A smart-sensing AI-driven platform for scalable, low-cost hydroponic units

D1.2 Report on Evaluation of Optimized Growth Environments in controlled Experiments

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ACRONYMS LIST

FW	Fresh Weight
DW	Dry Weight
ANT	Anthocyanin
CRT	Carotenoids
PP	Photoperiod
GP	Growing Period
LED	Light Emitting Diode
H	Hour
NS	Nutrient Solution
PPFD	Photosynthetic Photon Flux Density
BY	Biomass Yield
SM	Secondary Metabolite
HE 24-V	High Efficiency 24-Volt
RL	Research Light

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EXECUTIVE SUMMARY

Microgreens are plants at the young phenological stage between sprouts and baby greens, with a growing period of around 6-16 days from germination to harvest. They are gaining increasing attention because of their short production cycle and their beneficial attributes such as high nutrient density, secondary metabolite content, and gastronomic applications, all of which contribute to their categorization and use as 'functional foods.' Hence, the objective of this deliverable was to investigate experimentally the effects of nutrient solution fertilization, environmental parameterization, and light spectrum combinations and intensities on Biomass Yield and Secondary Metabolite Accumulation in five microgreen species and varieties: *Ocimum basilicum* (Basil var. Green Tesla), *Sinapis alba* (Mustard var. White Candy), *Brassica oleracea* var. sabellica L. (Kale var. Black Mandingo), *Brassica oleracea* (Radish var. Daikon Panzer), and *Brassica oleracea* convar. *acephala* var. *gongylodes* L. (Kohlrabi var. Red Cardinal). Our objective was to evaluate 12 different growth environments in controlled experiments conducted in a Climate Chamber in the University of Copenhagen's Taastrup Campus, Taastrup, Denmark. These growth environments were designed to test microgreens production along a spectrum of environmental conditions, from low-input to high-input, which will inform our larger GOhydro goals and later work packages. With this in mind, we chose to vary Relative Humidity (40 or 70%), Nutrient Solution (Yes or No), Light Type (Expensive or Cheap), and Light Quality (Recipe for Biomass Yield Max or Secondary Metabolite Max). The results of these experiments will provide important criteria for selecting optimal growth environments for microgreens within the context of GOhydro's project goals. Because of supply chain disruptions due to the Coronavirus, our original light and climate chamber supplier experienced critical chip shortages resulting in indefinite supply delays. We therefore had to change our procedure by finding a new supplier and acquiring both new lights and a grow system from separate companies. This has resulted in an unavoidable delay in beginning the experimental stage of this deliverable given the equipment's unavailability. In *Deliverable 1.2: Evaluation of optimized growth environments in controlled experiments*, we have therefore presented the deliverable's background, our objectives, methods, results template, and the results of our analyses that were conducted on the dataset that was generated from our literature review conducted in *D1.1: Review on nutrient and production parameters and light requirements*. This analysis was undertaken via multilinear regression models, which will be used for the final version of D1.2. Our initial analysis showed that more variation in FW, DW, Carotenoid, Phenols, and Anthocyanin outcomes was explained by the light spectrum combinations than light intensity; this informed our experimental parameters that are set for D1.2. Given that we have already undertaken a similar analysis that has refined our methodology, including having ready R-code for analysis, D1.2 only needs data from our experiments to be completed.

1 INTRODUCTION

Microgreens are plants at the young phenological stage between sprouts and baby greens, with a growing period of around 6-16 days from germination to harvest. They are increasingly gaining attention because of their short production cycle and their beneficial attributes such as high nutrient density, secondary metabolite content, and gastronomic applications, all of which contribute to their categorization and use as 'functional foods.' Among the environmental parameters for microgreen production under controlled conditions, light spectrum combinations and intensities are key factors of contemporary interest affecting Biomass Yield (BY) or Secondary Metabolite (SM) accumulation. Furthermore, ambient air conditions such as temperature and Relative Humidity (RH) are also important factors to consider for optimal growth. Finally, the choice to fertilize microgreens with a Nutrient Solution (NS) or rely on water can also affect microgreen growth. Hence, the objective of this deliverable was to investigate experimentally the effects of NS fertilization, environmental parameters, and light spectrum combinations and intensities on BY and SM in five microgreen species and varieties. The five microgreen species and varieties are: *Ocimum basilicum* (Basil var. Green Tesla), *Sinapis alba* (Mustard var. White Candy), *Brassica oleracea* var. sabellica L. (Kale var. Black Mandingo), *Brassica oleracea* (Radish var. Daikon Panzer), and *Brassica oleracea* convar. *acephala* var. *gongylodes* (Kohlrabi var. Red Cardinal).

Our objective was to evaluate 12 different growth environments in controlled experiments conducted in a Climate Chamber in the University of Copenhagen's Taastrup Campus, Taastrup, Denmark. As shown in Table 1, by varying four environmental factors viz. light spectrum recipes (Secondary Metabolite Maximization, or Biomass Yield Maximization), Relative Humidity (40 or 70%), light type (high-cost LED research lights vs. low-cost grow lights), and Nutrient Solution (yes or no), we can track the influence of these parameters on downstream outcomes such as Fresh Weight and Dry Weight (kg/m^2), and SM such as Phenols (mg/kg Dry Weight). Our 12 different growth environments will be evaluated for the maximization of both BY and SM accumulation. Given the inherent biological tradeoff between maximizing BY and SM accumulation, we will identify growth environments that produce maximum BY, maximum SM accumulation, or a balanced production of BY and SM accumulation. The identification of these impactful environmental conditions can be used by microgreen producers to help make production decisions based on their species of choice and their desired outcomes. We have constructed the environmental recipes in Table 1 by varying these four parameters, while maintaining the same Photoperiod at 16 hours, Growing Period at 10 days, and Day/Night Temperature at 21/17 °C. Our other parameters, CO_2, Electrical Conductivity, and pH will be measured, but they will not be 'set' as the other parameters will be.

Table 1. List of 12 Growth Environment Recipes for optimal microgreen production for selected species. Nut. Sol. = Nutrient Solution; Temp. = Temperature; RH = Relative Humidity; EC = Electrical Conductivity; PP = Photoperiod; 'x' represents environmental parameters that are measured but not set.

Recipe	Nut. Sol. (Yes/No)	Temp. (℃)	RH (%)	pH	EC (mS)	CO_2 (PPM)	PP (hours)	Growing Period (days)	Light Quantity (PPFD)	Light Recipe Goal	Light Quality (Recipe)
1	Yes	21/17	45	x	x	x	16	10	HE 24V LED	""	""
2	Yes	21/17	65	x	x	x	16	10	HE 24V LED	""	""
3	Yes	21/17	45	x	x	x	16	10	250	SM Max	UV2.5:B20:G0.5:R74.5:FR2.5
4	Yes	21/17	65	x	x	x	16	10	250	SM Max	UV2.5:B20:G0.5:R74.5:FR2.5
5	Yes	21/17	45	x	x	x	16	10	250	BY Max	UV0.5:B10:R84.5:G2.5:FR2.5
6	Yes	21/17	65	x	x	x	16	10	250	BY Max	UV0.5:B10:R84.5:G2.5:FR2.5
7	No	21/17	45	x	x	x	16	10	HE 24V LED	""	""
8	No	21/17	65	x	x	x	16	10	HE 24V LED	""	""
9	No	21/17	45	x	x	x	16	10	250	SM Max	UV2.5:B20:G0.5:R74.5:FR2.5
10	No	21/17	65	x	x	x	16	10	250	SM Max	UV2.5:B20:G0.5:R74.5:FR2.5
11	No	21/17	45	x	x	x	16	10	250	BY Max	UV0.5:B10:R84.5:G2.5:FR2.5
12	No	21/17	65	x	x	x	16	10	250	BY Max	UV0.5:B10:R84.5:G2.5:FR2.5

Our initial parameters and growth environment values in Table 1 were derived from our literature review undertaken in *D1.1: Review on nutrient and production parameters and light requirements.* This literature review gathered information from around 70 peer-reviewed research papers on microgreen light and growth environment conditions. In D1.1, we created average tables of values to illustrate the starting point for designing optimal growth environments based on species selection, which can be used by microgreens producers. Our literature review overall showed that there is much inherent microgreen biological variation in physiological responses to these environmental conditions based on the species or variety selected. Here in D1.2, we investigated our growth environment recipes on five different species or varieties of microgreens and presented results demonstrating relationships between growth environments and production outputs (BY and SM). Importantly, D1.2 differs from the research projects in our literature review in that we operate under GOhyrdo's objectives of producing low-cost hydroponic units with a smart-sensing AI-driven platform, which demands different experimental goals. For instance, our growth environment parameters were set to represent similar conditions to those found in office environments. One of the largest differences between office environments and hydroponic experimental conditions is the relative humidity, which in buildings is generally around 40-60%; we therefore chose to use 40% and 70% RH to determine the impact of humidity on our production outputs. This is an important experimental consideration, as too low RH values can diminish nutrient transport, and can cause increased transpiration that stresses the plants into closing their stomata. Ensuring that microgreens can grow optimally in low-tech environments is a key threshold for project output success.

We therefore chose to vary RH (40 or 70%), Nutrient Solution (Yes or No), Light Type (Expensive or Cheap), and Light Quality (Recipe for BY Max or SM Max). This will provide important criteria for selecting optimal growth environments for microgreens within the context of GOhydro's project goals.

Because of supply chain disruptions due to the Coronavirus, our original light and climate chamber supplier experienced critical chip shortages that resulted in their indefinite delay in order fulfilment. We therefore had to change our procedure and find new suppliers to acquire both new lights and a grow system from separate companies. This has resulted in an unavoidable delay in beginning the experimental stage of this deliverable given the equipment's unavailability. In *Deliverable 1.2: Evaluation of optimized growth environments in controlled experiments*, we have therefore presented the deliverable's background, our objectives, methods, results template, and the results of our analyses that were conducted on the dataset that was generated from our literature review in D1.1. These results are partially derived from a paper that we are presenting at the International Society for Horticultural Science (ISHS) annual conference in Angers, France. In this work, we carried out a literature review from 36 different studies to collect, harmonize, and synthesize information on the effects of light spectrum combinations and intensities on 12 commonly grown Brassicaceae microgreens. We have presented results on the influence of light intensities and spectrum combinations on microgreen BY and SM generation. This analysis was undertaken via multilinear regression models which showed that more variation in FW, DW, Carotenoid, Phenols, and Anthocyanin outcomes was explained by the light spectrum combinations than light intensity, which informed our experimental parameters that are set for D1.2. The results from our conference paper, and those that will be generated in D1.2, provide a summarized overview on light intensity and spectrum combination effects on microgreens with a focus on accounting for variety- and species-specific variation in microgreens which can inform microgreen producers' future research designs and production environments based on desired outcomes. Given that we have already undertaken a similar analysis that has refined our methodology, including having ready R-code for analysis, D1.2 only needs data from our experiments to be completed.

2 MICROGREENS PRODUCTION BACKGROUND

Many plants cultivated for human consumption at a mature stage can also be harvested at a younger stage as a microgreen. In practice, there are currently around 80-100 commonly cultivated species of microgreens (Ying et al., 2020a, 2020b, 2020c). However, there exists a large variety of microgreens suitability to the specific conditions associated with indoor hydroponic production. For instance, some species are cultivated more often than others, with the most commonly cultivated species being from the Brassicaceae family, which includes broccoli, cabbage, arugula, kale, and mustard, to name a few examples (Björkman et al., 2011). In general, microgreens production ideally takes place indoors in a climate controlled environment. The most important factors to control are day-length (photoperiod), which can be augmented by lights to ensure 'growth' cueing via photoreceptor stimulation; having a relatively stable Day/Night temperature around 21/17 °C for optimal physiological function; and the type of light quality (Red/Blue proportions, UV, or Far-Red inclusion, for example). These environmental parameters can be manipulated relatively cheaply, for instance if paired with low-cost 24-Volt LED light strips, especially if the hydroponic system can be placed in spaces that are already climate controlled (offices, homes, etc.). Controlling for these environmental factors offers key advantages: firstly, it allows year-round production, as there is no dependence on natural photoperiod or light cues; secondly, the disease and competition burden is lessened; thirdly, it allows targeted delivery of resources viz. water, nutrients, and light. Delivery of these resources can be achieved by any of the six most common hydroponic systems that are used today, although the most commonly used systems for microgreens are 'drip' and 'ebb and flow' systems because microgreens have smaller roots than mature plants and grow at a much higher density, thereby making these hydroponic systems optimal:

1. **Ebb and flow** uses an external reservoir that is pumped and drained through the growing medium at regular timed intervals to deliver water/nutrients to the plants; the ebb and flow allows for oxygenation of roots and the medium when pumps are not running, as roots are exposed to the air
2. **Drip** systems are most appropriate for large-scale industrial processes; they apply the NS at regular or nearly continuous intervals in the root zone through narrow pipes that allows for nutrient uptake and ample oxygenation of the roots
3. **Nutrient film technique**, uses a nutrient delivery system with pumps, where a thin film of nutrient is flowing at regular intervals in pipes, making the nutrients available for easy uptake and ample oxygenation of roots
4. **Wicking** system constitutes a medium that can absorb the nutrient solution and make it available to the plants and hence the medium 'wicks' or moves water via diffusion from the solution to the growing substrate and the plants
5. **Deep water culture** uses a reservoir of nutrient solution (NS) where the plants are grown in a floating medium and roots are immersed in NS. Root oxygenation is an issue due to limited spaces between the floating medium and the nutrient solution

6. **Aeroponics**, consists of spraying nutrient solutions in fine mist form to suspended plant roots with a spraying pump to supply the nutrients and oxygenate roots for optimal plant growth.

Beyond the movement of water, oxygen, and nutritional resources via hydroponic systems, the microgreens have to be grown in an appropriate medium that has enough porosity, strength, durability, and will not leach harmful compounds to ensure effective growth and food-safe production. Some examples of ideal substrates are coconut coir, polyethylene terephthalate, peat moss, cellulose, or even gauze, to name a few examples, which we have shown in Table 2 below.

Table 2. List of substrates used in the literature review seen in D1.1.

Substrate Type	Example Reference
30% compost, 30% peat, 30% coir, 10% perlite	Gerovac et al., 2016
Sphagnum peat moss	Samuoliene et al., 2017
Coconut fiber (coir)	Kong et al., 2020
Polyethylene terephthalate fiber	Craver et al., 2015
Gauze	Zhang et al., 2019
Peat moss and Rockwool	Kamal et al., 2020
General purpose soil	Lobiuc et al., 2017

Besides the hydroponic system and grow medium, the next important component for microgreens production is the Nutrient Solution (NS), which should contain concentrations of macro- and micro-nutrients that generally follow Hoagland's solution of nitrogen, phosphorous, potassium, calcium, sulfur, magnesium, chlorine, salt, manganese, zinc, copper, molybdenum, and iron, the concentrations of which can be seen in Table 3. The nitrogen source is important to consider as well; ideally, the NS should contain a ratio of ammonium (NH4) and nitrate (NO3); this can be seen in more detail in previous research (e.g., Palmitessa et al., 2020). The NS should also be kept around 21 °C with a pH of 6.0. The amount and timing of NS depends on production goals and growing period, as microgreens generally don't need fertilization until after the first week, depending on the particular species or variety's needs. In this deliverable, we will investigate the differences between microgreens grown with and without NS.

Table 3. Hydroponic fertilization regimes derived from (Hoagland & Snyder, 1933) and (Hoagland & Arnon, 1950). Values for general elements are presented in parts per million (ppm), while molecular nutrient source is presented in concentration.

Nutrient	Elemental Acronym	Parts per million (ppm)	Molecular Nutrient Source	Concentration
Nitrogen	N	210 ppm	$NH_4H_2PO_4$	115 g/L
Phosphorous	P	31 ppm	*See Nitrogen*	
Potassium	K	235 ppm	KNO_3	202 g/L
Calcium	Ca	160 ppm	$Ca(NO_3)_2$	472 g/L
Sulfur	S	64 ppm	$MgSO_4$	493 g/L
Magnesium	Mg	48.6 ppm	*See Sulfur*	
Chlorine	Cl	0.65 ppm	$MnCl_2$	1.81 g/L
Salt	Na	1.2 ppm	NaCl	
Boron	B	0.5 ppm	H_3BO_3	2.86 g/L

Manganese	Mn	0.5 ppm	*See Chlorine*	
Zinc	Zn	0.05 ppm	$ZnSO_4$	0.22 g/L
Copper	Cu	0.02 ppm	$CuSO_4$	0.08 g/L
Molybdenum	Mo	0.011 ppm	H_2MoO_4	0.02 g/L
Iron	Fe	5 ppm	$C_{12}H_{12}Fe_2O_{18}$	5 g/L

Beyond the hydroponic system, substrate type, and fertilization, the microgreens should have other environmental parameters controlled, such as air temperature, RH, growing period (days), photoperiod (imitating day length), and CO2 if possible. These are controlled to ensure optimal physiological conditions, health, and successful production of microgreens. Microgreens production can be achieved on a continuum of technological and capital input; a shelf or series of shelves in an office with ambient climate control, a grow tent with circulating fan, an industrial warehouse with fixed parameters, and a climate-controlled research growth chamber, are all possible methods of keeping the environmental parameters in check to greater or lesser degrees. More detail on the ranges of these parameters is discussed in greater detail in *D1.1: Review on nutrient and production parameters and light requirements*.

The final important category of growth environment parameterization is light. This is currently the focus of most recent research, as the correlation between different spectra (quality) and amount (quantity) of light and production outputs are current trends in research. The outcomes of this previous research on microgreens production are discussed in D1.1. Furthermore, the results of our analysis on the dataset derived from the literature review in D1.1, portions of which are presented below in Section 4, show that light spectrum has more explanatory power than light quantity. Therefore, we will use a standard common middle-range light intensity value for our experiments, while varying light spectrum to generate more data on this novel avenue of research on manipulating the physiology of microgreens via photomorphogenesis. Overall, because of the goals of the GOhydro project, we will be varying Nutrient Solution (Yes or No), Relative Humidity (40 or 70%), Light Type (Cheap or Expensive), and Light Recipe (BY Max or SM Max). These growth environment parameterizations affect production outcomes (such as FW, DW, and Phenols) of five different species or varieties of microgreens, which will allow tailored production recommendations to be made that are in line with the overall goals of the GOhydro project.

3 MATERIALS AND METHODS

After we obtained our general parameter requirements for the growth environment conditions from our literature review in D1.1, we then designed our experimental conditions. Our first step was acquiring a growth chamber here at the University of Copenhagen's Taastrup Campus, Taastrup, Denmark. We are using a 'Conviron CMP5090' climate chamber with programmable conditions set to daily (24-hour) schedules (Figure 1). In this climate chamber, we can control the temperature, airflow, and the power inputs via this external panel. We also have access to an external NS dispenser which is routed to the inside of the chamber as needed; for our initial experiment, we will use the fresh water from the hose seen in Figure 1. Inside the climate chamber we are using an Instagreen Professional Cultivation System (Instagreen.eu), seen in Figures 2 and 3. This grow rack system can hold up to 12 trays on six layers (2 trays per layer), with a total intended growing space of 7.26 m^2. Each layer has four High Efficiency 24-volt (HE 24-V) LED lights equidistant from one another. The Instagreen grow system is 217.5 cm in height, 120 cm wide, and 112 cm deep. The Instagreen setup is a modular system with variable layers that are tilted slightly to direct water flow via gravity after being pumped to the top from a reservoir, which descends from one layer to the next via perforations in the bottom-most section of each tray and falls over a slide into the top of the next layer. The reservoir holds approximately 41 liters of solution at a maximum. Our experimental design with this modular, integrated setup is to use the upper three layers as growing levels, with the top-most layer using the Osram Research Lights (RLs) and the second and third layer using the Instagreen 24-volt LED grow light strips. This allows us to compare outputs from these different lights given that they have the same background environmental conditions. The bottom three layers will be used as germination layers to ensure a seamless transition for germination, growth, harvest, and starting the next generation. Our top-most layer, which is under our Osram RL LED banks, is required to be 40 cm from the light emitting surface (LEDs). Therefore, we have increased the height of the uppermost layer and shortened the lower-most layer. The four remaining middle layers are each approximately 32 cm in height. The vertical dimensions of the bottom three layers are functionally flexible, as they are germination layers, and the lights on those layers are not turned on in our current trial period.

Each growing tray has the dimensions of 110 (L) x 55 (W) x 5 (H) cm. 40 cups fit per tray, with each cup having a dimension of 10 x 14.5 x 5 cm; the trays and cups can be seen in Figure 2. We can therefore produce 104 cups of microgreens for our experiments per harvest cycle, as we cannot use around 36% of the growing space at the uppermost layer due to the light footprint effect of the Osram RL, which is approximately 110 x 70 cm. This means we have overall around 3.2 m^2 of usable microgreen production area across the three layers. We have an additional 3.63 m^2 used for germination trays in the lower three layers of the grow system, although we will only use 3.2 m^2 of this space to provide a 1:1 parity for our production's spatial needs. Therefore, we have a total usable production space of approximately 6.4 m^2 in our grow rack system. In the bottom three germination layers, two trays are stacked one on the other to offer a light-free and high humidity environment; the uppermost tray has no holes as a blackout tray. This setup can be seen in Figure 2. Detailing the spatial dimensions of our growing space allows us to provide metrics for production of microgreens per unit area. We

also believe it is useful to provide metrics on the amount of water used by the plants, as well as the amount of energy for the lights per unit area as well. We will therefore measure these inputs to also offer resource use metrics for microgreen production. Ultimately, we will be able to track the water use, energy use, and light use per unit of microgreens produced per unit area (BY in kg/m^2), with corresponding concentrations of valuable

secondary metabolites (mg/kg DW). This will allow for determinations of optimal scenarios for producing microgreens.

Figure 1. Exterior image of the Conviron CMP5090 Climate Chamber at the University of Copenhagen's Taastrup Campus, Taastrup, Denmark.

Figure 2 (Left). Interior image of the Instagreen Professional Cultivation System with 6 layers of grow trays inside our Climate Chamber in Taastrup, Denmark.

Figure 3 (Right). Example photo the Instagreen Professional Cultivation System with various microgreens species nearing harvest time (photo credit: Instagreen.eu).

In our Climate Chamber we have a centralized utility box to ensure compliance with Danish safety regulations (Figure 4). Within this utility box we have two 'Paladin pro4 Bluetooth' timers for the 'Water Master 1800 l/h' water pump and the High Efficiency 24-volt (HE 24-V) LED grow light regulation. Our grow system is watered two times a day for 15 minutes, 12 hours apart, using this timer. The HE 24-V LED lights are controlled using the second 'Paladin pro4 Bluetooth' timer, which ensures that the light will be on for 16 hours, and off for 8 hours during every 24 hour period. Within our climate chamber we also have a 'Plus Zap' infrared bug zapper to control pest incidence. We are also using a sanitizing floor mat that is filled with 'Rodalon' (active ingredients: chloride-based compounds), a chlorine-free disinfectant to avoid damaging plastics, which will ensure that unwanted pathogens are not tracked into our climate chamber. Furthermore, in this regard, all users wear standard protective lab coats when working with the climate system, which are seen hanging adjacent to the door in Figure 1. To measure and record RH (%) and air temperature (°C) we are using two 'TinyTag View2 Loggers', one for the general Climate Chamber air, and the second to ensure proper germination humidity in the lower three levels. We are also using a 'HOBO MX CO_2 Logger' to measure CO_2 in our climate chamber.

Finally, we have a 'HOBO Onset Pendant temp/light Logger' to measure the amount of light (in lux units); this is useful to verify settings for the Osram RLs, to determine the intensity of the HE 24-V LEDs, and also verify that the blackout trays for the three germination layers are functioning properly.

Figure 4. Regulation utility box housing our two timers, three power supplies for the Osram RLs, our MOXA port, the HE 24-V LED power supply, and green network cable connecting to our router for remote control.

Our primary experiments concerning light spectrum effects on microgreens are made possible by utilizing the Osram Research Light (Osram.com), the recipes of which have the capacity to be customized via the Phytofy software. This software allows for highly precise light recipes to be made, as each spectral peak, at 385 nm (UV), 450 nm (Blue), 521 nm (Green), 660 nm (Red), and 730 nm (Far Red), can be exactly set as a % of the total output, or tuned as total PPFD (µmol/m^2/s). The Osram RL has a color temperature of 2,700 Kelvin, which is classified as between 'Sunrise and Sunset.' The different spectral components and their corresponding maximum PPFD are listed in Table 4 and shown in Figure 5. Each individual spectral component is dimmable from 10-100% as well, allowing for many light recipe possibilities. The lights themselves, and the corresponding footprint, can be seen in Figure 6.

Table 4. List of the Osram Research Light LED colors, spectral peaks, and maximal PPFD

Name	UV	Blue	Green	Hyper-Red	Far Red
Spectral Peak (nm)	385	450	521	660	730
Max PPFD (µmol/m²/s)	50	250	100	250	100

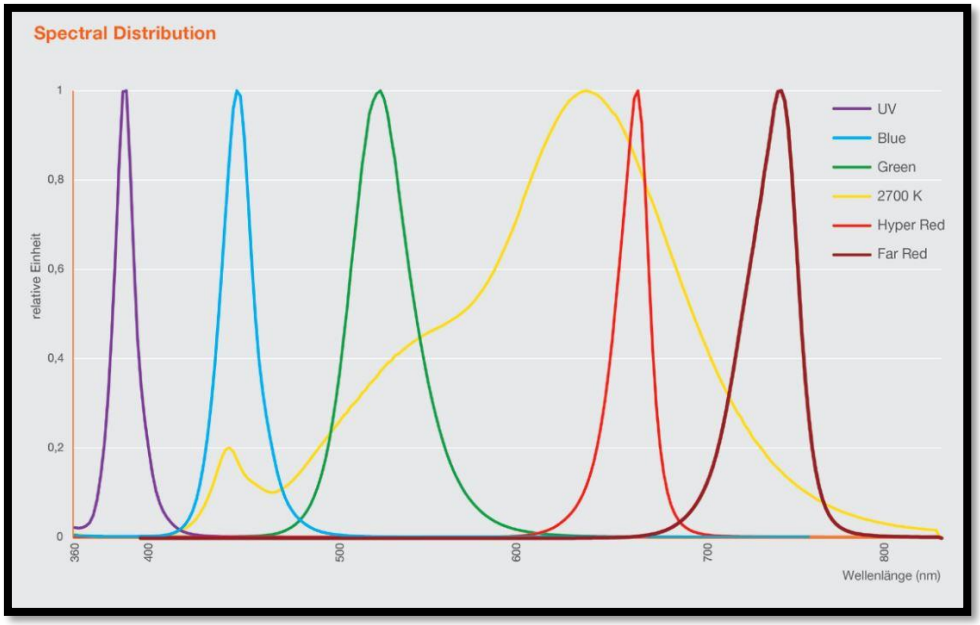

Figure 5. Spectral distribution for different LED components in the Osram Research Light (figure credit from the Osram RL pamphlet).

Figure 6. Interior image of Climate Chamber detailing the placement of the three Osram RLs.

Our second level of lights uses four 'Fullwat DOMOX line high efficiency 24-volt' LED light strips. The HE 24-V lights have a light color temperature of 4,000 Kelvin, which is classified as near direct sunlight (4,800 K). We will record the HE 24-V LED spectral signature using a spectrometer (e.g., Sekonic Spectomaster Meter c-800). This will allow us to make another figure that replicates the one seen above in Figure 5. This will allow us to compartmentalize the spectral peaks into different light spectrum effects for use in our multilinear regression models. The methodology surrounding these models and their use of compartmentalized light spectrum effects is described below in Section 3.1.

Table 5 demonstrates the five different microgreen species and varieties we have selected to study in our experiments. These were chosen from a larger subset of eight different microgreen species and varieties because they represent the most common Brassicaceae microgreen species, as well as including Basil, which is the microgreen of choice for all three partner countries (Greece, Romania, and Denmark). Five of the eight total microgreens species were selected because of budget constraints according to the required expense of measuring the secondary metabolites. This selection of microgreens will generate cross-country data that can be used for comparison purposes. This will allow us to include other countries' Basil data into our multilinear regression model analyses for a wider effect comparison, as they will have different environmental conditions. We can therefore include wider environmental effects from three different countries, with more samples also increasing the power of our multilinear regression model analyses.

Table 5. List of the five microgreens species and varieties used in our experiment

Family	Genus	Species	Common Name
Lamiaceae	*Ocimum*	*basilicum*	Basil (var. Green Tesla)
Brassicaceae	*Sinapis*	*alba*	Mustard (var. White Candy)
Brassicaceae	*Brassica*	*oleracea* var. *sabellica* L.	Kale (var. Black Mandingo)
Brassicaceae	*Brassica*	*oleracea*	Radish (var. Daikon Panzer)
Brassicaceae	*Brassica*	*oleracea* convar. *Acephala* var. *gongylodes* L.	Kohlrabi (var. Red Cardinal)

3.1 DELIVERABLE PROGRESS REPORT METHODS

The dataset that constitutes this deliverable's initial analysis was derived from a literature review conducted in the summer of 2021, the outcome of which is a paper that has been submitted to the International Society for Horticultural Science Annual Conference 2022 in Angers, France (ishs.org/symposium/640). We used the Royal Danish Library's online catalogue (kb.dk) to search for Brassicaceae microgreens research within the context of light experiments that used LEDs. More elaborate explanation on methodology can be found in D1.1.

Once the literature review was completed, we had collected detailed information on 36 articles with LED specific information and selected outcomes specifically for Brassicaceae microgreens production.

Beyond the presentation of collated literature review data showing average results across studies for many varieties of microgreens within the Brassicaceae family, we conducted an inter-study comparison of the influence of light quality and quantity on our seven selected yield and secondary metabolite parameters. All analyses were done in R (version 4.1.3). Our data were log transformed to have better model validity, and we tested the assumptions of our multilinear regression via Normal Q-Q, Residual vs Fitted, Scale-location, and Residuals vs Leverage plots. After testing the assumptions of our five yield and secondary metabolite parameters (FW, DW, Carotenoids, Phenols, and Anthocyanins), we ran a variety of models and used the Akaike's 'An Information Criterion' (AIC) values to determine which models were best. We conducted our analysis using multilinear regressions that exploited the compositional nature of the light recipe (every light recipe sums up to 100%) by splitting up each recipe into its component parts as a model effect (e.g. B13R87 = 13% Blue and 87% Red as two separate model effects). We also incorporated Cumulative Light Integral (CLI = daily light integral * growing period) as an effect to account for differences in study growing period lengths and daily light integral values. Instead of comparing different models for light quality and quantity, we decided to incorporate both CLI and light spectrum effects into the same model. Finally, our model incorporated the variation present from the different varieties of microgreens, as a 'Common Name' effect in the model, as we assumed that the biological reality of species-specific and variety-specific variation was crucial to incorporate. We then tested the model with the 'Common Name' effect; the AIC showed the best values (lowest) for the complete model that included individual light spectrum effects, a Common Name effect, and the light quantity effect (CLI). To investigate the different effect sizes within our multilinear regressions, we calculated the relative sum squares (effect sum square / total sum square), which were derived from multi-way ANOVAs for each of the seven parameters. For comparing differences between species or varieties, we used a two-way ANOVA for FW and DW (as they both have a combined units effect), and used a one-way ANOVA for the other five parameters. We then used the Tukey correction for multiple comparisons to obtain compact letter displays (CLD) for each of our seven parameters. This allowed for determinations of significant difference between species and varieties. Equation 1 demonstrates an example form of our multilinear regression models that we used in this review paper, and that we will use for our experiments for D1.2.

Equation 1. Example multilinear regression format for Fresh Weight (FW), which was derived from the literature review dataset in D1.1. UV = Ultraviolet; B = Blue; G = Green; Y = Yellow; R = Red; Fr = Far-Red; β_0 is the intercept; and ε is the error term.

$$FW = \beta_0 + \beta_1(FW\_TYPE) + \beta_2(UV) + \beta_3(B) + \beta_4(G) + \beta_5(Y) + \beta_6(R) + \beta_7(Fr) + \beta_n X_n + \varepsilon$$

4 DELIVERABLE RESULTS

4.1 INTERSPECIFIC DIFFERENCES

One result from our initial literature review dataset analysis concerns differences between species for our outcomes of interest (BY and SM accumulation). In order to better understand the amount of variability present between species or varieties of microgreens, we conducted one-way ANOVAs for each of the five secondary metabolite parameters, and two-way ANOVAs for FW and DW. We then used Compact Letter Display (CLD) to display significant differences between species and varieties. This method uses the estimated marginal means (emmeans) to give an estimate as to weighted means in this regard. The results from these ANOVAs and subsequent CLDs are presented in Table 6. This Table shows that all ANOVAS were significant, indicating that there are significant differences between species or varieties of microgreens for every parameter tested. All p-values were <0.001 except for the DW_TYPE test, which was p=0.016, indicating that there was a less significant difference between g/10 plants and kg/m^2 for DW than for FW. For all other parameters, however, the differences were highly significant; this is likely the result of experimental differences as well as the inherent biological variation present. Our multilinear models, presented below, illustrate some of this variability.

Table 6. Illustration of the Compact Letter Display (CLD) for significant differences between microgreens varieties and species derived from our literature review. This was done for our primary yield and secondary metabolite parameters of interest.

Common Name	FW (kg/m2 and g/10 plants)		DW (kg/m2 and g/10 plants)		Carotenoids (mg/kg FW)		Phenols (mg/kg FW)		Anthocyanins (mg/kg FW)	
	Emmean	CLD	Emmean	CLD	Emmean	CLD	Emmean	CLD	Emmean	CLD
Arugula	-0.38	BC	-3.00	A	4.52	AB	7.20	CD	3.52	A
Brassica	-0.30	BC								
Broccoli	-0.23	BC	-2.94	AB	4.90	B	6.96	CD	5.81	BC
Cabbage	-0.23	BC	-2.88	A						
Cress	-0.21	BC	-2.79	AB			5.58	AB		
Kale	-0.05	C	-2.82	AB	4.35	AB	6.17	ABCD	5.08	B
Kohlrabi	-0.22	BC			4.25	AB	6.23	ABCD	6.91	CDEF
Mizuna	-0.11	C	-2.20	B	4.10	A	5.74	ABC		
Mustard	-0.38	BC	-2.98	A	4.66	AB	6.87	D	6.30	CD
Radish							5.77	A	7.28	EF
Red Cabbage					4.58	AB			6.01	BCDE
Red Pak Choi	-1.46	A			6.30	C	6.65	BCD	6.67	DEF
Red Russian Kale									7.12	F
Tatsoi	-0.99	AB			6.12	C	6.70	CD	6.53	DEF
ANOVA p-value	Common Name	< 0.001	Common Name	< 0.001	Common Name	< 0.001	Common Name	< 0.001	Common Name	< 0.001
	FWTYPE	< 0.001	DWTYPE	0.016						

4.2 MULTILINEAR REGRESSION MODELS

In our initial analysis, we used multilinear regression models to investigate the relationship between light quality and quantity with yield and secondary metabolite production. This was done in order to parse apart some of the production relationships that may not be immediately evident with descriptive formatting. Our primary analytical tool that we used in D1.2, which has been shown to be effective in our initial analysis shown below, is the use of multilinear regression models. These models can illustrate both effect sizes (Relative Sum Squares) and directional influence (positive or negative coefficients) of different model components. This allows us to compare different effects, such as the amount of light compared to light spectrum, or different types of light spectrum (e.g., Red vs. Blue) and using the lens of our outcomes as a threshold indicator.

4.2.1 FRESH WEIGHT

The results of our Fresh Weight (FW) multilinear model showed that there was a highly significant ($p<0.001$) association between independent and dependent variables. This model did an excellent job explaining the variation, with an adjusted R^2 of 0.93. As Table 7 shows, there were highly significant light effects on FW outcomes; for instance, Green had a highly significant p-value of $p<0.001$, while blue, red 638 nm, and red 660 nm were significant at $p<0.05$. The coefficients for these light quality effects were all positive, indicating that increasing these proportions of light can result in greater FW outcomes. Green was the light quality effect most positively associated with FW increases, with a coefficient of 0.018; Blue and Red 638 nm both had a coefficient of 0.005; and Red 660 nm had a coefficient of 0.004. It is also worth mentioning that Far Red light had a near 0 coefficient and was not significant. Furthermore, the amount of light, as CLI, was also highly significant ($p<0.001$), with a small positive coefficient of 0.001. Therefore, both light quantity and quality were significant positive predictors of FW outcomes in Brassicaceae microgreens. We also included the 'Common Name' effect in the model to account for the inter-variety variation present biologically. Only Kale ($p=0.002$) and Tatsoi ($p<0.001$) varieties were significant predictors of FW outcomes. Kale was positively associated, while Tatsoi was negatively associated with FW outcomes. Because there were two different types of units in the research seen for FW (g/10 plants and kg/m^2), we included this in the model as a 'FW_TYPE' effect instead of running two different models for each unit. Unsurprisingly, as a result, the FW_TYPE effect explained around 80% of the variation. To better demonstrate the variation for light spectrum, quantity, and Common Name, we transformed the remaining SS variation into proportional relative SS. Therefore, Green light explained 7.67%, Red 660 nm explained 6.93%, and CLI explained 5.95% of the model's remaining variation in FW outcomes. Therefore, comparing the coefficients, the p-values, and the relative SS of the different significant model components, we have shown that light quality effects are overall greater predictors of FW outcomes compared to overall pure light quantity, as their total summed relative SS for light spectrum effects were 24.08% compared to 5.95% of the light quantity effect, CLI. The varieties of microgreens together explained around 39.02% of the model's remaining variation, indicating a very strong influence of the type of microgreen used on FW accumulation.

Table 7. Fresh Weight multilinear regression summary for model design, adjusted R^2 (ADJ. R^2), p-value, degrees of freedom (DOF), model effects with estimates, standard error (Std. Error), relative sum squares (Relative SS), p-value, and significance code (Sig. Code). FW_TYPE is the unit effect (kg/m² and g/10 plants), B=Blue, G=Green, Rb=Red 660 nm, Ra=Red 638 nm, Fr=Far-Red, UV=Ultraviolet, and HPS=high pressure sodium.

FRESH WEIGHT (KG/M² AND G/10 PLANTS)

MODEL	log(FW) = FW_TYPE + B + G + Rb + Ra + Fr + UV + HPS + CLI + Common Name				
ADJ. R^2	0.93				
P-VALUE	<0.001				
DOF	106				
EFFECTS	**Estimate**	**Std. Error**	**Relative SS**	**p-value**	**Sig. Code**
INTERCEPT	-1.721	0.190		< 0.001	***
FW_TYPE	1.439	0.080	79.65%	< 0.001	***
UV	0.056	0.034	4.23% *	0.097	.
BLUE	0.005	0.002	1.03% *	0.021	*
GREEN	0.018	0.005	7.67% *	< 0.001	***
RED 638 NM	0.005	0.002	3.05% *	0.028	*
RED 660 NM	0.004	0.002	6.93% *	0.026	*
FAR RED	0.000	0.002	0.20% *	0.973	
HPS	0.001	0.003	0.98% *	0.674	
CLI	0.001	0.000	5.95% *	< 0.001	***
BRASSICA	0.048	0.173		0.784	
BROCCOLI	0.029	0.153		0.852	
CABBAGE	0.103	0.087		0.242	
CRESS	-0.122	0.156		0.435	
KALE	0.282	0.087		0.002	**
KOHLRABI	0.050	0.119	39.02% *	0.677	
MIZUNA	0.136	0.103		0.191	
MUSTARD	-0.012	0.070		0.866	
RED PAK CHOI	-1.088	-1.088		< 0.001	***
TATSOI	-1.306	0.188		< 0.001	***
RESIDUALS			6.31%		
R^2			0.94		

*=proportional relative sum squares

4.2.2 DRY WEIGHT

The results of our Dry Weight (DW) multilinear regression showed that there was a highly significant ($p<0.001$) association between independent and dependent variables. This regression did an excellent job explaining the variation, with an adjusted R^2 of 0.92. There were highly significant light effects on DW outcomes; for instance, Red 638 nm and Red 660 nm both had highly significant associations ($p<0.001$); Green had a p-value of 0.002; and Blue had a p-value of 0.047. All four of these light effects had positive coefficients, with Green having the highest of 0.018; Red 638 nm having 0.008; Red 660 nm having 0.005; and Blue having 0.003. UV had a highly negative coefficient (-0.246), and was also highly significantly associated with DW outcomes ($p<0.001$). The presence of HPS lights also had a significant ($p<0.001$) effect on DW, with a positive coefficient of 0.010. The two unit types for DW (g/10 plants and kg/m²) explained a large amount of the model's variation, at 61% (DW_Type effect). To better demonstrate the variation for light spectrum, quantity,

and Common Name, we transformed the remaining SS variation into proportional relative SS. The four significant, positively associated light effects all had small relative SS values, with Green explaining 1.21%; Red 638 nm explaining 1.87%; Red 660 nm explaining 2.11%; and Blue explaining 0.15% of the model variation. Furthermore, HPS, Common Name, CLI, and UV had relatively large relative SS values (20.49%, 16.02%, 15.30%, and 15.20%, respectively). Broccoli (p=0.002), Kale (p=0.028), and Mizuna (p<0.001) were all significantly associated with DW outcomes; however, only Broccoli had a negative coefficient, while Kale and Mizuna were both positive (-0.429, 0.132, and 0.65, respectively). The DW model was sensitive to increases in outcomes according to the amount of light, as shown via CLI's values of p<0.001, relative SS of 5.96%, and positive coefficient of 0.002. The UV having a highly significant negative association with DW, and FR having a non-significant, and near-zero coefficient are consistent biologically. Overall, the light spectrum effects explained more variation in the multilinear regression model, with a summed remaining relative SS of 50.81%, and CLI only explaining around 15.30% of the remaining relative SS. This indicates the better explanatory power of light spectrum when predicting DW outcomes during microgreens production.

Table 8. Dry Weight multilinear regression summary for model design, adjusted R^2 (ADJ. R^2), p-value, degrees of freedom (DOF), model effects with estimates, standard error (Std. Error), relative sum squares (Relative SS), p-value, and significance code (Sig. Code). DW_TYPE is the unit effect (kg/m² and g/10 plants), B=Blue, G=Green, Rb=Red 660 nm, Ra=Red 638 nm, Fr=Far-Red, UV=Ultraviolet, and HPS=high pressure sodium.

DRY WEIGHT (KG/M² AND G/10 PLANTS)					
MODEL	log(DW) = DW_TYPE + B + G + Rb + Ra + Fr + UV + HPS + CLI + Common Name				
ADJ. R^2	0.92				
P-VALUE	<0.001				
DOF	77				
EFFECTS	**Estimate**	**Std. Error**	**Relative SS**	**p-value**	**Sig. Code**
INTERCEPT	-3.941	0.145		< 0.001	***
DW_TYPE	0.623	0.086	61.05%	< 0.001	***
UV	-0.246	0.029	15.20% *	< 0.001	***
BLUE	0.003	0.001	0.15% *	0.047	*
GREEN	0.018	0.006	1.21% *	0.002	**
RED 638 NM	0.008	0.002	1.87% *	< 0.001	***
RED 660 NM	0.005	0.001	2.11% *	< 0.001	***
FAR RED	0.000	0.002	9.78% *	0.929	
HPS	0.010	0.002	20.49% *	< 0.001	***
CLI	0.002	0.000	15.30% *	< 0.001	***
BROCCOLI	-0.429	0.134		0.002	**
CABBAGE	0.072	0.059		0.223	
CRESS	-0.042	0.116	16.02% *	0.717	
KALE	0.132	0.059		0.028	*
MIZUNA	0.65	0.11		< 0.001	***
MUSTARD	0.02	0.05		0.711	
RESIDUALS			6.96%		
R^2			0.93		

*=proportional relative sum squares

4.2.3 CAROTENOIDS

The results of our Carotenoids multilinear regression showed that there was a highly significant ($p < 0.001$) association between independent and dependent variables. This model also did a good job explaining the variation, with an adjusted R^2 of 0.81. The Carotenoids multilinear model showed that Red 638 nm had a highly significant association ($p<0.001$) and a large positive coefficient (0.025) with a high relative SS which explained 42.55% of the model variation. The Common Name effect explained 18.66% of the model variation, with Mizuna having a significant ($p=0.007$) but negative coefficient, and Tatsoi and Red Pak Choi having a highly significant ($p=0.002$ and $p<0.001$, respectively) and very large positive coefficients of 0.696 and 0.869, respectively. Overall, the Carotenoid multilinear regression model had more variation explained by the light spectrum effects (65.33%) compared to the CLI of 0.04%. The CLI effect having a near zero coefficient, NS p-value, and low 0.04% relative SS is consistent biologically, as light intensities >300 µmol/m^2/s destroy Carotenoids.

Table 9. Carotenoids multilinear regression summary for model design, adjusted R^2 (ADJ. R^2), p-value, degrees of freedom (DOF), model effects with estimates, standard error (Std. Error), relative sum squares (Relative SS), p-value, and significance code (Sig. Code). B=Blue, G=Green, Rb=Red 660 nm, Ra=Red 638 nm, Fr=Far-Red, UV=Ultraviolet, and HPS=high pressure sodium.

CAROTENOIDS (MG/KG FW)					
MODEL	log(Carotenoids) = B + G + O + Y + Rb + Ra + Fr + HPS + CLI + Common Name				
ADJ. R^2	0.81				
P-VALUE	<0.001				
DOF	77				
EFFECTS	**Estimate**	**Std. Error**	**Relative SS**	**p-value**	**Sig. Code**
INTERCEPT	4.238	0.281		< 0.001	***
B	0.003	0.004	2.11%	0.482	
G	0.002	0.008	5.45%	0.804	
Y	0.020	0.046	3.63%	0.665	
O	-0.024	0.046	1.90%	0.599	
RA	0.025	0.004	42.55%	< 0.001	***
RB	0.004	0.003	8.96%	0.164	
FR	-0.003	0.004	0.53%	0.493	
HPS	0.005	0.004	0.20%	0.147	
CLI	0.000	0.001	0.04%	0.983	
BROCCOLI	-0.482	0.263		0.070	.
KALE	-0.299	0.209		0.156	
KOHLRABI	-0.382	0.195		0.053	.
MIZUNA	-0.537	0.195	18.66%	0.007	**
MUSTARD	-0.190	0.157		0.232	
RED CABBAGE	-0.070	0.209		0.738	
RED PAK CHOI	0.869	0.215		< 0.001	***
TATSOI	0.696	0.215		0.002	**
RESIDUAL			15.98%		
R^2			0.84		

4.2.4 PHENOLS

The results of our Phenols multilinear model showed that there was a highly significant ($p < 0.001$) association between independent and dependent variables. This model also did an acceptable job explaining the variation, with an adjusted R^2 of 0.60. Blue, Green, and Red 660 nm light effects were significantly associated with Phenolic outcomes (p=0.013, 0.011, and 0.009, respectively) with negative coefficients (-0.006, -0.095, and -0.006, respectively). Red 660 nm explained the most model variation with a Relative SS of 17.82%; Blue explaining 5.74%; and Green explaining 6.37%. CLI was also significantly associated with Phenolic content (p=0.026) with a negative coefficient of -0.002 and explaining 0.06% of the model variation. The Common Name effect explained 24.75% of the model variation, with Kale (p=0.013 and -1.023 coefficient), Radish (p<0.001 and -1.781 coefficient), Red Pak Choi (p=0.022 and -0.655 coefficient), and Tatsoi (p=0.021 and -0.665 coefficient) having significant but negative influences.

Table 10. Phenols multilinear regression summary for model design, adjusted R^2 (ADJ. R^2), p-value, degrees of freedom (DOF), model effects with estimates, standard error (Std. Error), relative sum squares (Relative SS), p-value, and significance code (Sig. Code). B=Blue, G=Green, Rb=Red 660 nm, Ra=Red 638 nm, Fr=Far-Red, UV=Ultraviolet, and HPS=high pressure sodium.

PHENOLS (MG/KG FW)

MODEL	log(Phenols) = B + G + O + Rb + Ra + Fr + UV + HPS + CLI + Common Name				
ADJ. R^2	0.60				
P-VALUE	<0.001				
DOF	150				
EFFECTS	**Estimate**	**Std. Error**	**Relative SS**	**p-value**	**Sig. Code**
INTERCEPT	7.582	0.281		< 0.001	***
UV	0.003	0.002	0.24%	0.163	
B	-0.006	0.002	5.74%	0.013	*
G	-0.095	0.037	6.37%	0.011	*
O	0.006	0.011	3.99%	0.595	
RA	-0.005	0.003	4.42%	0.120	
RB	-0.006	0.002	17.82%	0.009	**
FR	-0.004	0.003	0.00%	0.241	
HPS	0.002	0.002	1.02%	0.360	
CLI	-0.002	0.001	0.06%	0.026	*
BROCCOLI	-0.520	0.321		0.107	
CRESS	-0.073	0.326		0.823	
KALE	-1.023	0.407		0.013	*
KOHLRABI	-0.458	0.333	24.75%	0.170	
MUSTARD	-0.256	0.275		0.352	
RADISH	-1.781	0.295		< 0.001	***
RED PAK CHOI	-0.655	0.283		0.022	*
TATSOI	-0.665	0.284		0.021	*
RESIDUAL			35.59%		
R^2			0.64		

4.2.5 ANTHOCYANINS

The results of our Anthocyanin multilinear regression showed that there was a highly significant ($p < 0.001$) association between independent and dependent variables. This model also did an acceptable job explaining the variation, with an adjusted R^2 of 0.74. All light effects were highly significant ($p<0.001$) with positive coefficients except for Orange, which had a slightly larger p-value of 0.040. CLI had a similar relationship, with a p-value of 0.003 and a positive coefficient. As is consistent biologically, UV had the highest model variation explanation, with a relative SS of 13.27%; Red 638 nm had the second highest value of 9.41%. The Common Name effect explained 43.37% of the model variation, indicating the highly sensitive variety-specific responsiveness of these different varieties. All varieties were highly significantly ($p<0.001$) associated with Anthocyanin content, with each having positive large coefficients. Radish and Red Russian Kale had the largest coefficients, indicating a strong likelihood of there being a variety-specific association there as well, which is unsurprising given their bright pigmentation, which can be utilized as a gastronomic goal.

Table 11. Anthocyanin multilinear regression summary for model design, adjusted R^2 (ADJ. R^2), p-value, degrees of freedom (DOF), model effects with estimates, standard error (Std. Error), relative sum squares (Relative SS), p-value, and significance code (Sig. Code). B=Blue, G=Green, Rb=Red 660 nm, Ra=Red 638 nm, Fr=Far-Red, UV=Ultraviolet, and HPS=high pressure sodium.

ANTHOCYANINS (MG/KG FW)					
MODEL	log(Antho) = B + G + O + Rb + Ra + Fr + UV + HPS + CLI + Common Name				
ADJ. R^2	0.74				
P-VALUE	<0.001				
DOF	198				
EFFECTS	**Estimate**	**Std. Error**	**Relative SS**	**p-value**	**Sig. Code**
INTERCEPT	0.026	0.505		0.959	
UV	0.058	0.005	13.27%	< 0.001	***
B	0.038	0.005	0.95%	< 0.001	***
G	0.035	0.005	0.18%	< 0.001	***
O	0.030	0.015	0.05%	0.040	*
RA	0.040	0.005	9.41%	< 0.001	***
RB	0.029	0.005	4.06%	< 0.001	***
FR	0.036	0.005	0.06%	< 0.001	***
HPS	0.030	0.005	4.59%	< 0.001	***
CLI	0.003	0.001	0.36%	0.003	**
BROCCOLI	2.166	0.344		< 0.001	***
KALE	1.421	0.269		< 0.001	***
KOHLRABI	2.943	0.321		< 0.001	***
MUSTARD	2.741	0.235		< 0.001	***
RADISH	4.078	0.364	43.37%	< 0.001	***
RED CABBAGE	2.494	0.284		< 0.001	***
RED PAK CHOI	3.275	0.235		< 0.001	***
RED RUSSIAN KALE	3.633	0.359		< 0.001	***
TATSOI	3.072	0.247		< 0.001	***
RESIDUAL			23.69%		
R^2			0.76		

4.3 DELIVERABLE 1.2 RESULTS

The above examples of multilinear regression model outputs, with corresponding interpretations, will be used as the format for Deliverable 1.2's analysis. This literature review dataset from D1.1 and subsequent paper presented at the ISHS 2022 Conference has primed our analysis for D1.2 by providing tested analytical protocols and methods. Although we experienced a setback due to unavoidable supply chain constrictions that impacted a critical chip supply shortage in a previous supplier's materials, our system is currently up-and-running. The following example in Table 12 is the initial collected microgreen production data format, but we will use it to make bar graphs with standard errors and CLDs showing differences between light recipes (1-12); the corresponding table (Table 13) paired with Table 12 is the same format as those shown above in sections 4.2.1 to 4.2.5. Currently we have the potential to measure FW (kg/m^2), DW (kg/m^2), and Phenols (mg/kg DW). The Total Phenols are measured via the 'Folin Ciocalteu' method. Each of these selected outcomes will have their own figure and multilinear regression table. As Table 13 shows, our model effects in the multilinear regressions are the five different light spectrum components (UV, B, G, R, Fr); the Nutrient Solution (Yes or No); Relative Humidity (40 or 70%); Light Type (Cheap or Expensive); CLI (Light Quantity for Cheap and Expensive); and Common Name (Species or Variety). This model should therefore allow us to make informed analyses concerning the impact of light, NS, and environmental conditions on microgreens BY and SM accumulation. This will inform future deliverables in WP4, for example, and will also allow for a thorough reporting on the likely outcomes associated with specific environmental conditions.

Table 12. Example Phenols table of means, ± standard error, for Basil, Mustard, Kale, Radish, and Kohlrabi for each of the 12 growth environment recipes. Upper case adjacent letters indicate significant differences using the Compact Letter Display (CLD).

	Phenols (mg/kg DW)				
Recipe #	**Basil**	**Mustard**	**Kale**	**Radish**	**Kohlrabi**
1	**.** ± ** X	""	""	""	""
2	**.** ± ** X	""	""	""	""
3	**.** ± ** Y	""	""	""	""
4	**.** ± ** X	""	""	""	""
5	**.** ± ** Y	""	""	""	""
6	**.** ± ** Z	""	""	""	""
7	**.** ± ** X	""	""	""	""
8	**.** ± ** X	""	""	""	""
9	**.** ± ** Y	""	""	""	""
10	**.** ± ** Z	""	""	""	""
11	**.** ± ** Y	""	""	""	""
12	**.** ± ** X	""	""	""	""

Table 13. Example Phenols multilinear regression summary for model design, adjusted R^2 (ADJ. R^2), p-value, degrees of freedom (DOF), model effects with estimates, standard error (Std. Error), relative sum squares (Relative SS), p-value, and significance code (Sig. Code). UV=Ultraviolet, B=Blue, G=Green, R=Red, Fr=Far-Red, Nut. Sol.=Nutrient Solution, Rel. Hum.=Relative Humidity, LT=Light Type, and CLI=Cumulative Light Integral.

PHENOLS (MG/KG DW)

MODEL	log(Phenols) = UV + B + G + R + Fr + NS + RH + LT + CLI + Common Name				
ADJ. R^2	**.**				
P-VALUE	**.**				
DOF	***				
EFFECTS	**Estimate**	**Std. Error**	**Relative SS**	**p-value**	**Sig. Code**
INTERCEPT	**.**	**.**	**.**	**.**	**.**
UV	**.**	**.**	**.**	**.**	**.**
B	**.**	**.**	**.**	**.**	**.**
G	**.**	**.**	**.**	**.**	**.**
R	**.**	**.**	**.**	**.**	**.**
FR	**.**	**.**	**.**	**.**	**.**
NUT. SOL.	**.**	**.**	**.**	**.**	**.**
REL. HUM.	**.**	**.**	**.**	**.**	**.**
LIGHT TYPE	**.**	**.**	**.**	**.**	**.**
CLI	**.**	**.**	**.**	**.**	**.**
BASIL	**.**	**.**	**.**	**.**	**.**
MUSTARD	**.**	**.**	**.**	**.**	**.**
KALE	**.**	**.**	**.**	**.**	**.**
RADISH	**.**	**.**	**.**	**.**	**.**
KOHLRABI	**.**	**.**	**.**	**.**	**.**
RESIDUAL			**.**		
R^2			**.**		

5 DISCUSSION

Results from our initial analysis, whose data was derived from D1.1, demonstrated the necessity of considering variety-specific variation when designing growth environments, as microgreens species and varieties respond differently to environmental conditions. This is something we will explore in more depth in D1.2. Based on this initial analysis, and the literature review in general, we expect that the high degree of species or variety-specific variation for microgreens will necessitate parameterization for each specific kind of microgreens that are grown. We will be able to show this via our analysis in D1.2 and make recommendations for the digital platform developed in later GOhydro work packages. We expect that there is no single optimal environmental recipe for all microgreens, let alone those five that we have chosen to experiment with here in D1.2. In this regard, we also want to contextualize the claim to optimality via different lenses; for instance, whether there are significant gains in expensive lights compared to cheaper ones should be contextualized with their degree of difference in accordance with their price. The same goes for energy usage and degree of labor input required for successful user operation. We therefore are primarily interested in establishing how different the low-input (No NS), low Relative Humidity (40%), and Cheap Light (HE 24-V, e.g.) environmental recipe performs compared to more intensive operations. We will also have information on water usage and energy usage as well. By presenting results on these different recipes' outcomes, we can begin to contextualize production regimes and make recommendations that are in line with the GOhydro project goals.

We believe that future research should continue in this avenue of comparing low-tech and high-tech inputs to determine what the gains are in FW, DW, nutrient accumulation, and secondary metabolite production per unit input (including water and energy), as current research focuses on highly sophisticated technologies and techniques, while some commercial farmers still rely on very simple setups. The large number (n=42) of total light papers from our literature review in D1.1 provides a useful reference of the existing breadth of research. Furthermore, 40 out of 42 light papers were published within the last 5 years, therefore indicating the novelty of the research undertaken, the lack of concrete established optimality, and the likely direction of further research. This therefore means that there is also much room for investigating and establishing optimal standards for major microgreens species, including variety-specific fertilization, light regimes, and environmental parameterization. Here in D1.2, we take this a step further by also considering the inputs of water, energy use, and light type per unit of microgreen product.

6 CONCLUSIONS

Although we experienced an unavoidable setback due to supply chain constrictions from Coronavirus on key chip components in our original climate chamber, we have since found a new supplier for lights and a climate chamber, and our operation is currently up-and-running. We have demonstrated in this deliverable the integration of previous research stemming from our D1.1 literature review and our paper for the ISHS 2022 Annual Conference in Angers, France. Our conference paper has demonstrated the potential of using multilinear regression models to determine significant relationships that can exist across species and varieties of microgreens when attempting to design tailored production environments. For instance, we showed that there are inter-study trends whereby the use of Green light has been positively and significantly associated with DW accumulation. For Carotenoids, only Red 638 nm light was significantly associated with their accumulation, and its incidence explained more than 40% of the model variation. For Anthocyanins, all spectra of light were significantly and positively associated with their accumulation. Importantly, we were also concerned with comparing light quality (spectrum) to light quantity (CLI) explanatory potential for yield and secondary metabolite accumulation. Our results showed that light quantity (CLI) explained much less variation compared to light spectra for Carotenoids and Anthocyanins (0.04% vs. 65.33%, and 0.36% vs. 32.57%, respectively). In a similar vein, for DW accumulation, light quantity had lower relative SS values than the spectrum values (15.30% vs. 50.81% respectively). This same pattern was also seen for Fresh Weight and Phenols as well, as all the five yield and secondary metabolite parameters had a greater influence of light spectrum than light quantity on accumulation. This previous work has established a functional methodology and analytical process that is ready for data input from our D1.2 experiments that are in progress.

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