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

D4.1 Trial protocols for microgreens production in hydroponic units

DELIVERABLE NUMBER	D4.1
DELIVERABLE TITLE	Trial protocols for microgreens production in hydroponic units
RESPONSIBLE AUTHOR	Teodor Rusu (USAMV)

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ACRONYMS LIST

CHSK	Crop Health Sensor Kit
MMSK	Multi-modal Sensor Kit
NCK	Nutrient-content Kit
DA	Degree of Attack
FW	Fresh Weight
DW	Dry Weight
ANOVA	Analysis of Variance

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

EXECUTIVE SUMMARY

The activity T4.1 Experimentation methodology for high-value nutrient design had as objective the elaboration of the deliverable D4.1 Trial protocols for microgreens production in hydroponic units (R, PU) [M15]. This is a report detailing the methods, materials and hydroponic units to be used for the GoHydro trials both in controlled and operational settings.

In order to meet the requirements of the project so that the datasets generated in each trial site are comparable and combinable into a single, common dataset, the following principles for the elaboration of the hydroponic microgreen production protocol are suggested:

- IN CONTROLLED SETTINGS – setting the optimal ranges of environmental parameters between the limits of favorability for each species, as seen in the literature review in D1.1, and validated in D1.2 experiments, for microgreens to highlight the effects of the GoHydro platform (Greece and Romania).
- IN OPERATIONAL ENVIRONMENTS SETTINGS – monitoring, but not controlling, environmental conditions (which will be different in the three centers: Greece, Denmark and Romania).

Except for the two differences above, the determination plan is still the same (in controlled settings an in operational environments settings):

Collecting data using the GoHydro platform and the sensor kit: Crop Heath Sensor Kit (CHSK) and the Nutrient Content Kit (NCK) are:

1. Air temperature
2. Water temperature
3. pH
4. Electrical Conductivity
5. Relative Humidity
6. Light
7. Nutrient content

Activities (procedures, parameters) related to microgreen growth, production and quality:

1. Obtaining the microgreens seedling
2. Measuring the morphology of plants (LAI or height)
3. Determining the health state of plants
4. Harvesting microgreens
5. Measuring Fresh Biomass Yield – Fresh Weight (FW, kg m^{-2})
6. Measuring Dry Matter Content - Dry Weight (DW, g m^{-2})
7. Secondary metabolite collection and analysis
8. Statistical analysis

Feedback from these trials will be used for the final validation of the analytics components of the GoHydro platform.

Link for collecting data in controlled settings:
https://docs.google.com/spreadsheets/d/1kxX_6DifZBtFC6J2_tahIVBhQch-Jtq1cvrgpTPQcnE/edit?usp=sharing

Link for collecting data in operational environments settings:
https://docs.google.com/spreadsheets/d/19puWTafm7GenD1zhaqFk73vxc-78xy-FK47QeIGIKMU/edit?usp=sharing

Additional information can be found in the published paper:
Moraru, P.I.; Rusu, T.; Mintas, O.S. Trial Protocol for Evaluating Platforms for Growing Microgreens in Hydroponic Conditions. Foods 2022, 11, 1327. https://doi.org/10.3390/foods11091327

1 INTRODUCTION

GoHydro aims at developing a cost-efficient smart-sensing ICT platform capable of monitoring hydroponically cultivated microgreens' environmental parameters and nutrient content in order to optimize the cultivation process and allow the harvest of the best possible products in many hydroponic installations. GOhydro aspires to culminate in the production of a radical platform that will be a shifting paradigm of how AI-driven technological innovation can become an affordable, accessible-by-all and user-friendly tool applicable to all forms of urban farming.

The purpose of the research made within WP4 concerns pilot trials in low-cost hydroponic units with a specific focus on the influence the environmental factors on the growth and development of microgreens under hydroponic conditions. The hydroponic technology is analyzed with the goal to highlight, under the conditions of the GoHydro platform, in what way different environmental and nutritional factors can influence the development of microgreens and can improve its production and quality.

Because of their lifecylce spanning early phenological stages from germination to first cotyledon or true leaf apperance, microgreens are very sensitive to changes in environmental parameters, available water regime, nutrition, pests, etc. Using hydroponic technology has the advantage of favouring the development of microgreens at this young stage by enabling the control of these important environmental parameters, thereby ensuring their quantity and quality. The purpose of this research is to analyze in detail certain elements relevant for the microgreens crop grown under hyroponic systems, such as: air temperature, water temperature, humidity, pH, electrical conductivity, different nutrient solutions, and the influence of light regimes (quantity, quality and photoperiods). This will situate growth regimes to allow for the control of diseases and pests and achieve desired growth and production trajectories. The goal of all these determinations is to contribute to the development of knowledge concerning the challenges of microgreens crops in a controlled hydroponic environment which will push forward discussions and practice in this segment of research and industry.

The trial protocols of Deliverable 4.1 are set up based on previous research seen in:

- Task 1.1 Review on nutrient and production parameters and light requirements (UCPH, SCiO, USAMV) [M1-M6], and
- Task 1.2 Evaluation of optimised growth environments in controlled experiments (UCPH & SCiO) [M4-M12] and on the selection of promising recipes.

Deliverable 4.1 (D4.1): Experimentation methodology for high-value nutrient design [M10-M15] [01.12.2021-31.05.2022] has the task of preparing the trial protocols in order to make microgreen production tests in GoHydro hydroponic units. Based on the findings of Task 1.1 and Task 1.2 (the selection of promising environmental recipes, in progress), D4.1 will prepare the trial protocols for carrying out production trials of microgreens in low-cost hydroponic units.

In formulating protocols, a minimum two of the four microgreens proposed by the project: basil (*Ocimum basilicum*), lettuce (*Lactuca sativa*), coriander (*Coriandrum sativum*) and mint (*Mentha sp.*), will be selected per partner for testing in low-cost hydroponics units established in Greece, Denmark and Romania.

Furthermore, the protocols of D4.1 will define the data collection procedures and data format organization to be followed, so that datasets generated in each trial site are comparable and combinable into a single, common dataset.

Partners' role: USAMV (lead), with participation of UCPH, SCiO, and NCSR-D.

Deliverable for D 4.1 Trial protocols for microgreens production in hydroponic units (R, PU) [M15]:

- A report detailing the methods, materials and hydroponic units to be used for the GoHydro trials both in controlled and operational settings.

2 TIMETABLE AND THE PRINCIPLES OF THE TRIAL PROTOCOLS

2.1 OBJECTIVES AND WORK PLAN FOR WP4

WP4 activity has 2 main objectives:

- To design the trial protocols for evaluating the GoHydro platform in realistic uncontrolled installations and in operational highly controlled settings;
- To carry out pilot trials and provide feedback for the refinement and calibration of the GoHydro platform.

Timetable of task components of WP4 are as follows:

Table 2.1: Timetable of task components of WP4

Task	Month	Partners' role	Activities	Deliverables
T4.1 Experimentation methodology for high-value nutrient design	[M10-M15] [01.12.2021-31.05.2022]	USAMV (lead), with participation of UCPH, SCiO, and NCSR-D	In formulating protocols, a minimum two of the four microgreens identified (basil, lettuce, coriander and mint) will be selected per location for testing in low-cost hydroponics units in Greece, Denmark and Romania. The protocols will define the data collection and data format to be followed, so that datasets generated in each trial site are comparable and combinable into a single, common dataset.	D4.1 Trial protocols for microgreens production in hydroponic units (R, PU) [M15] A report detailing the methods, materials and hydroponic units to be used for the GoHydro trials both in controlled and operational settings.
T4.2 Experimentation in controlled realistic installations	[M13-M24] [01.03.2022-28.02.2023]	SCiO (lead), with participation of USAMV	The low-cost hydroponic units to be used will be capable of supporting the growth of approximately 80-100 plants per production cycle. The trials will take place in two sites, Greece and Romania, where each partner will test at least two crops to sample the different growing environments.	D4.2 Evaluation results from experiments in semi-controlled realistic installations (R, PU) [M21] A report presenting and analysing the results and insights gained from the pilots under controlled settings.
T4.3 Validation of performance of low-cost hydroponic units in operational settings	[M19-M24] [01.09.2022-28.02.2023]	USAMV (lead), with participation of UCPH, SCiO, and NCSR-D	Production trials on the two identified microgreens in low-cost hydroponic units, will be carried out in Greece, Denmark and Romania in operational environments. The operational environments include office and living spaces for everyday use; the production parameters are not controlled. Feedback from these trials will be used for the final validation of the analytics components of the GoHydro platform.	D4.3 Report on validation of microgreens production in operational settings (R, PU) [M24] A report presenting and analysing the results and insights gained from the pilots under operational settings.

2.2 PRINCIPLES OF THE TRIAL PROTOCOLS FOR EVALUATING THE GOHYDRO PLATFORM

In order to meet the requirements of the project so that the datasets generated in each trial site are comparable and combinable into a single, common dataset, the following principles for the elaboration of the hydroponic microgreen production protocol are suggested:

- IN CONTROLLED SETTINGS – setting the optimal ranges of environmental parameters between the limits of favorability for each species, as seen in the literature review in D1.1, and validated in D1.2 experiments, for microgreens to highlight the effects of the GoHydro platform (Greece and Romania).
- IN OPERATIONAL ENVIRONMENTS SETTINGS – monitoring, but not controlling, environmental conditions (which will be different in the three centers: Greece, Denmark and Romania).
- Use of identical GoHydro prototypes - for comparable data:
  - Each pilot site (GREECE, DENMARK and ROMANIA) is responsible for setting up a hydroponic installation on its own by following the guidelines of deliverable D2.1 (Figure 2.1);
  - GoHydro will provide identical sensor kits (Crop Heath Sensor Kit and Nutrient Content Kit from Task 2.4). By Month 18 (August 2022) three sensor kits will be integrated and sent to the partners' sites for testing within the framework of WP4.
- It is also important to use the same type of substrate for seed germination and growth, and to use the same variety of our common species (basil) in the three partner sites.
- Common protocols will be set for data collection, data formatting, and collection intervals.
- Data collection will be done using 3 repetitions, which will depend on the controlled or operation setting of the GoHydro platform (e.g. seen in Fig. 2.1).

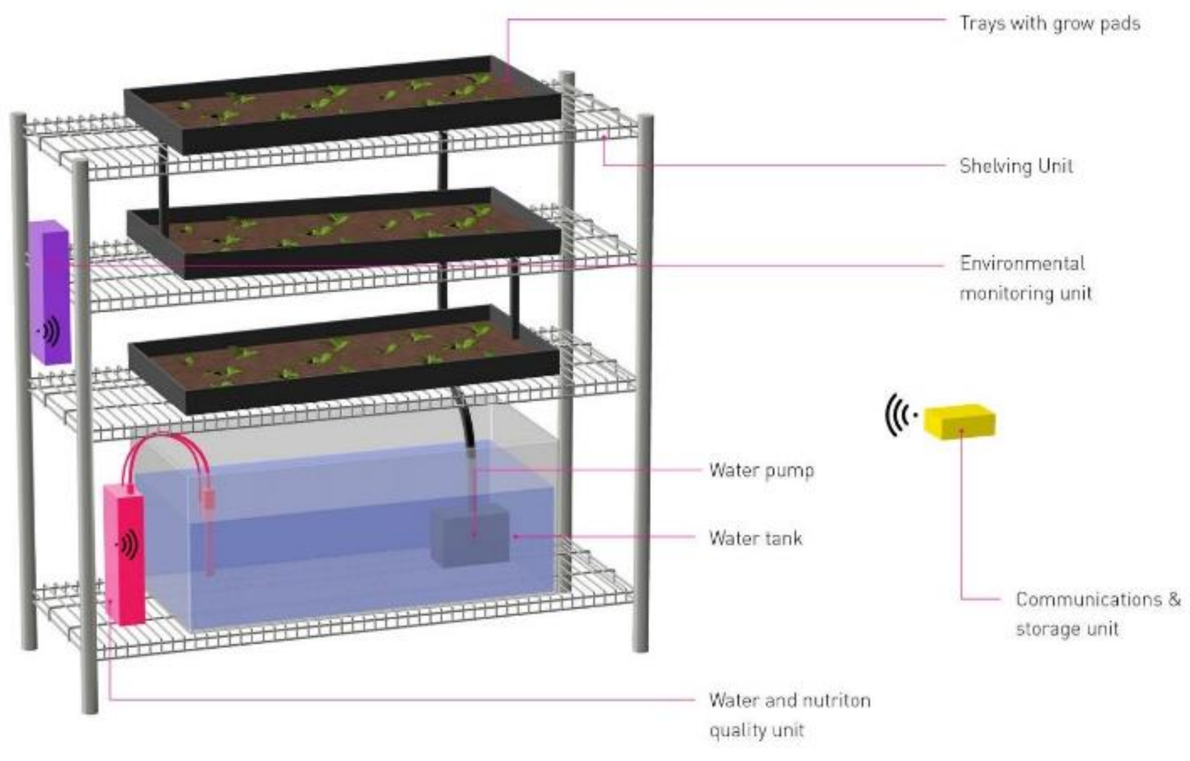

Figure 2.1: Schematic Representation of the two sub-units of the Crop Health Sensor Kit (D2.1)

3 SYNTHESIS OF THE RESULTS OBTAINED IN TASK 1.1 REVIEW ON NUTRIENT AND PRODUCTION PARAMETERS AND LIGHT REQUIREMENTS

Task 1.1 conducted a literature review on nutrient requirements, production parameters, and lighting needs for hydroponically grown microgreens, examining different production parameters like pH, electrical conductivity, humidity, dissolved oxygen, CO_2, temperature (of air and water) and nutrients. We also collected literature data on the effect of light parameters on crop growth, nutrient profile and yield. Relevant variables include light quality, light intensity and photoperiod effects on microgreen growth and development. The synergistic effects of such factors were organized to identify the most promising growth environments for enhanced growth and development for nutrition-rich and high yields in the examined microgreens. This is the fundament of D4.1 and other protocols for experimentation as they are derived from a synthesized literature review of real experimental practices.

Synthesis of the results obtained until now (Task 1.1) and elaboration of input conclusions for T4.1.
Microgreen reviewed:
SCiO: Basil, Brussels sprouts in particular, but all microgreens in general
UCPH: Basil, Lettuce, Coriander, Mint in particular, but all microgreens in general
USAMV: Basil, Lettuce in particular, but all microgreens in general

The main results obtained during this activity, as a scientific basis for the following activities of the project are:

- Completing the GoHydro online database for hydroponics microgreens such as basil (*Ocimum basilicum*), lettuce (*Lactuca sativa*), coriander (*Coriandrum sativum*), mint (*Mentha sp.*), parsley (*Petroselinum crispum*), beets (*Beta vulgaris*), peas (*Pisum sativum*), broccoli (*Brassica oleracea var. italica*), mustard (*Sinapis sp.*), etc.

- **Journal papers:**

1. Rusu, T., R.J. Cowden, P.I. Moraru, M.A. Maxim, B.B. Ghaley, 2021. Overview of multiple applications of basil species and cultivars and the effects of production environmental parameters on yields and secondary metabolites in hydroponic systems. Sustainability, 13(20), Article number 11332. https://doi.org/10.3390/su132011332, (FI: 3.521).

2. Rusu, T., P.I. Moraru, O.S. Mintas, 2021. Influence of environmental and nutritional factors on the development of lettuce (*Lactuca sativa* L.) microgreens grown in a hydroponic system: A review. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 49(3), Article number: 12427. https://doi.org/10.15835/nbha49312427, (FI: 1.444).

- **Zenado papers:**

1. Cowden, R., G., Bhim Bahadur, T. Rusu, 2021. GOhydro Literature Review Light Database (1.0) [Data set]. Zenodo. https://doi.org/10.5281/zenodo.5512207

2. Cowden, R., G., Bhim Bahadur, T. Rusu, 2021. Report on nutrient and production parameters and light requirements for microgreen production in hydroponic units. Zenodo. https://doi.org/10.5281/zenodo.5509620

- **Conference papers:**

1. Rusu, T., P. I. Moraru, M. A. Maxim, 2021. Influencing factors in obtaining microgreens in hydroponic conditions. The 20th International Conference Life Sciences for Sustainable Development, 23-25 September 2021 (online), Program Booklet, p. 36, Book of Abstracts No. 8/2021, p. 174, USAMV Cluj-Napoca, https://symposium.usamvcluj.ro.

2. Rusu, T., 2021. Evaluation of the growth and quality of lettuce microgreens (*Lactuca sativa* L.) in the hydroponic system: A review. 7th International Conference on “Multidisciplinary Academic Research & Global

Innovation" (MARGI-2021). Abstract Proceeding Book, August 28-29, 2021, Amsterdam, Netherlands, ISBN: 978-969-695-070-7, pag. 27-28.

- **Magazine article (in Romanian):**

1. Rusu, T., 2021. GoHydro – O nouă perspectivă în producția microplantelor (GoHydro - A new perspective in the production of microplants). Agricultura 365, Anul IX, nr. 43, November-December 2020, pag. 34-35. ISSN 2343-9580, ISSN-L 2343-9580, Inkorporate Print Publishing House Bucharest.

- **Deliverables from WP1:**

D1.1 Report on nutrient and production parameters and light requirements for microgreen production in hydroponic units [M6]. This report summarising the findings of the experimental literature and estbalished baselines for testing in controlled experiments that this project will adopt.

The results obtained, synthesized and presented in these papers lead to the following **conclusions:**

1. The literature review has demonstrated that the production of microgreens using hydroponic systems must be organized with care for controlling the many environmental and production parameters to achieve desired outputs that are of an adequate quality and quantity. This is in comparison to more conventional production methods using soil, considering all the controllable factors in hydroponic systems that have been shown to influence the accumulation of bioactive substances, the harvest timeframe, and the quality of the finished product. Furthermore, the lack of a soil's microbiome in hydroponic systems is also important to consider, as unsuccessful parameterization leaves the plants vulnerable to harmful spoilage by microorganisms.
2. However, the high degree of environmental control necessary for optimal growth, which can limit uptake by some users, can also be beneficial to hydroponic systems, as the sophisticated organization can lead to the elaboration of certain protocols to influence factors which can positively influence plants in order to obtain a crop as uniform as possible throughout the year, with higher concentrations of active substances and nutrients valuable for human health. This is why it is necessary to standardize certain cultivation protocols to ensure their quality, while also keeping in mind uptake likelihood (i.e. comparing operational and controlled experiments to understand how outcomes vary between the different systems); this is also why future research will benefit from syntheses of a wide variety of data on the value and production trends of hydroponically grown microgreens. For instance, there is a wide variation of environmental impact factors and their relationship to downstream microgreens outputs, which means that there is no single prescription that will guarantee perfect results. This is even more so when considering species and varieites of microgreens: we will ideally establish variety-specific protocols to accomodate these biological realities.
3. The literature review has demonstrated that there are optimal ranges within which one can begin the task of designing effective prescriptions for successful microgreen production. And given that we want to improve the GoHydro hydroponic platform, we consider it appropriate to ensure the optimal ranges environment for microgreens (in controlled settings: Greece, Denmark, and Romania).
4. The literature review has shown that the nutritional solution, temperature, and light regime have the most important role in seed germination and development, while also discussing the recent research on the many promising research trends in refining microgreen production to achieve optimal outputs along its phenological stages. The nutritional solution, air, and water temperature, light regime, pH, electrical conductivity, dissolved oxygen, CO_2 concentration, and relative humidity (Figure 3.1) are all important factors which influence secondary metabolism from an incipient phase, which in the final stages increases both the perceived and actual value of the plants by contributing to human health and nutritional fortification.
5. This literature review has shown that microgreen producers must integrate specific systematic hydroponic strategies to obtain high quality microgreens and high quantity and quality bioactive substances, while also avoiding the potential for spoilage and low-quality production when moving too far beyond the noted parameter ranges summarized here.
6. Many authors in the literature review have noted that best practices have not been developed for microgreens, as it is a relatively novel practice; this literature review has therefore gathered critical information regarding hydroponically grown microgreen production that can be used by researchers and producers to improve the chances of successful production of yields and valuable secondary metabolites.

7. Microgreens are currently considered among the five most profitable crops, along with mushrooms, ginseng, saffron and goji berries (Kyriacou et al., 2020). Therefore, developing species-specific protocols to support year-round production and to enhance valuable antioxidant components is of the utmost importance for the microgreens industry. Running experiments in both operational and controlled settings also allows for the comparison of cost and inputs measured against desired outputs.

8. It is particularly important that the fundamental research into ensuring the safety and quality of this new addition to healthy diets is done so that the produce industry can avoid some of the problems that have challenged the mature produce and sprout industries during the past several decades (Turner et al., 2020).

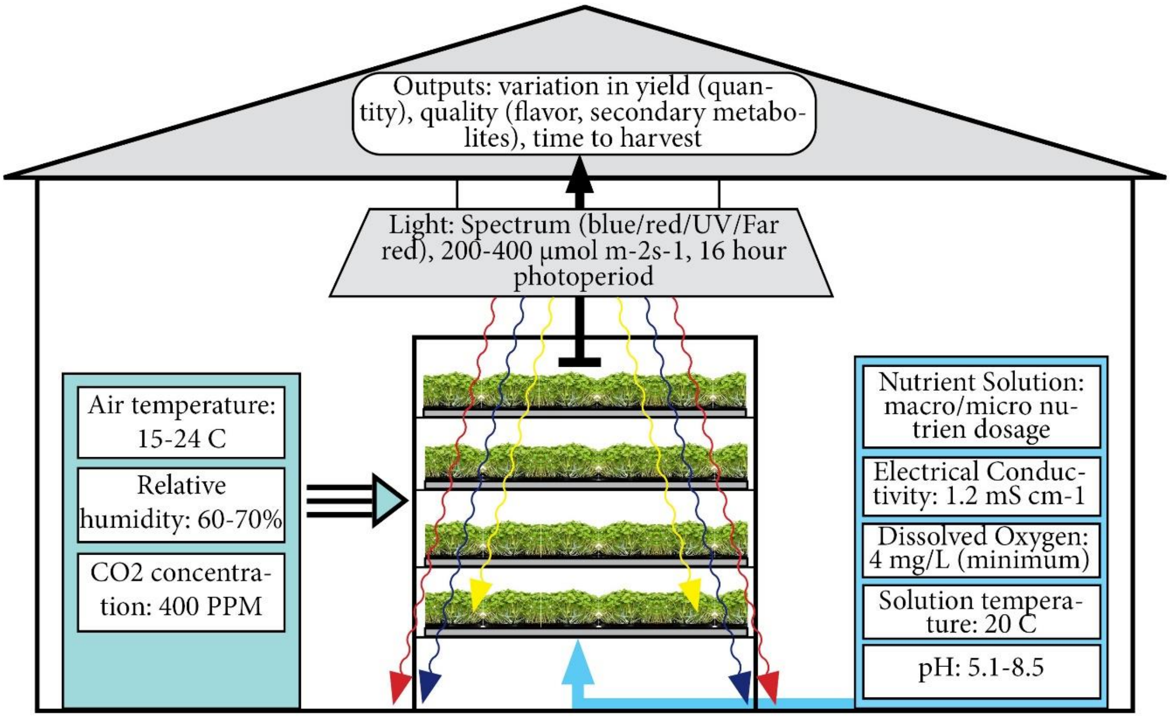

Figure 3.1: Schematic representation of the influence of environmental factors

4 INPUT FROM OTHER ACTIVITIES

T1.2 Evaluation of optimised growth environments in controlled experiments [M4-M12] [01.06.2021-28.02.2022], UCPH and SciO: Based on the controlled experiment results, a list of promising climate recipes will be identified for testing in actual living environments in office and indoor spaces in WP4.

Deliverables in progress in WP1:

D1.2 Report on evaluation of optimized growth environments in controlled experiments [M12] [28.02.2022]. A report summarising the findings from the controlled experiments for optimising growth environment to be further validated in realistic installations is in the process of being produced which will offer required feedback and determination of ideal protocols to be designed before comparing operational and controlled experiments in later WP4 tasks.

Deliverable 2.1 has already selected the components of the hydroponic installation which costs in total ~720 € (Table 4.1).

Table 4.1 List of the pilot GOHYDRO hydroponic installation components and their cost

Component #	*Site for on-line ordering*	*Cost*
Shelving Unit	https://www.ikea.gr/proioda/kouzina/rafieres-rafia-kouzinas/rafieres/omar-monada-rafiwn/69829083?gclid=Cj0KCQjw9YWDBhDyARIsADt6sGZGanXE8FuOkjTptS3ZdY2aLGMp-sd1-GC-3yUZIwLR664b8JwArXMaAqkNEALw_wcB	79.90 €
Hydroponic Tent	https://www.gadget-shop.gr/idroponiki-skini-esoterikou-chorou-kalliergias-fiton-120-x-60-x-150-cm-outsunny-845-263-845-263?skr_prm=WyJhNTcyZTgyYi1mOTFhLTQ0Y2EtYTBkZC0xNWVmNGNmNjQ3 MGQiLDE2MTcwMTgwNDkwNDQseyJhcHBfdHlwZSI6IndlYiIsImNwIjoiYiIs InRhZ3MiOiIifV0#prettyPhoto	99.95 €
Trays (x4)	https://aeroponic.gr/kanali-kalliergeias-1m.html	4×11= 44 €
Grow Pads (x100 –minimum order)	https://www.alibaba.com/product-detail/Hot-Sale-Outdoor-Biodegradable-Jute-Felt_1600072664839.html?spm=a2700.7724857.normal_offer.d_title.6f097281m2Q8ty	199 €
Pump	https://aeroponic.gr/boyu-fp-1000-adjustable-pump-1000l-hr-eu-plug.html	21,90 €
Airstone	https://www.growit.gr/%CF%80%CF%81%CE%BF%CE%B9%CE%BF%CE%BD/%ce%ba%cf%85%ce%ba%ce%bb%ce%b9%ce%ba%ce%b7-%ce%b1%ce%b5%cf%81%ce%bf%cf%80%ce%b5%cf%84%cf%81%ce%b1-175mm/	9,50 €
Tubes	Generic hardware store	~ 10 €
Sweet Basil Seeds (1kgr minimum order)	https://www.alibaba.com/product-detail/Top-Quality-Ocimum-basilicum-Sweet-Basil_1600131992747.html?spm=a2700.pc_countrysearch.main07.37.657f4462cF63Jc	9.90 €
Water Tank	Any aquarium type acrylic water tank is suitable and will be purchased from a pet shop	~ 30 €
Hydroponic Lamp	https://www.amazon.com/Sun-Blaze-Fluorescent-Hydroponic-Greenhouse/dp/B009GCQWX2/ref=pb_allspark_session_sims_desktop_2?pd_rd_w=N9otb&pf_rd_p=bfefd6e2-acb1-463d-94d0-38a6e00f41d3&pf_rd_r=P2KT6ZQXZGDRK31XSV4R&pd_rd_r=3db928d6-530f-4d77-a274-fbf74edea30c&pd_rd_wg=P6zAh&pd_rd_i=B009GCQWX2&psc=1	~ 70 €
Nutrient Powder	Commercially available at several sites	~50 €
	TOTAL COST	718.20 €

WP2 Multi-modal sensor kits [M1-M18], design specifications, requirements and specific protocols of the two basic components of the multi-mode sensor kit:

- **Nutrient-content Kit** - which will be based on the novel immersible photonic probe system.
- **Crop-health Kit** - environmental monitoring kit - which we will be based on off-the-shelf components.

These two systems will include in their design the necessary components for data collection, transmission and driving through the mobile application.

T2.4 Multi-modal sensor kit [M10-M18] [01.12.2021-31.08.2022], NCSR-D, nr21 DESIGN, SCiO: The first prototype will remain in Greece for the final evaluation in WP4. By M18 [31.08.2022], two more systems (in total) three will be integrated and sent to the partners' site for testing within the framework of WP4. Throughout Task 2.4 and until M21, NCSR-D will be fabricating photonic probes and mail them to the testing sites.

Deliverables WP2:

D2.5 Procurement of 2 additional Multi-modal Sensor Kits (M18, Nr21 DESIGN). Two additional integrated GoHydro systems will be produced and sent to Denmark and Romania for the final demonstrations of WP4.

5 OBJECTIVES FOR TASK 4.1. EXPERIMENTATION METHODOLOGY FOR HIGH-VALUE NUTRIENT DESIGN

The general objective of **Task 4.1. Experimentation methodology for high-value nutrient design [M10-M15] [01.12.2021-31.05.2022]** targets the set-up and development of scientifically-argumented techniques with immediate applicability to obtain microgreens in a hydroponic system.

The following objectives have been established in order to obtain conclusive results:

- Based on the findings of Task 1.1 and Task 1.2, Task 4.1 will prepare the trial protocols for carrying out production trials of microgreens in low-cost hydroponic units.
- In formulating protocols, a minimum two of the four microgreens identified (basil, lettuce, coriander and mint) will be selected per location for testing in low-cost hydroponics units in Greece, Denmark and Romania.
- The protocols will define the data collection and data format to be followed, so that datasets generated in each trial site are comparable and combinable into a single, common dataset.

In the protocol we consider that microgreen growth could be characterised by four variables that are not necessarily correlated (Jans-Singh et al., 2019):

(1) number of growing days to reach desired size,
(2) yield per area (while considering seeding density),
(3) crop health (percentage of crops diseased),
(4) secondary metabolite accumulation (ascorbic acid, carotenoids, chlorophyl, etc.)

These four criteria will be used to evalute and compare microgreens production outcomes and success between the controlled and the operational (uncontrolled) settings. These variables should be researched and highlighted if there are any differences within the experiments of the GoHydro platform to be used in the feedback and final validation process of the analytics components of the GoHydro platform.

6 TRIAL PROTOCOLS FOR CARRYING OUT PRODUCTION TRIALS OF MICROGREENS IN LOW-COST HYDROPONIC UNITS

We present below the methods, materials and hydroponic units to be used for the GoHydro trials both in controlled and operational settings.

A first set of trials will be held in controlled rooms, while the second pilot session will be deployed in operational (uncontrolled) settings.

6.1 IN CONTROLLED SETTINGS (GREECE AND ROMANIA)

T4.2 Experimentation in controlled realistic installations [M13-M24] [01.03.2022-28.02.2023].

The task will carry out production trials in hydroponic units installed in sites where the parameters defined by the plant development model are continuously monitored and controlled. The low-cost hydroponic units to be used will be capable of supporting the growth of approximately 80-100 plants per production cycle. The trials will take place at two sites, Greece and Romania, where each partner will test at least two crops to sample the different growing environments.

Partners' role: SCiO (lead), with participation of USAMV.

The crops tested will be:

Greece_SCiO: Basil, Brussels sprouts

Romania_USAMV: Basil, Lettuce

The determination plan comprises:

a. Setting the optimal ranges (between the limits of favorability for each species) for microgreens to highlight the effects of the GoHydro platform, based on D1.1 and D1.2 inputs.

b. Collecting data using the GoHydro platform and the sensor kit: Crop Heath Sensor Kit (CHSK) and the Nutrient Content Kit (NCK) are:

1. Air temperature
2. Water temperature
3. pH
4. Electrical Conductivity
5. Relative Humidity
6. Light
7. Nutrient content

c. Activities (procedures, parameters) related to microgreen growth, production and quality:

1. Obtaining the microgreens seedling
2. Measuring the morphology of plants (LAI or height)
3. Determining the health state of plants
4. Harvesting microgreens
5. Measuring Fresh Biomass Yield – Fresh Weight (FW, kg m^{-2})
6. Measuring Dry Matter Content - Dry Weight (DW, g m^{-2})
7. Secondary metabolite collection and analysis
8. Statistical analysis

6.2 IN OPERATIONAL ENVIRONMENTS SETTINGS (GREECE, DENMARK AND ROMANIA)

T4.3 Validation of performance of low-cost hydroponic units in operational settings [M19-M24] [01.09.2022-28.02.2023].

The operational environments include office and living spaces for everyday use. In these production trials, the final validation of GoHydro platform will take place in fully operational settings, where the production parameters are not controlled. The collection and analysis of collected data will occur at regular intervals. Feedback from these trials will be used for the final validation of the analytics components of the GoHydro platform.

Partners' role: USAMV (lead), with participation of UCPH, SCiO, and NCSR-D.

The crops tested will be:
Greece_SCiO: Basil, Brussels sprouts
Denmark_UCPH: Basil, and one of either Parsley, Lettuce, Mint, Beet, Cress, or Mustard
Romania_USAMV: Basil, Lettuce

The determination plan comprises:

a. Weather conditions in the area/ testing period.

b. Collecting data using the GoHydro platform and the sensor kit: Crop Heath Sensor Kit (CHSK) and the Nutrient Content Kit (NCK) are:

1. Air temperature
2. Water temperature
3. pH
4. Electrical Conductivity
5. Relative Humidity
6. Light
7. Nutrient content

c. Activities (procedures, parameters) related to microgreen growth, production and quality:

1. Obtaining the microgreens seedling
2. Measuring the morphology of plants (LAI or height)
3. Determining the health state of plants
4. Harvesting microgreens
5. Measuring Fresh Biomass Yield – Fresh Weight (FW, kg m^{-2})
6. Measuring Dry Matter Content - Dry Weight (DW, g m^{-2})
7. Secondary metabolite collection and analysis
8. Statistical analysis

6.3 DATA COLLECTION AND DATA FORMAT

a(6.1). Setting the optimal ranges, in controlled settings (Greece and Romania) (between the limits of favorability for each species) for microgreens to highlight the effects of the GoHydro platform. The threshold values of optimal environmental conditions for the microgreens grown in GoHydro platform were first identified through a literature review, but they can be adapted by observing the development of microgreens cultures. As reported in literature, special attention must be addressed to the choice of growth medium, which represents one of the key factors in the production process and could influence microgreens yield and quality (Di Gioia et al., 2016).

Data collection and data format to be followed:

Parameters defined for different species of microogrens continuously monitored and controlled are presented in table 6.1. (Rusu et al., 2021; El-Nakhel et al., 2021). The spectral output of the lighting system must be quantified using a spectrometer, at various points of growth of the trays of the GoHydro platform.

Table 6.1: Parameters defined for different species of microgreens continuously monitored and controlled

No.	Parameter	Unit of measurement	Average value of parameters for example species***		
			Basil	Lettuce	Brussels sprouts
1	Light	W	400	400	400
1.1	Photoperiodicity	h	06:30-21:30 (15h) (10-20h)	07:00-20:00 (12h)	07:00-20:00 (12h)
1.2	Light intensity	$\mu molm^{-2}s^{-1}$	300 (200-400)	500	300 ± 15
1.3	Color spectrum	nm	440-460 (260-780)	440-460	400-700
1.4	Distance from light	cm	150 – Lamps HPS* 40 - Lamps LED*	150 – Lamps HPS 40 - Lamps LED	150 – Lamps HPS 40 - Lamps LED
2	Ambient air temperature	°C	21±2 Day/17 Night	20±2	24 Day/18 Night ± 2
3	Relative humidity	%	65±5 (50-60)	80±5	70/80% ± 5
4	Nutrient concentration	N-P-K : 3-2-3 (%)	changed every 10 days**	changed every 10 days**	changed every 10 days**
5	pH	pH units	6.8±0.4	6.3±0.4	6.0 ± 0.2
6	Electrical conductivity	mS	1.2±0.2	1.8±0.2	1.8±0.2
7	Solution temperature	°C	20±2	18±2	20±2

Note: *HPS-High Pressure Sodium; LED-Light emiting diodes. **8 o'clock in the morning; *** monitor daily at 8 o'clock in the morning in 3 repetitions;

Table 6.2 presents measurement units, methods and possible equipment to be used complementary to GoHydro platform equipment (for tests performed before receiving the sensor kit set).

Table 6.2: Recommended measurement methods and equipment				
No.	Parameter	Unit of measurement	Methods	Equipment for measuring (example)
1	Light	W	HPS/LED	Parameter specific
1.1	Photoperiodicity	h	Soft setting	Clock
1.2	Light intensity	$\mu molm^{-2}s^{-1}$	Number of photons	Digital device (Luxmeter, spectroradiometer)
1.3	Color spectrum	nm	Light spectrum	Spectrometer
1.4	Distance from light	cm	Adjustment	Ruler
2	Ambient temperature	°C	Temperature sensor	Temperature sensor
3	Humidity	%	Relative humidity	Hygrometer sensor
4	Nutrient	N-P-K : 3-2-3 (%)	Type of solution	Standard
5	pH	pH units	Solution reaction	Laboratory pH meter
6	Electrical conductivity	mS	Electrical conductivity in water	Digital electrical conductivity measurement water conductivity sensor
7	Solution temperature	°C	Temperature sensor	TMCx-HD Water Temperature Sensor

The vegetation chamber is controlled by a system operated through a software program. The environmental factors (temperature, humidity, light) are controlled and monitored throughout the entire experimental period for the controlled experiments; for the operational experiments, they will only be monitored, not controlled. Thus, for example, in the case of basil, the environment factors from the vegetation chamber shall be set as follows: temperature 21±2 Day/17 Night; humidity 65±5%, additional light by lamps of 400W, photoperiodism: 06:30-21:30 (15h), automatic airing at ±2°C, compared to the programmed temperature.

a(6.2). Weather conditions in the area/ testing period (Table 6.3), in operational environments settings (Greece, Denmark and Romania). In order to determine if the weather conditions in the area/ testing period have an influence on the operation of the GoHydro platform, important atmospheric data will be recorded daily (Jans-Singh et al., 2019). The growing medium plays a very important role in determining the microgreens' yield and quality (Di Gioia et al., 2016), and the sustainability of the production process.

Table 6.3: Weather conditions in the area/period testing									
Country	Species	Parameters	Day 1	Day 2	Day 3	...	...	.....	Day n
Greece_SCiO	Basil	Minimum temperature, °C							
		Maximum temperature, °C							
		Mean temperature, °C							
		Minimum atmospheric humidity, %							
		Maximum atmospheric humidity, %							
		Mean atmospheric humidity, %							
	Brussels sprouts	Minimum temperature, °C							
		Maximum temperature, °C							
		Medium temperature, °C							

		Minimum atmospheric humidity, %							
		Maximum atmospheric humidity, %							
		Mean atmospheric humidity, %							
Denmark _UCPH	Basil	Minimum temperature, °C							
		Maximum temperature, °C							
		Medium temperature, °C							
		Minimum atmospheric humidity, %							
		Maximum atmospheric humidity, %							
		Mean atmospheric humidity, %							
	XXX	Minimum temperature, °C							
		Maximum temperature, °C							
		Medium temperature, °C							
		Minimum atmospheric humidity, %							
		Maximum atmospheric humidity, %							
		Mean atmospheric humidity, %							
Romania_ USAMV	Basil	Minimum temperature, °C							
		Maximum temperature, °C							
		Medium temperature, °C							
		Minimum atmospheric humidity, %							
		Maximum atmospheric humidity, %							
		Mean atmospheric humidity, %							
	Lettuce	Minimum temperature, °C							
		Maximum temperature, °C							
		Medium temperature, °C							
		Minimum atmospheric humidity, %							
		Maximum atmospheric humidity, %							
		Mean atmospheric humidity, %							

b. Parameters related to the growth environment (Table 6.4) – environmental conditions and nutrient solution:

- **Data collected using the GoHydro platform and the sensor kit**:
  - Crop Heath Sensor Kit (CHSK)
  - Nutrient Content Kit (NCK)

GoHydro hydroponic platform have specific characteristics, like the layer of crop used – nutrient solution, type of irrigation – closed, method of irrigation – immersing, irrigation level – root level. Plants are cultivated in a substrate membrane, over which nutrient solution passes periodicially.

The high-capacity tank helps to maintain a constant pH. The colour of the tank is white on the outside to maintain a constant temperature of the nutrient solution, and it is not affected by solar radiation. The water pump is in the tank, and the nutrient solution reaches the surface of containers through a pipe system. The pump recirculates the whole solution within 30 minutes of a fertilization regime, and the result is the mixing of the solution in the system.

Microgreens can germinate and grow without any fertilizer application, up to the capacity of the specific seed's capacity. However, providing mineral nutrients to microgreens will increase yields and secondary metabolite concentration.

Deliverable 2.1 shows that the microgreen GOHydro hydroponic installations contain "Multi-modal Sensor Kits (MMSK)", components that will be responsible for data collection and data communication; it is upon these data that the AI-based platform of the e-agronomist is going to be built around.

Table 6.4: Parameters collected related to the growth environment

No.	Component / Parameter	Model (Deliverable 2.1)	Unit of measurement*	Greece_SciO		Denmark_UCPH		Romania_USAMV	
				Basil Day 1-n	Brussels sprouts Day 1-n	Basil Day 1-n	xxx Day 1-n	Basil Day 1-n	Lettuce Day 1-n
A	**Monitoring the environmental conditions – by Crop Heath Sensor Kit (CHSK)**								
1	Air Temperature Sensor**	Adafruit Si7021 Temperature & Humidity Sensor Breakout Board	°C						
2	Air Humidity Sensor**	Adafruit Si7021 Temperature & Humidity Sensor Breakout Board	%						
3	Light Sensor (UV)	SI1145 Digital UV Index / IR / Visible Light Sensor	µmolm⁻²s⁻¹						
B	**Monitoring the water and nutrient quality – by Crop Heath Sensor Kit (CHSK) and Nutrient Content Kit (NCK)**								
4	Water Temperature Sensor**	Temperature Sensor – Waterproof (LM35)	°C						
5	Digital pH Meter	Liquid PH Value Detection Sensor Module	pH units						
6	Electroconductivity Sensor	Analog TDS Sensor Water Conductivity Sensor	mS						
7	Nutrient Content Kit (NCK)	Spectrometer, Cartridges	Nutrient content						

Note: *The collection interval depends on the setting of the GoHydro platform; For data comparison it is very important that the GoHydro hydroponic platforms are identical; **Air and water temp and RH should be measured min, max, and mean.

The nutrient solution shall be changed every 10 days (at 8 o'clock in the morning) in order to provide the need of macro- and micro-elements. After each change, the systems and all the devices used shall be disinfected. The nutrient solution shall be monitored daily and manipulated in order to be maintained at the best parameters for the development of plants. The level of the nutrient solution must be kept constant.

Measurements of the oxygen dissolved into water shall be made daily. These measurements shall record the quantity of oxygen dissolved into water, the temperature of the solution, the date, time and temperature from the atmosphere.

Artificial lighting shall be measured on all the experimental surface, in different points, both from the point of view of intensity, and from the point of view of the quality of light. Light intensity shall be measured with a digital device which determines the number of photons relatively to surface and time ($\mu mol\ m^{-2}\ s^{-1}$). The light spectrum shall be determined by using a spectrometer. These measurements shall be made above each tray and at differences of 10, 20, 30 and 40 cm above their canopy.

c. Activities (procedures, parameters) related to microgreen growth, production and quality:

Obtaining the microgreens seedling

In the case of hydroponic crops, the production of the seedling is essential in order to obtain uniform and quality microgreens.

To ensure the possibility of comparing data, the same variety will be used in Greece, Denmark and Romania (in the case of basil). Varieties with rapid seed germination and not requiring low temperature treatments to stimulate it are preferred (Lettuce may require precooling). This is preferable so as not to have an additional factor influencing the results.

The seeds are in seminal rest until the best medium allows for germination. The cultivation of microgreens requires an ample supply of neutral to slightly acidic water (Turner et al., 2020). Seeds of some varieties are soaked overnight to enhance germination.

The crop substrates, humidity, temperature and light regime have the most important role for the seed germination. The germination of microgreens seeds will be done in darkness at 20-24°C (depending on the species) and 100% relative humidity. For basil seed germination occurred in a climatic chamber in the dark at 24 °C for 3 days (Bulgari et al., 2017). After approximately 3 days, the plants are exposed to light and watered daily until the first set of true leaves begin to emerge.

Three distinctive phases are undergone in the process of seed germination (Giurgiu, 2016):

- water soaking,
- reinitiating metabolic activities from the seed,
- appearance of the radicle and its elongation.

Substrates. Among common substrates used for the microgreens production, peat-based media are the most utilized, followed by coconut coir and several synthetic media (Bulgari et al., 2021). Recently, natural fiber-based media - such as jute, cotton, cellulose, etc. - have gained increasing popularity since they could represent a sustainable alternative (Kyriacou et al., 2020).

The seeds are placed directly in the sterile sublayer. The sterile substrate, jute felt for microgreen production (or another substrate) must be identical in all 3 GoHydro platforms (the main characteristics must be known: air porosity, water-retention capacity, etc.). Bulgari et al., 2021, investigate the influence of three growing media (vermiculite, coconut fiber, and jute fabric) on yield and quality parameters of two basil varieties (Green basil – *Ocimum basilicum* L., Red basil – *Ocimum basilicum* var. *Purpurecsens*) and rocket (*Eruca sativa* Mill.) as microgreens. The results showed that the choice of the substrate significantly affected the yield, the dry matter percentage, and the nitrate concentration of microgreens, while the other qualitative parameters were most influenced by the species.

Seeds may require sterilization. Seed contamination is a well-known problem in the microgreens industry. If seeds are contaminated, pathogens can become internalized from the beginning of the growing process and once incorporated are very difficult to remove (Wang and Kniel, 2016). During seed germination, the seed releases a mixture of carbohydrates and peptides that can attract surrounding bacteria in the rhizosphere (Turner et al., 2020). Access to inner apoplastic space is restricted by protective border cells on the root surface. However, bacteria can enter via germinating radicals or secondary roots and can persist in localized sites (Warriner et al., 2003). In mature plants, bacteria localized in apoplastic fluid surrounding root cells cannot enter the xylem because of the Casparian strip: a thickened cell wall containing the water-insoluble substance, suberin. However, in immature plants protective structures are not fully formed, enabling entry of bacteria into xylem (Warriner et al., 2003). It is recommended that the saturation with oxygen of the nutrient solution be maintained above 6.5 mg L-1, in order to eliminate the risk of the appearance of pathogens and for an optimal development of the root system (Giurgiu, 2016).

Recently, studies have demonstrated that microgreen growing systems, especially hydroponic systems, are vulnerable to pathogen proliferation when seeds are contaminated, highlighting the importance of seed sanitation (Turner et al., 2020):

- Xiao et al. (2015) showed that E. coli O157:H7 were able to survive and proliferate significantly on radish microgreens in both soil-substitute and hydroponic production systems, with higher populations reported in the hydroponic production system.
- Di Gioia et al. (2016) reported lower microbial populations in recycled fiber mats and on microgreens growing on them than in peat-based mixes and microgreens grown in pure peat. They suggested that recycled fiber mats may be safer growth media than peat.
- Reed et al. (2018) reported that the type of growth medium played an important role in serovar-dependant Salmonella survival and growth on microgreens irrigated with contaminated water. Of the different growth media tested, hydroponic pads resulted in the highest percentage of Salmonella-positive samples and the highest Salmonella population level on microgreens.
- Wang et al. (2015) examined the survival and proliferation of seed-borne Listeria monocytogenes and other members of the seeds microbiota on microgreen plants grown in soil substitute and hydroponic production systems. During microgreen growth for 10 days, Listeria monocytogenes counts on the seed coats increased by 0.7 and 1.3 log, respectively, for soil and hydroponic systems. Similar increases were observed on the edible portion of the microgreens. Seed coats, roots, and cotyledons were most heavily contaminated.
- Wang and Kniel (2016) evaluated the capability of the human norovirus surrogate, murine norovirus (MNV), to internalize from roots to edible tissues of kale and mustard microgreens, as well as virus survival in recirculated water without disinfection. They found constant high levels of viral RNA in edible tissues. MNV remained infectious in previously contaminated hydroponic systems for up to 12 days and was translocated in edible tissues via roots. Examination of the spatial distribution of bacterial cells on different parts of microgreen plants showed that contaminated seeds led to systematic contamination of whole plants, including both aerial parts and roots.
- Kim et al. (2016) reported that there is a potential for LED light in the UV and blue ranges to enhance food safety of hydroponically grown microgreens by treating the water as it circulates. Light in blue and UV wavelengths is able to kill bacteria.

Sanitization of harvested product is not likely to be an effective control strategy (Turner et al., 2020). Once contaminated, it is almost impossible to eliminate pathogens from living plant tissues. Microgreens are very delicate and can be easily damaged by harsh sanitizing treatments.

Seeds should receive precautionary sanitary treatments for eliminating pathogenic bacteria such as those recommended for sprouts production by the U.S. Food and Drug Administration (Kyriacou et al., 2016). Tavan et al., 2021, proposes that Tuscan black kale (*Brassica oleracea var. acephala*) seeds be first sterilized by soaking for 2 min in 80% ethanol, rinsed twice with distilled water, and then oven-dried at 45°C for 40 min.

A textile material will be placed over the seeds to stop the light. After moistening and the beginning of the germination process (3 days), the textile material shall be removed for growing seedlings (Puccinelli et al., 2022).

Bulgari et al., 2017, recommend in the case of hydroponic cultivated basil, on pepolystyrene cell trays filled with vermiculite, a crop density of approximately 21,700 plants m^{-2} (about eight plants per cell).

To determine the density, depending on the species of microgreens, the amount of seeds can be calculated according to the size and shape of the trays using Microgreens Seed Density Calculator (Created by Francesco Di Gioia): https://extension.psu.edu/microgreens-seed-density-calculator.

Seeding density impacts microgreens yield; as the seeding density increases, the weight per individual plant decreases due to competition among seedlings, while the total yield increases from the increased number of seedlings in each area, up to an equilibrium production capacity.

Measurements on the morphology of plants (Table 6.5)

The determination is performed in the juvenile vegetative phase before harvesting the microgreens. The surface of the leaves (leaves area) will be determined with a planimeter on 10 plants per tray (Bulgari et al., 2017). 10 plants representative for each tray of the GoHydro platform will be harvested on the diagonals of the tray. Another possibility is to calculate the Leaf Area Index (LAI) by employing the formula of Fang et al., 2019.

$$\text{LAI} = \frac{Leaf\ area\ per\ plant\ (cm^2)}{Land\ area\ occupied\ by\ a\ plant\ (cm^2)}$$

Table 6.5: Measurements on the morphology of plants

Country	Species	Repetition	Stem length	Internode length	Number of leaves	Leaves area
Greece_SciO	Basil	1				
		2				
		3				
	Brussels sprouts	1				
		2				
		3				
Denmark_UCPH	Basil	1				
		2				
		3				
	XXX	1				
		2				
		3				
Romania_USAMV	Basil	1				
		2				
		3				
	Lettuce	1				
		2				
		3				

Determining the health state of plants (Table 6.6)

Due to the short crop time for microgreens, there are few severe pests or physiological disorders.

The most significant disease in microgreens production is damping off of recently germinated seedlings. Seed can be sterilized prior to planting to minimize disease incidences.

Determining the health state of plants shall be made through continuous monitoring and all the symptoms appeared shall be noted. The health state of plants shall be noted in ascending order with grades from 1 to 9, with the maximum grade corresponding to a perfect health state. The results shall be presented as average values of repetitions, graphically represented compared to time.

Table 6.6: Determining the health state of plants

Country	Species	Repetition	Day 1	Day 2	…..	……	Day n
Greece_SciO	Basil	1					
		2					
		3					

	Brussels sprouts	1					
		2					
		3					
Denmark_UCPH	Basil	1					
		2					
		3					
	XXX	1					
		2					
		3					
Romania_USAMV	Basil	1					
		2					
		3					
	Lettuce	1					
		2					
		3					

We consider it appropriate to assess the average intensity of the disease attack using the FAO grades (using grades 1 to 9; ISTIS, 2008):

Note 1 – if the attack is not observed
Note 2 – when the attack is incipient, with less obvious symptoms
Note 3 – if the stains occupy up to 5% of the surface
Note 4 – when the stains cover between 5-15% of the surface
Note 5 – when the stains cover between 15-25% of the surface
Note 6 – when the stains cover between 25-40% of the surface
Note 7 – when the stains cover between 40-50% of the surface
Note 8 – when the stains cover between 50-75% of the surface
Note 9 – when the stains cover between 75-100% of the surface

Next we can calculate the degree of attack that represents the expression of the influence and severity of microgreens health.

The degree of attack (DA, %) is calculated according to the relation (ISTIS, 2008):

DA, % = F, % x I, % / 100
Where:
F, % - attack frequency of a phytopathogen
F, % = N x 100 / Nt
N = number of plants (organs) attacked
Nt = total number of plants (organs) observed (controlled)

I, % - attack intensity of a phytopathogen
I, % = Σ (i x f) / n
i = percentage of grade awarded
f = number of plants (organs) marked with the respective note
n = total number of attacked plants (organs) analyzed

Microgreens Harvesting

Microgreens are ready for harvest when they reach the first true leaf stage, usually at about 2 inci (5.08 cm) tall (Treadwell et al., 2020). Recommended maximum height limit of 6 cm (Senevirathne et al., 2019). Time from seeding to harvest can vary greatly by crop from 7 to 21 days (Treadwell et al., 2020), typically around 14 days (Fig. 6.1; Riggio et al., 2019). Use of seedling height as a harvesting index can be recommended, as it can be determined easily (Senevirathne et al., 2019). However, leaf area can also be used as a harvesting stage index (Senevirathne et al., 2019). As different parts of the plants such as seeds, cotyledons and leaves may have different health promoting properties, the ideal time of consumption to benefit by their phytochemicals vary, which shows the importance of determining antioxidant activity at different stages.

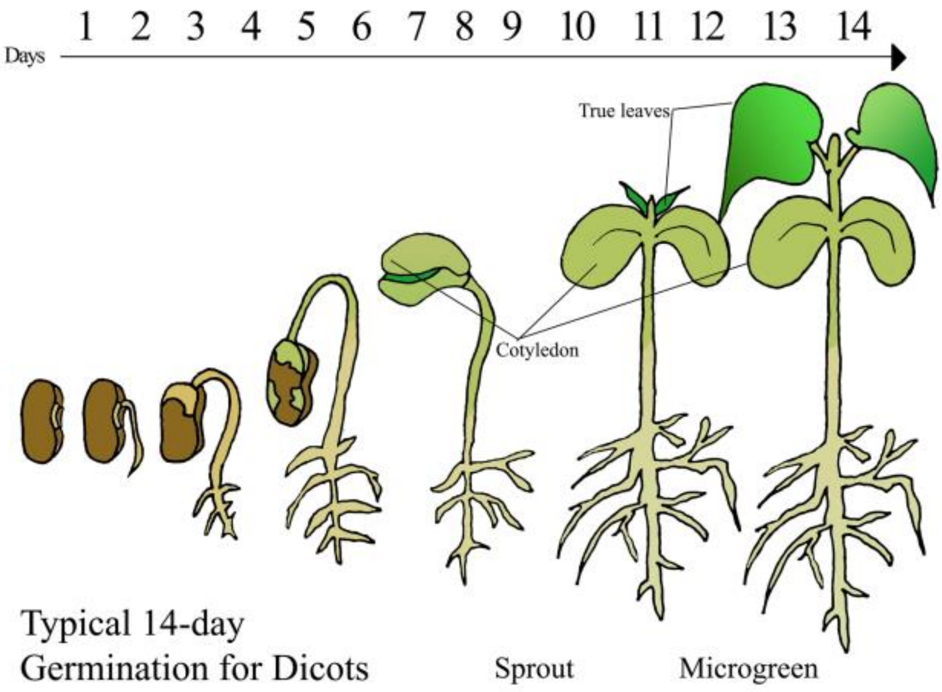

Figure 6.1: Microgreens and sprouts differ by age at harvest

No formal studies in the literature were found about harvest age effects on shelf life of microgreens.

Production in small trays will likely require harvesting with scissors.

The majority of vegetable varieties grown as microgreens are ready for harvest in about 2 weeks. Pannico et al., 2020, proposes for lettuce microgreens harvesting at 16 days after sowing, upon the appearance of the first two true leaves.

They are weighed to determine the fresh vegetable mass, then they are dried (lyophilization) in the oven at 65°C during 3 days, or 4 days (Puccinelli et al., 2022), to constant weight (Toscano et al., 2021). Lyophilization is considered to be the best dehydration method for both storage and sample pre-treatment, since it does not cause thermal degradation of carotenoids (Saini and Keum, 2018). Drying the vegetable material is an important process when it comes to a correct characterization of plants and the active substances accumulated by them.

Time of the day for harvesting may have significant implications for the bioactive composition (Hasperue et al., 2016) and shelf-life of microgreens (Garrido et al., 2015). We propose that the microgreens be harvested at 7 o'clock in the morning, so as to avoid exposure to light, opening of the stomata and possible tendencies to lose weight of the preserved samples (Noichinda et al., 2007).

Careful harvesting is required and quick cooling to remove vital heat and suppress the rate of respiration, spoilage and senescence (Kyriacou et al., 2016). Samples collected from the 3 trays of the GoHydro platform will be stored at –20◦C until analyzed (Paradiso et al., 2020) if necessary.

Current dip/wash and drying procedures significantly reduce the quality of the microgreens since microgreens are very delicate. Improved wash/drying technologies are necessary to provide ready-to-eat microgreens with better quality and longer shelf life (Kou et al., 2015). The postharvest wash step can be avoided when the microgreens are grown under controlled settings to minimize the microbial contamination. Microgreens crops usually are grown indoors. Thus, the materials used for propagation can be easily decontaminated to maintain compliance with food safety regulations.

Fresh Biomass Yield – Fresh Weight (FW, kg m^{-2}) (Table 6.7)

Fresh Biomass Yield – Fresh Weight (FW, kg m^{-2}). All the microgreens within each tray wil be cut right above the substrate level (cutting them at the base, excluding the substrate) and collected to determine FW (Bulgari et al., 2021).

Dry Matter Content – Dry Weight (DW, g m^{-2}) (Table 6.7)

Dry Matter Content – Dry Weight (DW, g m^{-2}) will be measured on an analytical balance following lyophilization until a constant weight was reached. Each sample shall be dried in an oven at 70°C during 3 days (Tavan et al., 2021) until constant weight is reached and DW shall be recorded (at 75°C, 4 days; Bulgari et al., 2021) . The dry samples will be finely ground to be utilized for chemical analysis.

Table 6.7: Determining Fresh Biomass Yield and Dry Matter Content

Country	Species	Repetition*	Fresh microgreens	65°C, 3 days
			Fresh Weight (FW), kg m^{-2}	Dry Weight (DW) g m^{-2}
Greece_SciO	Basil	1 2 3		
	Brussels sprouts	1 2 3		
Denmark_UCPH	Basil	1 2 3		
	xxx	1 2 3		
Romania_USAMV	Basil	1 2 3		
	Lettuce	1 2 3		
		Total / species		
		Average / species		

Water Use Efficiency (WUE) – can be an important indicator of the efficiency of the GoHydro platform. if necessary it can be calculated based on total harvested biomass (Tavan et al., 2021):

WUE = TFW / ΣW

Where:

TFW – total harvested biomass, g

ΣW - total water added to each growing container of GoHydro platform

Bioactive compound analysis (Table 6.8)

The most important bioactive compounds in microgreens include vitamins (vitamin C), minerals (copper – Cu, zinc – Zn, and selenium – Se), and phytochemicals (e.g., carotenoids and phenolics) (Zhang et al., 2021).

Vitamin C, also known as ascorbic acid, is a potent antioxidant and is essential for a variety of biological functions, such as wound healing, collagen synthesis, and immune system regulation. As microgreens are usually consumed fresh, Vitamin C can be largely retained without cooking (Yadav et al., 2019). For the total ascorbic acid (TAA, g kg^{-1}) analysis, an UV-Vis spectrophotometer will be used.

Several trace minerals, i.e., Cu, Zn and Se, as cofactors or components of antioxidant enzyme, play an essential role in the endogenous antioxidant defense system of human body, and are therefore referred to as antioxidant minerals (Wołonciej et al., 2016). The content of chemical elements (Ca, Mg, Na, K, Mn, Fe, Zn, Cu, Se) (g kg^{-1}) and volatile oils (mg L^{-1}), shall be determined by specific HPLC methodology (chromatographic).

Phytochemicals (e.g., carotenoids and phenolics) are found in significant amounts in microgreens. Carotenoids possess antioxidant activity and play important physiological roles in human body (Rodriguez-Amaya, 2015). Phenolic compounds are the most abundant secondary metabolites of plants ranging from small molecules, e.g., phenolic acids, to flavonoids with multiple rings, and to highly polymerized compounds, e.g., tannins (Dai and Mumper, 2010). Phenolics are antioxidants for plants to repair damage caused by free radicals and have shown many health benefits for human (Dai and Mumper, 2010).

Bioactive compound: carotenoids (μg mL^{-1}) and total polyphenols (μg mL^{-1}) will be analyzed by HPLC methodology.

Table 6.8: Bioactive compound analysis

Country	Species	Repetition	Vitamin C (ascorbic acid) (TAA, g kg^{-1})	Vitamin content in general (g kg^{-1})	Carotenoids (μg mL^{-1})	Total phenols (μg mL^{-1})	Glucosinolates / Anthocyanins	Macro and micronutrient content (NPK, Cu, Zn, etc.)
Greece_SciO	Basil	1						
		2						
		3						
	Brussels sprouts	1						
		2						
		3						
Denmark_UCPH	Basil	1						
		2						
		3						
	XXX	1						
		2						
		3						

Romania_USAMV	Basil	1						
		2						
		3						
	Lettuce	1						
		2						
		3						

Statistical analysis

All data will be analyzed for differences using SPSS software and will be presented as average ± SE (standard error). Average values must be separated by LSD test ($p < 0.05$).

The data will be analyzed in combination and compared with each other to obtain significance and establish optimal environmental conditions, which must be provided by the GoHydro platform.

Data collection will be done in 3 repetitions (representing the 3 trays of the GoHydro platform).

This ensures the accuracy of the data, real feedback, and the possibility to improve the accuracy of the GoHydro platform.

7 CONCLUSIONS

One of the major limitations of the expansion of microgreen consumption is the rapid deterioration of their quality, which occurs immediately after harvest, thus limiting their marketing. From this point of view, the GoHydro platform represents an optimal cultivation solution, microgreens being very suitable for hydroponic production on different substrates, indoors, representing a sustainable alternative to conventional agriculture.

Many studies have shown that the variation in the content of bioactive compounds in microgreens is based on several factors, such as genetic material (species), cultivation conditions and light parameters (spectral quality and intensity), but also other variables (including nutrition / biofortification and growth medium) have implications for shaping the nutritional and phytochemical composition of microgreens. Despite the short growing cycle, special attention should be paid in the testing protocol to establishing growth media for microgreens, which is one of the most important factors in the production process that influences the quality of microgreens and highlights the characteristics of the GoHydro platform.

The determination plan comprises in trial protocols for microgreens production in GoHydro hydroponic units are:

a. IN CONTROLLED SETTINGS - Setting the optimal ranges (between the limits of favorability for each species) for microgreens to highlight the effects of the GoHydro platform, based on D1.1 and D1.2 inputs. IN OPERATIONAL ENVIRONMENTS SETTINGS – monitoring, but not controlling, environmental conditions.

b. Collecting data using the GoHydro platform and the sensor kit: Crop Heath Sensor Kit (CHSK) and the Nutrient Content Kit (NCK) are:

1. Air temperature
2. Water temperature
3. pH
4. Electrical Conductivity
5. Relative Humidity
6. Light
7. Nutrient content

c. Activities (procedures, parameters) related to microgreen growth, production and quality:

1. Obtaining the microgreens seedling
2. Measuring the morphology of plants (LAI or height)
3. Determining the health state of plants
4. Harvesting microgreens
5. Measuring Fresh Biomass Yield – Fresh Weight (FW, kg m^{-2})
6. Measuring Dry Matter Content - Dry Weight (DW, g m^{-2})
7. Secondary metabolite collection and analysis
8. Statistical analysis

Feedback from these trials will be used for the final validation of the analytics components of the GoHydro platform.

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