impact of hemp tissue culture facility design, light spectrum, intensity, electricity costs and propagation productivity could be vital to the success or failure of a business at both small and large scale. Finding the right spectrum and intensity could offer a competitive advantage in quality of explants produced while providing cost saving efficiencies.

Light Quality

The generally accepted spectrum for Photosynthetically active radiation (PAR) is the wavelength range 400-700 nm. Blue light is defined as 450-495 nm. White light consists of multiple colors on the spectrum when combined are defined as White light encapsulating the full PAR 400-700. White light may exhibit peaks and valleys of intensity across the PAR spectrum. Red light is defined as 620-700 nm. Far Red is defined as 700-750 nm. Far red falls outside of the range of the standard PAR, although it does appear to have photomorphogenic effects on plants (Pepe et al., 2021).

Light intensity and spectrum play important roles for in vitro morphogenic and developmental processes (Batista et al., 2018). Different photoreceptors recognize the quality and quantity of light (e.g., phytochromes absorb red and far-red, phytotropins and cryptochromes absorb blue light), and subsequently use this information to direct photomorphogenic functions (Li et al., 2012).

In this study, Blue light treatments were the most important factor for shoot length, shoot number, and node number. Greatest shoot length was achieved from 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$ W + 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$Fr. Plantlets developing the most nodes came from 33 $\mu mol \cdot m^{-2} \cdot s^{-1}$ each from R + B + Fr. The largest canopy surface area was attained by plantlets grown under 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ each from R + B. Greatest root length was achieved with 33 $\mu mol \cdot m^{-2} \cdot s^{-1}$ each from R + B + Fr. Red light was the most important spectra for governing canopy development (Pepe et al., 2021).

Light Quantity

Optimal light quantity will vary across species in alignment with common knowledge, which is easily observed in nature, and verifiable by taking measurements with light meters. Most micropropagation systems use light levels ranging from 40-80 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR (Murphy & Adelberg, 2021).

Cannabis grows best in vivo under higher light levels (Murphy and Adelberg, 2021; Wróbel et al., 2020), with yields available under a Precision tissue culture of Cannabis sativa increasing linearly up to at least 1600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR depending on the culture system (Chandana et al., 2018; Pepe et al., 2021).

The validation experiment in this study, by Pepe et al, demonstrates that cannabis responded to high light levels. Similarly, in the initial experiment, it was observed that higher intensities of R in combination with equal or lower intensity of B or W to be beneficial to canopy development (Pepe et al., 2021).

MATERIALS AND METHODS

The goal of this experiment is to determine if there is a quantitative or qualitative benefit to the addition of far-red light in the micropropagation of hemp and influence of rooting hormone of three hemp cultivars in tissue culture.

Culture Media

The basal medium used for this experiment was Murashige and Skoog (MS, Murashige and Skoog 1962). MS and 3%sucrose were combined. The pH of the medium was adjusted to 5.8 with 0.1 N KOH. For Stage II media 0.11 mg/liter BA (0.5 uM) was added. For Stage III media 0.5 mg/l (2.5 µM IBA) was added. The medium was solidified with the addition of 0.7% (w/v) agar. Magenta™ GA-7 vessels (Magenta LLC., Lockport, IL) containing 60 mL of media and capped with non-ventilated lids. The culture vessels were autoclaved at 121°C and 1.03421 Bar for 20 minutes.

Plant Material

The cultivars ‘TJ’s CBD’, ‘A2’, and ‘Janets’ G’, were used in this experiment. These explants existed in Stage I tissue culture at the Cornell University Long Island Horticultural Research Center in Riverhead Long Island prior to being sub cultured onto newly prepared media. 48 replicate explants of similar size and quality were sub cultured into individual Magenta GA-7 vessels for Stage II and Stage III respectively.

Growing Conditions

Cultures were maintained in a growth room at approximately 28°C for 56 days. (See Figures 1 & 2). The experiments were installed on two separate racks in the same room. The temperature was controlled by a window installed air conditioner with thermostat controls set to 28°C/82°F. The temperature was logged hourly for the duration of the experiment via *SensorPush*

Lighting Treatments

24 Stage II explants and 24 Stage III explants were cultured under the control lighting treatment, a 24-hour light photoperiod at 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provided by LED White 400-700 nm (2x Barrina 4-foot LED T8).

24 Stage II explants and 24 Stage III explants were cultured under the far-red lighting treatment, a 24-hour light photoperiod at 66 $\mu mol \cdot m^{-2} \cdot s^{-1}$White 400-700 nm + 33 $\mu mol \cdot m^{-2} \cdot s^{-1}$ FR 700-750 nm

Hormone Treatments

Within each lighting treatment half of the plants received Stage III media, 0.5 mg/l (2.5 µM IBA) and half received, Stage II media, 0.11 mg/liter BA (0.5 µM). Overall there were eight replicate explants per cultivar, per lighting and hormone treatment combination (for a total of 48 explants).

Data Collection

After 56 days in culture data was collected from all explants. The tools for measurement were a digital caliper and digital balance. The same measurements were made for both Stage II and Stage III. The measurements were explant wet weight, length of the largest shoot, canopy width, number of lateral nodes, root length. In the absence of fully formed roots, the presence of callus was recorded, where 2= large callus 1= some callus and 0= no callus. Each explant was individually removed from its vessel, rinsed in warm water to remove excess media, patted dry with paper towels then measured. The temperature data was aggregated from the *SensorPush* (Temperature/Humidity Smart Sensor) for both the control and far-red environments.

Statistical Analysis

Data for all experiments was analyzed using JMP statistical software (SAS Institute Inc., 2005). Data for all experiments were collected on a per explant basis. Data for this trial was analyzed with a generalized linear model (GLM) with a binomial distribution. Within each cultivar, fixed factors with main effects were compared using Tukey's Honestly Significant Difference (HSD) at $P < 0.05$ for mean separation.

In planning this project bubbl.us was used to map out controls and variables of this experimental design. (See Figure 3)

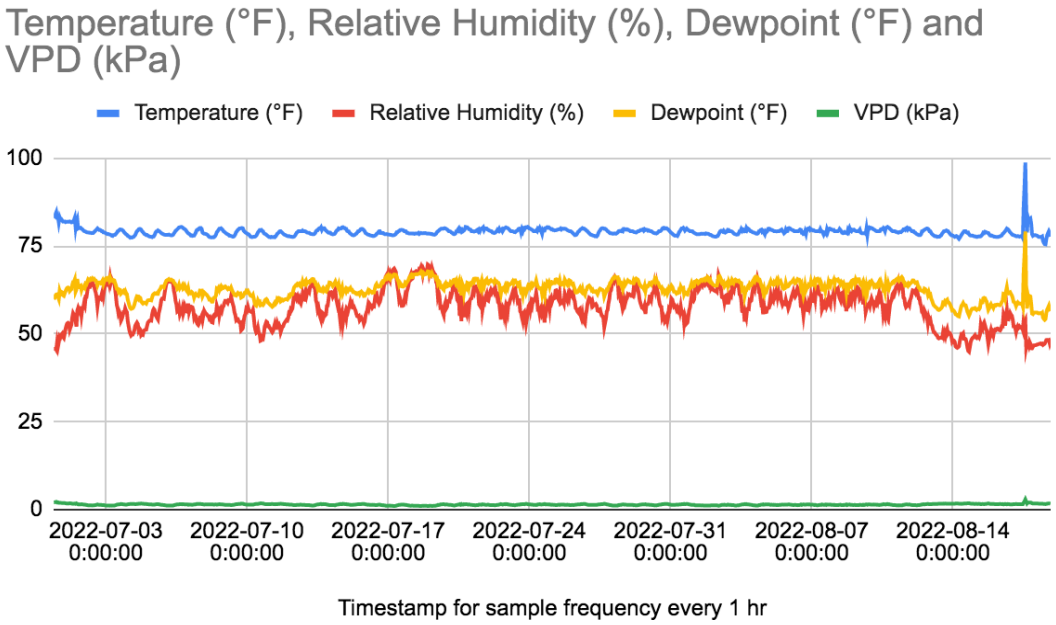

Figure 1. Temperature data from the *SensorPush in the* control lighting treatment environment

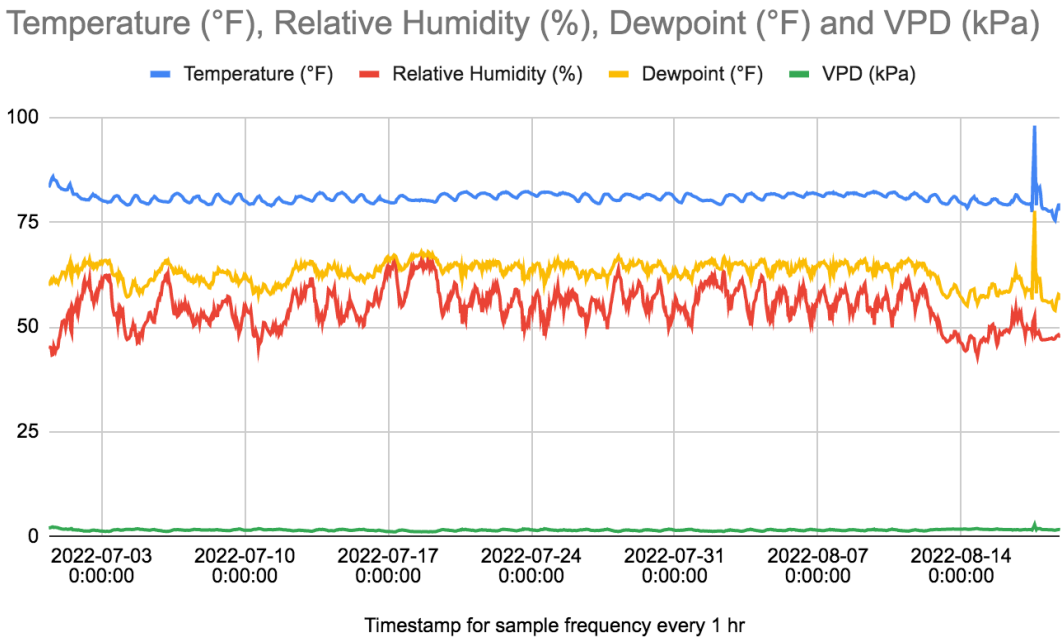

Figure 2. Temperature data from the *SensorPush in the* far-red lighting treatment environment.

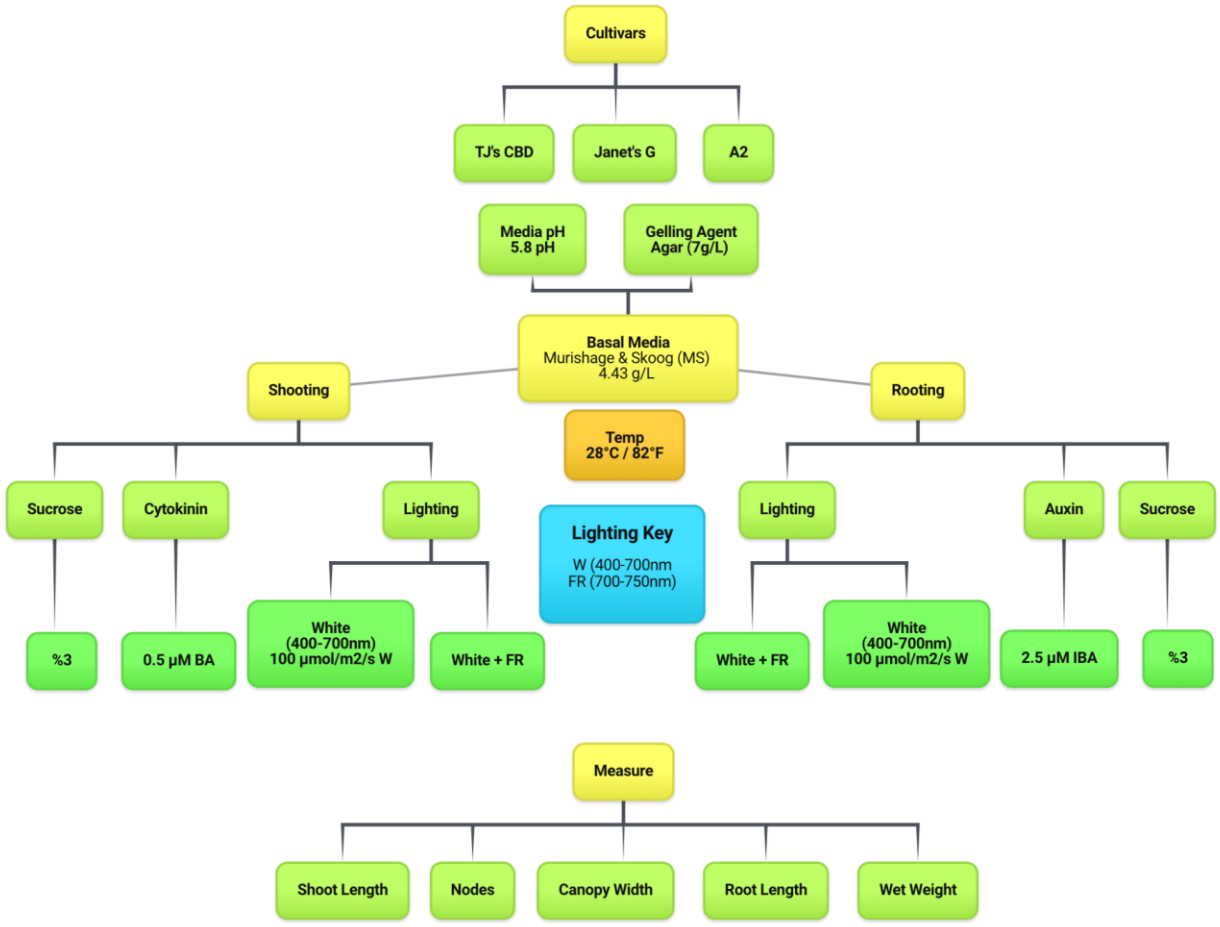

Figure 3. Experimental design diagram for *Experimentation with Far-Red Spectrum in Micropropagation of Hemp in Tissue Culture*

RESULTS

Wet weight was significantly greater under the control lighting treatment in 2 out 3 cultivars. In ‘A2’ IBA yielded the greatest wet weight. In ‘TJ’s’ BA yielded the greatest wet weight. The lighting treatment was the same but different hormone treatments produced the greatest wet weight in different cultivars.

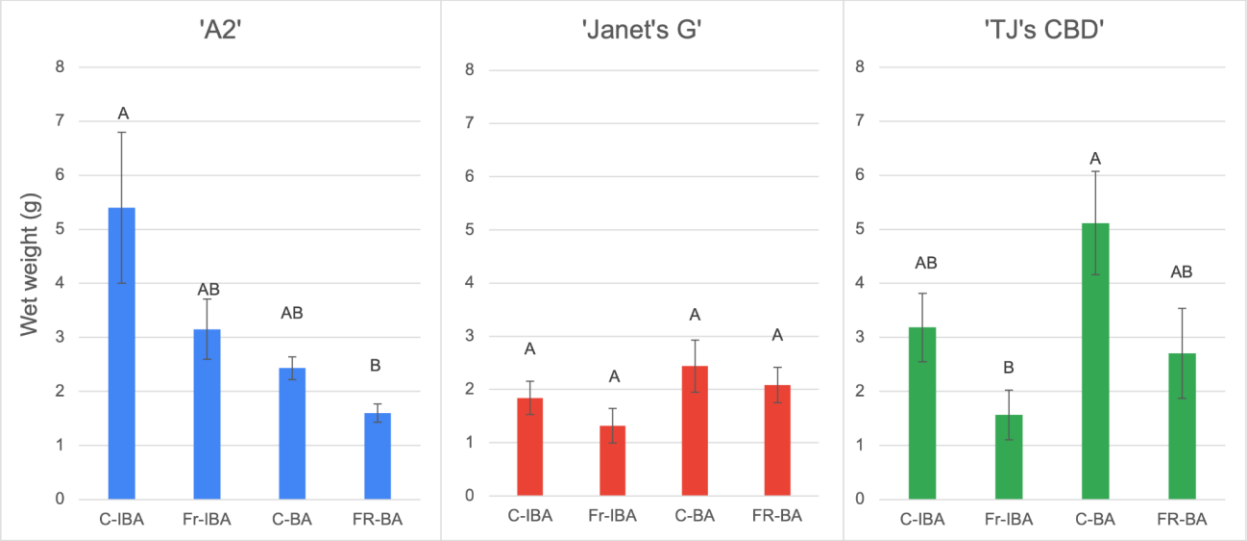

Figure 4. Wet weight (g) of three hemp cultivars in tissue culture in response to lighting treatment, control (C) and far-red (FR) and two different media hormones (IBA and BA). Data are means ± std. err. And letters represent mean separation comparison within each cultivar using Tukey’s Honestly Significant Difference (alpha = 0.05).

Height was significantly greater under the far-red lighting treatment in 2 out of 3 cultivars. In ‘A2’ IBA produced the tallest explants. In ‘Janets’ G’ BA produced the tallest explants.

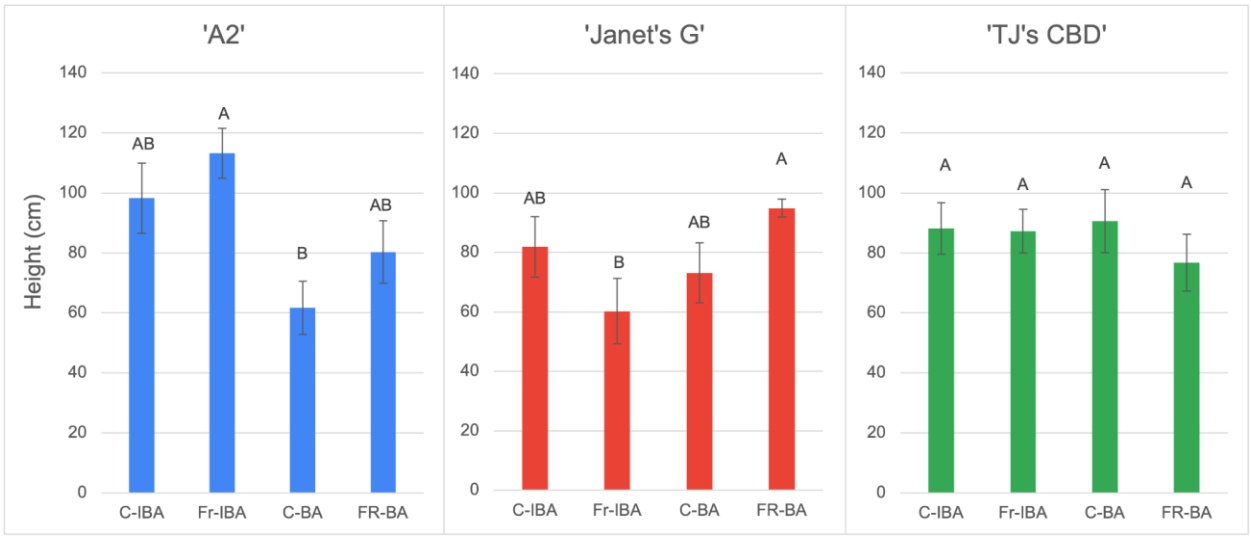

Figure 5. Height (cm) of three hemp cultivars in tissue culture in response to lighting treatment, control (C) and far-red (FR) and two different media hormones (IBA and BA). Data are means ± std. err. And letters represent mean separation comparison within each cultivar using Tukey's Honestly Significant Difference (alpha = 0.05).

Canopy Width was significantly greater under the control lighting treatment for 1 of 3 cultivars. All three cultivars produced the widest canopy in the IBA medium. This a worthwhile discovery because hemp and cannabis cultivators are necessarily concerned with canopy size and uniformity.

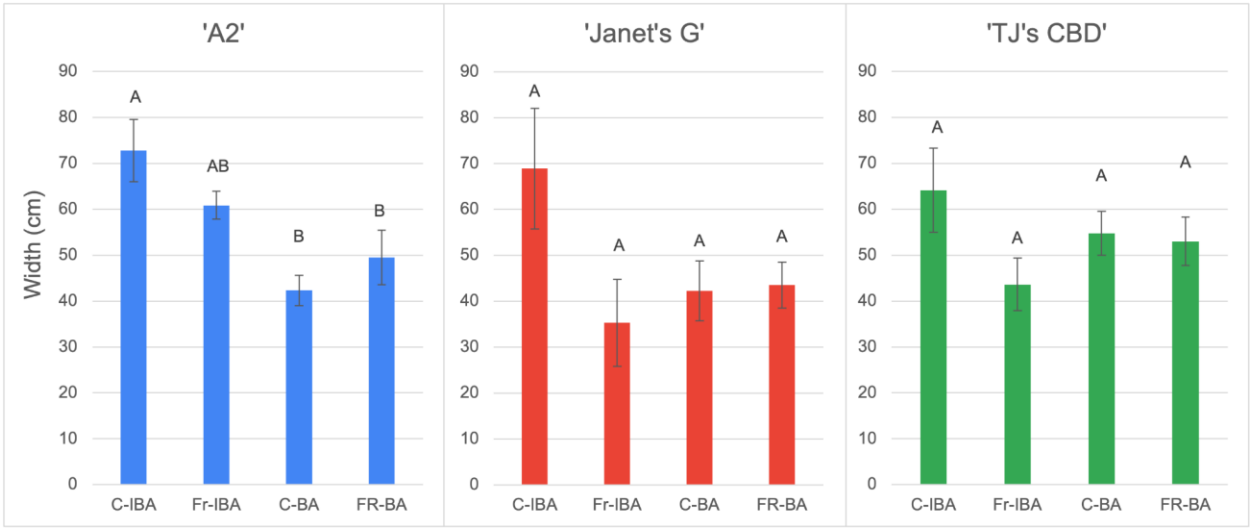

Figure 6. Width (cm) of three hemp cultivars in tissue culture in response to lighting treatment, control (C) and far-red (FR) and two different media hormones (IBA and BA). Data are means ±

std. err. And letters represent mean separation comparison within each cultivar using Tukey's Honestly Significant Difference (alpha = 0.05).

The Root Length of 'A2' was significantly greater under the far-red lighting treatment in the IBA medium. Numerically, the greatest root length overall was found in TJ's under the Control lighting treatment in the IBA medium. However, TJ's did not show significant differences in root length across hormone and lighting treatments. In general, it produced medium to long roots in all conditions.

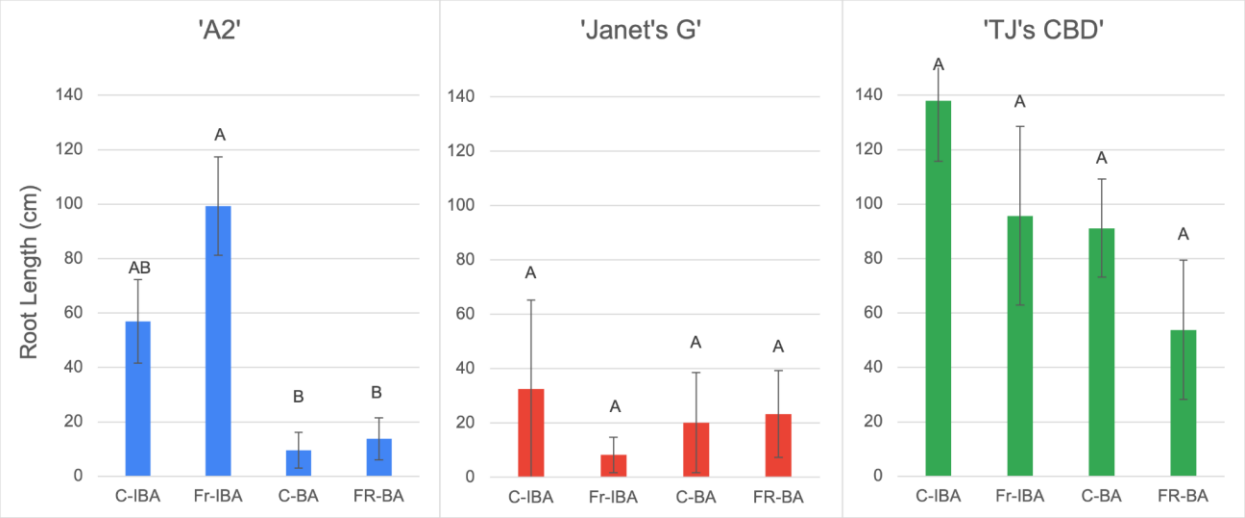

Figure 7. Root Length (cm) of three hemp cultivars in tissue culture in response to lighting treatment, control (C) and far-red (FR) and two different media hormones (IBA and BA). Data are means ± std. err. And letters represent mean separation comparison within each cultivar using Tukey's Honestly Significant Difference (alpha = 0.05).

Cultivar 'A2' produced significantly more nodes under the control lighting in the IBA medium than control with BA medium. The far-red lighting treatment produced a similar node number with IBA or BA media. The other two cultivars did not have significant differences across lighting and hormone treatments in regard to node number.

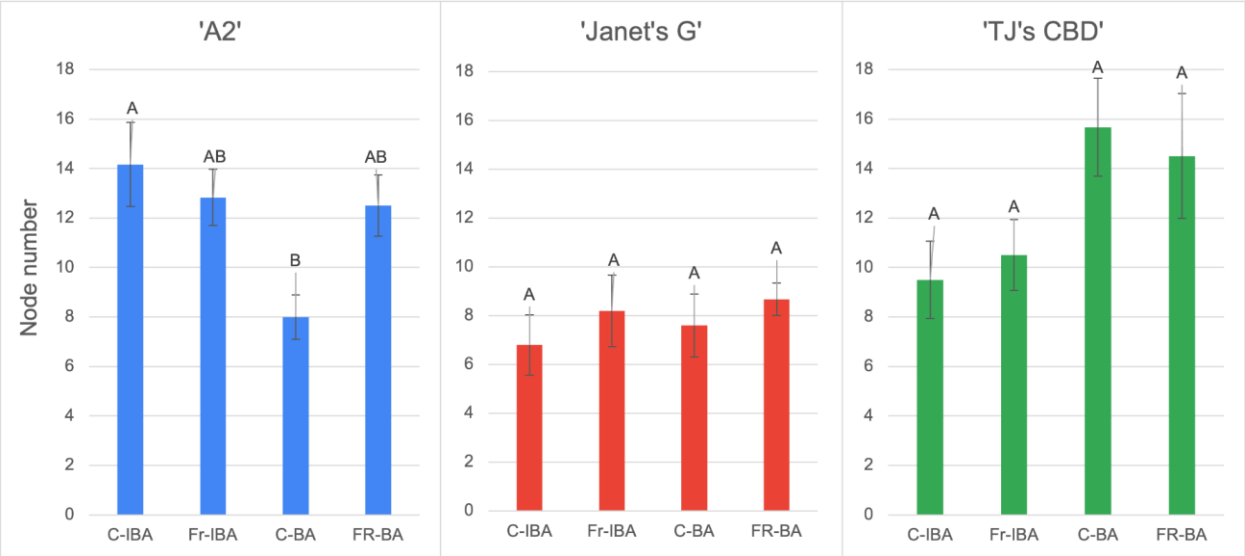

Figure 8. Node Number of three hemp cultivars in tissue culture in response to lighting treatment, control (C) and far-red (FR) and two different media hormones (IBA and BA). Data are means ± std. err. And letters represent mean separation comparison within each cultivar using Tukey's Honestly Significant Difference (alpha = 0.05).

In plants where roots were not produced or barely produced, the presence of callus was recorded. There were no significant differences in the amount of callus across cultivars, lighting treatments or medium except for TJ's which had less callus with the FR BA treatment vs. the other treatments.

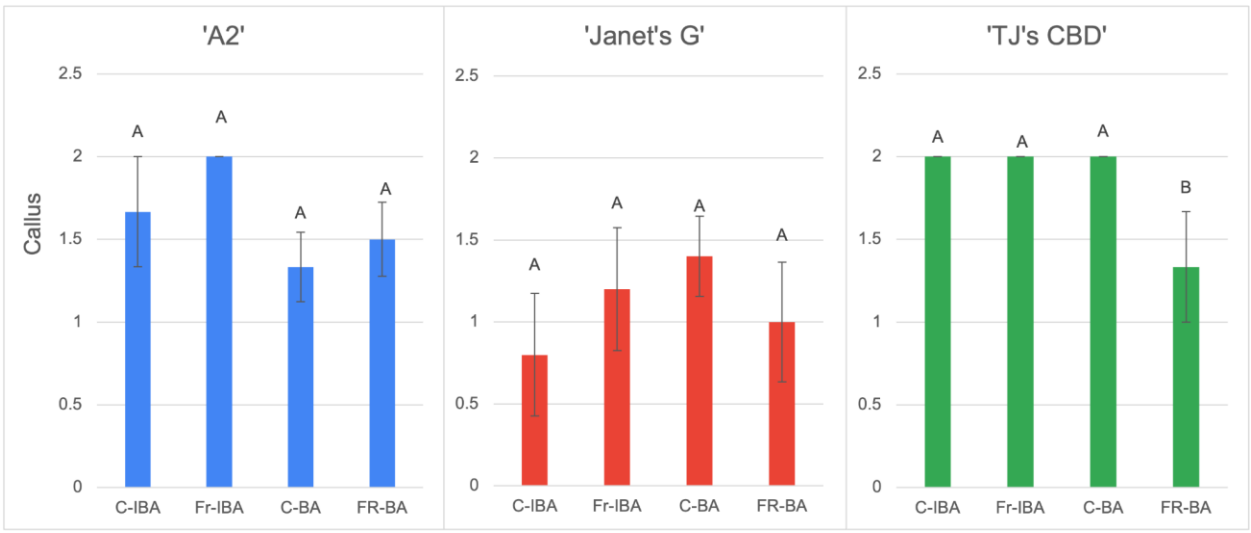

Figure 9. Callus of three hemp cultivars in tissue culture in response to lighting treatment, control (C) and far-red (FR) and two different media hormones (IBA and BA). Data are means ± std. err. And letters represent mean separation comparison within each cultivar using Tukey's Honestly Significant Difference (alpha = 0.05).

For internode length I don't have full confidence that the data accurately reflects the true internode length. The data was derived from the division of height by the number of nodes recorded in previous figures. I'm not satisfied with this methodology. To accurately record this data in the future, I would dissect each explant at the node, measure each, and graph that data.

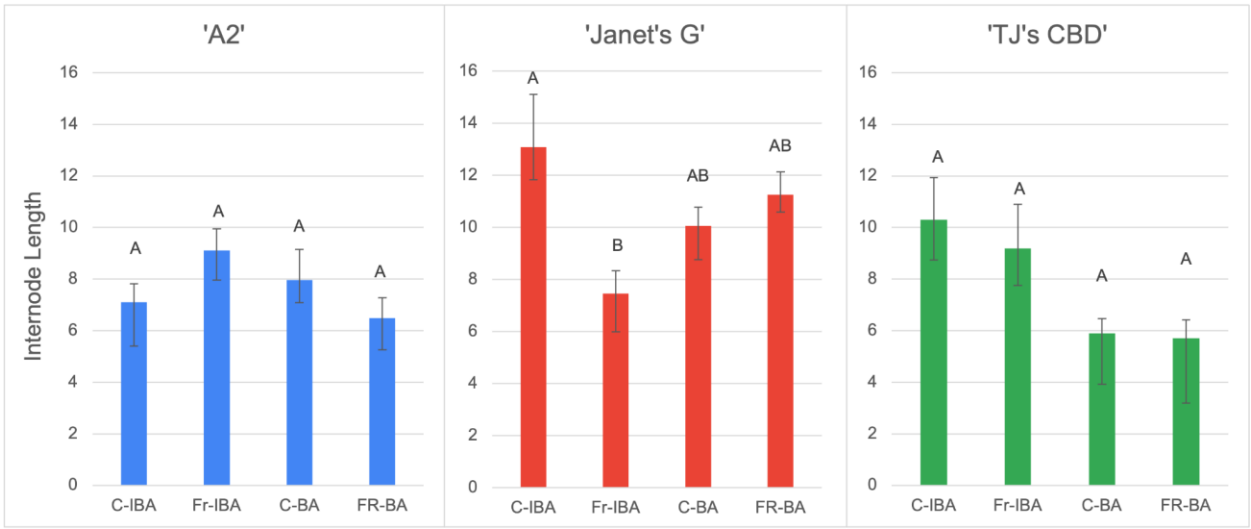

Figure 10. Internode Length of three hemp cultivars in tissue culture in response to lighting treatment, control (C) and far-red (FR) and two different media hormones (IBA and BA). Data are means ± std. err. And letters represent mean separation comparison within each cultivar using Tukey's Honestly Significant Difference (alpha = 0.05).

DISCUSSION

Across the three different cultivars used in this experiment the far-red Lighting treatment had a significant impact on the elongation of cell growth observed as taller explants or longer shoot length in two of the cultivars. In ‘A2’ the greatest height was measured in combination with the IBA hormone. In ‘Janets’ G’ The greatest height was recorded in combination with the BA hormone. Cultivar ‘TJ’s’ did not show a significant difference across lighting and hormone treatments. These findings are consistent with other scientific literature suggesting far-red induces cell elongation in different tissues within the plant (Sharma et al., 2018).

The root length of Cultivar ‘A2’ was significantly greatest under the far-red treatment in the presence of hormone IBA. Although the difference was not significant, ‘TJ’s’ produced its longest roots under the control lighting treatment in the presence of IBA. In general, ‘TJ’s’ exhibited the most prolific root growth regardless of hormone and lighting treatment. The cultivar ‘Janets’ G’ had the shortest roots across all treatments.

In general, the media performed as expected based on the available research outlined in this paper. The IBA hormone treatment outperformed the BA hormone treatment in cultivar ‘A2’ for the parameter of node number. Typically, BA is used for producing shoots and nodes, so these results lie outside of convention.

Cannabis sativa is genetically diverse due to its mostly diecious nature, whereby each successful pollination of the female inflorescence creates the recombination of millions of gene pairs. For this reason, we limited the number of variables to two

hormone treatments and two lighting treatments. The results yielded great variability across cultivars in each of the parameters measured for our data collection.

CONCLUSION

Overall, we found that hemp cultivars responded differently to far-red light and hormone treatment. This suggests that tissue culture methodology should be optimized for each cultivar.

Based on this experiment, it is not quantitatively beneficial to add far-red to the tissue culture micropropagation of hemp and cannabis for most of the parameters measured. The academic and scientific literature in hemp and cannabis is still very limited.

Based on the results for height and root length under the far-red treatment, I would recommend future research using the same control lighting treatment for 42 days and the far-red lighting treatment for 14 days. The far-red lighting treatment produced the tallest plants and longest roots. The control lighting treatment produced the widest canopy, greatest wet weight and greatest number of nodes. Potentially a combination of the two, timed properly yield may result in optimum rooting and subsequent canopy growth. In a second iteration of this research, I would track and record qualitative data to complement the quantitative data.

Given the lack of research in this field, there are many areas left to explore. With further exploration we have the potential to better understand the interaction at play between genetic markers within each cultivar, their relation to photoreceptors, light quality, and plant growth hormone signaling in the tissue culture micropropagation of hemp (*Cannabis sativa*). As technology continues to advance exponentially, mapping for genetic markers may become more accessible. Using this information in conjunction

with tissue culture and advanced breeding techniques holds limitless possibilities for discovery.

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