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Scriptwriter Name: Poornima G

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Title: Building and Operating a Low-Cost Elevated Carbon Dioxide Growth Chamber to Evaluate Microgreen Physiology under Spaceflight-Relevant CO₂ Levels

Authors and Affiliations:

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Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**

- 3. Filming location:** Will the filming need to take place in multiple locations? **Yes**
If **Yes**, how far apart are the locations? **3.2 miles**

- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No.**

Current Protocol Length

Number of Steps: 24

Number of Shots: 54 (6 SC)

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Aurora Toennisson:** We are interested in how extreme carbon dioxide levels at 3000 ppm affect plant growth and nutrition.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Aurora Toennisson:** We wanted to develop a low-cost extreme carbon dioxide chamber because currently few growth chambers can maintain extreme carbon dioxide concentrations.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.3. **Aurora Toennisson:** Our method could be used by researchers interested in space biology, microgreen physiology, or plant response to elevated carbon dioxide.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Aurora Toennisson:** Attention to sterility and sanitation is critical while performing this protocol to prevent diseases. The chambers should be checked regularly to ensure proper functioning.

- 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Aurora Toennisson**: These chambers could be used to investigate how a range of carbon dioxide concentrations affect the physiology of other small plants.
- 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Preparation of Media and Materials for the Experiment

Demonstrator: Aurora Toennisson

- 2.1. To begin, install and turn on the equipment required for the procedure [1]. Wipe all non-electronic surfaces of the chamber, including the walls, ceiling, and door, with SA-20 disinfectant prepared at 7.8 milliliters per liter of water [1]. Leave the chamber door open and allow the chamber to air dry overnight [2].
 - 2.1.1. WIDE: Talent showing the installed equipment and turning them on.
 - 2.1.2. Talent wiping the interior walls, ceiling, and door of the chamber with disinfectants.
 - 2.1.3. Talent leaving the chamber door open to allow the interior to air dry.
- 2.2. Prepare 0.25x MS basal salt media without agar by dissolving 4.3 grams of MS salts and 1 gram of 2-N-morpholino ethane-sulfonic acid in 1 liter of deionized water [1-TXT]. Use 1 Molar potassium hydroxide to adjust the media pH to 5.7 [2]. Dilute the solution to a final volume of 4 liters [3] and dispense 700 milliliter aliquots into 1-liter bottles [4].
 - 2.2.1. Talent adding MS salts and 2-(N-morpholino)ethanesulfonic acid to a container of 1 lit deionized water. **TXT: MS medium: Murashige and Skoog medium**
Added shot: Talent adding liquid to the MS media.
 - 2.2.2. Talent adding deionized water to bring the solution to a final volume to 2 liters in 2 flasks.
 - 2.2.3. Talent dispensing 700 milliliters of the prepared media into 1 liter bottles.
- 2.3. Then, fill autoclavable bottles with distilled or deionized water for seed sterilization and rinsing [1]. Autoclave the MS media and the prepared water using a liquid cycle for 30 minutes [2].
 - 2.3.1. Talent filling autoclavable bottles with distilled or deionized water.
 - 2.3.2. Talent placing bottles of MS media and water into the autoclave.

2.4. Wrap the hydroponic boxes, tools, and containers in aluminum foil [1]. Autoclave the wrapped materials on a dry cycle with a 30-minute dry time [2]. After autoclaving, keep the materials in a laminar flow hood [3].

2.4.1. Talent wrapping hydroponic boxes, tools, and containers in aluminum foil.

2.4.2. Talent placing the wrapped materials into the autoclave.

2.4.3. Talent placing the still-wrapped sterilized materials inside a laminar flow hood.

3. Radish Seed Sterilization and Addition to the Medium

3.1. Inside the hood, add radish seeds to a 2 percent bleach solution containing 0.05 percent Triton X-100 [1] and shake the seed suspension gently for 5 minutes at room temperature [2].

3.1.1. Talent adding seeds into a container holding the bleach and Triton X-100 solution inside the laminar flow hood.

3.1.2. Talent gently shaking the container holding the seeds in solution.

3.2. Decant the bleach solution from the seeds [1] and rinse the seeds three times with sterile water [2]. After rinsing, soak the seeds overnight in sterile water to promote uniform germination [3].

3.2.1. Talent decanting the bleach solution from the seed container.

3.2.2. Talent adding water and rinsing the seeds with sterile water.

3.2.3. Talent placing the seeds in sterile water for soaking.

3.3. Next, fill each hydroponic box with sterile 0.25x MS medium until the felt surface is covered with approximately 1 to 2 millimeters of liquid [1]. Verify that no air gap exists between the felt and the liquid [2].

3.3.1. Talent pouring sterile 0.25 times MS medium into a hydroponic box containing the felt insert.

3.3.2. Shot of pointing to the interface between the felt and the liquid surface inside the hydroponic box.

- 3.4. Using sterile forceps, place up to 30 radish seeds onto the felt in each hydroponic box [1-TXT]. Press the seeds gently into the felt with the forceps [2] and ensure that the seeds are centered above the tip holes on the insert [3].
 - 3.4.1. Talent placing seeds onto the felt surface of the hydroponic box using sterile forceps. **TXT: Place 60 seeds if using Amaranth**
 - 3.4.2. Talent pressing the seeds gently into the felt with forceps.
 - 3.4.3. Close-up of seeds positioned on the felt directly above the tip holes of the insert.
- 3.5. Cover each planted hydroponic box with cling wrap or sterile lids to maintain humidity during germination [1].
 - 3.5.1. Talent covering the hydroponic box with cling wrap or placing a sterile lid on top.

4. Running the Microgreen Experiment

- 4.1. Set the primary carbon dioxide regulator to approximately 10 pounds per square inch [1]. Now, slowly open the main valve on the carbon dioxide tank [2]. Adjust the secondary step-down regulator between 1 and 3 pounds per square inch [3-TXT].
 - 4.1.1. Talent adjusting the primary carbon dioxide regulator on the gas tank.
 - 4.1.3., Talent slowly opening the main valve on the carbon dioxide tank. **NOTE: This was shot with 4.1.1 and should come right after 4.1.1. VO is altered**
 - 4.1.2. Talent adjusting the secondary step-down regulator to the specified pressure.
TXT: CAUTION: Air pressures above 5 psi will damage the solenoids!
- 4.2. Turn on the benchtop incubator chambers [1] and set the internal temperature to 23 degrees Celsius [2].
 - 4.2.1. Talent switching on the benchtop incubator chamber.
 - 4.2.2. Talent adjusting the chamber temperature control to 23 degrees Celsius.
- 4.3. Verify that the internal fan is functioning and ensure that the fan is unobstructed to maintain proper air circulation [1].
 - 4.3.1. Shot of the internal chamber fan operating.

- 4.4. Now, open the Control_chamber_JoVE.py (*control chamber Jove dot pie*) code in Thonny [1]. Confirm the carbon dioxide and light settings [2]. Run the program [3] and then, verify that the solenoids, sensors, and lights respond properly [4].
 - 4.4.1. SCREEN: 70050_screenshot1.mp4. 00:03 – 00:13
 - 4.4.2. SCREEN: 70050_screenshot1.mp4. 00:14-00:24
 - 4.4.3. SCREEN: 70050_screenshot1.mp4. 00:24-00:42
 - 4.4.4. Shot of the chamber system showing lights activating and indicators responding.
- 4.5. Once the environment is stable, place the covered hydroponic boxes inside each chamber [1]. Close the chamber door [2] and confirm that the carbon dioxide concentration returns to the set point within 3 to 5 minutes [3].
 - 4.5.1. Talent placing covered hydroponic boxes into the incubator chamber.
 - 4.5.2. Talent closing the chamber door.
 - 4.5.3. SCREEN: 70050_screenshot4.mp4 00:02 – 00:17
- 4.6. Cover the chamber door panels with aluminum foil or blackout film to prevent light interference [1]. Flip open the cover to check the plants daily [2] and re-adhere the cover with tape after inspection [3].
 - 4.6.1. Talent covering the chamber door panel with aluminum foil or blackout film.
 - 4.6.2. Talent lifting the foil cover on the chamber door to observe the plants inside.
 - 4.6.3. Talent taping the foil or blackout cover back onto the chamber door.
- 4.7. Monitor the chamber readings one to three times daily using Real-VNC or locally through a high-definition multimedia interface display [1].
 - 4.7.1. Display the chamber monitoring interface accessed through RealVNC or an HDMI-connected monitor showing environmental readings.
- 4.8. Allow the seeds to germinate with the humidity covers in place for 3 to 4 days until cotyledon emergence is visible [1]. Remove the humidity covers once most seedling roots have penetrated the felt substrate [2].
 - 4.8.1. Close-up of seedlings in the hydroponic box with humidity covers in place.

4.8.2. Talent removing the humidity covers from the hydroponic boxes after root penetration through the felt substrate. **Authors: Please prepare these samples beforehand**

4.9. Respond promptly to any email alerts generated by the system and record any anomalies or manual interventions [1-TXT].

4.9.1. Talent documenting anomalies or manual interventions in a laboratory record while looking at the chamber or software interface. **TXT: Continue the incubation until the plant growth reaches the desired stage**

5. Plant Harvest, Data Collection and Chamber Shutdown

5.1. At the end of the growth cycle, open the chamber door [1] and remove the plants from the chamber. Process the plants as required for the experimental goals such as biomass, chlorophyll, or nutrient analysis [2-TXT].

5.1.1. Talent opening the chamber door.

5.1.2. Talent removing hydroponic boxes with plants from the chamber and cutting out a few plants. **TXT: 7-day old plants are shown for demonstration purpose**

5.2. Stop the control script [1] and power down the benchtop incubator and lights [2].

5.2.1. SCREEN: 70050_screenshot2.mp4. 00:04 – 00:14

5.2.2. Talent switching off the benchtop incubator and lighting system.

5.3. Rename the chamber_data.csv (*chamber data C-S-V*) environmental data file and copy the renamed file to a flash drive [1].

5.3.1. SCREEN: 70050_screenshot3.mp4. 00:03 – 00:25

5.4. Remove condensate from the chamber interior [1] and wipe the non-electronic interior surfaces with 70 percent ethanol [2].

5.4.1. Talent wiping condensation from the interior surfaces of the chamber.

5.4.2. Talent wiping non-electronic chamber surfaces with ethanol.

5.5. Leave the chamber door open [1] and run the air pump until the chamber and tubing are dry [2].

5.5.1. Talent leaving the chamber door open after cleaning.

5.5.2. Shot of the air pump operating while the chamber remains open.

5.6. Finally, turn off the power strips [1] and close the carbon dioxide tank valve to prevent leakage and conserve gas [2].

5.6.1. Talent switching off the power strip supplying the chamber equipment.

5.6.2. Talent closing the valve on the carbon dioxide tank.

Results

6. Results

6.1. Radish microgreens reached typical development by 10 days after planting [1].

6.1.1. LAB MEDIA: Figure 13A.

6.2. The carbon dioxide regulation system maintained target concentrations near 400 parts per million for the ambient condition and about 3000 parts per million for extreme carbon dioxide condition [1].

6.2.1. LAB MEDIA: Figure 14

6.3. Mean carbon dioxide concentrations during radish experiments were 618 parts per million for the ambient treatment [1] and 3061 parts per million for the extreme condition treatment [2].

6.3.1. LAB MEDIA: Figure 14A. *Video editor: Zoom in on the lower blue line*

6.3.2. LAB MEDIA: Figure 14A. *Video editor: Zoom in on the higher yellow line*

6.4. Carbon dioxide levels in the radish chambers became more variable during the daytime as plant mass increased, requiring frequent injections to maintain concentrations [1].

6.4.1. LAB MEDIA: Figure 14A. *Video editor: Highlight the fluctuating blue and yellow lines in the white background regions between days 3 to 10 on the X-axis.*

6.5. Nighttime plant respiration increased carbon dioxide concentrations in the ambient radish chamber to peaks above 1200 parts per million [1].

6.5.1. LAB MEDIA: Figure 14A. *Video editor: Highlight the blue lines between days 8 to 10 only in the grey shaded regions.*

6.6. Carbon dioxide levels declined to near 400 parts per million shortly after the light period began in the ambient chamber, indicating rapid daytime uptake [1].

6.6.1. LAB MEDIA: Figure 14A. *Video editor: Highlight the blue lines between days 8 to 10 only in the white background regions.*

6.7. Chamber temperatures remained near 25 degrees Celsius during the daytime [1] and close to the 23 degrees Celsius set point during the night in both the conditions [2].

6.7.1. LAB MEDIA: Figure 14B. *Video editor: Highlight the two graph lines (blue and yellow) in white background*

6.7.2. LAB MEDIA: Figure 14B. *Video editor: Highlight the two graph lines (blue and yellow) in grey shaded regions.*

6.8. Relative humidity increased gradually over the course of the radish experiment as plant transpiration increased [1].

6.8.1. LAB MEDIA: Figure 14C. *Video editor: Highlight the upward trend in the humidity lines from the (left) beginning to the end of the experiment towards the right.*

6.9. Air pressure remained constant and nearly identical between the ambient and extreme carbon dioxide treatments throughout the experiment [1].

6.9.1. LAB MEDIA: Figure 14D.

1. Disinfectant

Pronunciation link: <https://www.merriam-webster.com/dictionary/disinfectant>

IPA: /ˌdɪs.ɪnˈfɛk.tənt/

Phonetic Spelling: dis·in·fek·tuht

2. Autoclave

Pronunciation link: <https://www.merriam-webster.com/dictionary/autoclave>

IPA: /ˈɔː.tuː.kleɪv/

Phonetic Spelling: aw·toe·klayv

3. Deionized

Pronunciation link: <https://www.merriam-webster.com/dictionary/deionized>

IPA: /diˈaɪ.ə.naɪzd/

Phonetic Spelling: dee·eye·uh·nyzd

4. Molar

Pronunciation link: <https://www.merriam-webster.com/dictionary/molar>

IPA: /ˈmoʊ.lər/

Phonetic Spelling: moe·ler

5. Potassium

Pronunciation link: <https://www.merriam-webster.com/dictionary/potassium>

IPA: /pəˈtæs.i.əm/

Phonetic Spelling: puh·tas·ee·uhm

6. Hydroxide
Pronunciation link: <https://www.merriam-webster.com/dictionary/hydroxide>
IPA: /haɪˈdrɔːk.saɪd/
Phonetic Spelling: hy·draak·side
7. Aliquots
Pronunciation link: <https://www.merriam-webster.com/dictionary/aliquot>
IPA: /ˈæl.i.kwɑːt/
Phonetic Spelling: al·ih·kwaat
8. Autoclavable
Pronunciation link: <https://www.merriam-webster.com/dictionary/autoclavable>
IPA: /ˌɔː.tuʊˈkleɪ.və.bəl/
Phonetic Spelling: aw·toe·klay·vuh·buhl
9. Laminar
Pronunciation link: <https://www.merriam-webster.com/dictionary/laminar>
IPA: /ˈlæm.ə.nə/
Phonetic Spelling: lam·uh·ner
10. Triton
Pronunciation link: <https://www.merriam-webster.com/dictionary/triton>
IPA: /ˈtraɪ.təːn/
Phonetic Spelling: try·taahn
11. Decant
Pronunciation link: <https://www.merriam-webster.com/dictionary/decant>
IPA: /dɪˈkænt/
Phonetic Spelling: dih·kant
12. Germination
Pronunciation link: <https://www.merriam-webster.com/dictionary/germination>
IPA: /ˌdʒɜː.məˈnei.jən/
Phonetic Spelling: jur·muh·nay·shuhn
13. Hydroponic
Pronunciation link: <https://www.merriam-webster.com/dictionary/hydroponic>
IPA: /ˌhaɪ.drəˈpɑː.nɪk/
Phonetic Spelling: hy·druh·pon·ik
14. Solenoids
Pronunciation link: <https://www.merriam-webster.com/dictionary/solenoid>
IPA: /ˈsoʊ.lə.nɔɪd/
Phonetic Spelling: soh·luh·noyd
15. Incubator
Pronunciation link: <https://www.merriam-webster.com/dictionary/incubator>
IPA: /ˈɪn.kjəˌbeɪ.tə/
Phonetic Spelling: in·kyuh·bay·ter
16. Cotyledon
Pronunciation link: <https://www.merriam-webster.com/dictionary/cotyledon>
IPA: /ˌkɑː.təˈliː.dən/
Phonetic Spelling: kaa·tuh·lee·duhn

17. Substrate

Pronunciation link: <https://www.merriam-webster.com/dictionary/substrate>

IPA: /'sʌb.streɪt/

Phonetic Spelling: sub·strayt

18. Respiration

Pronunciation link: <https://www.merriam-webster.com/dictionary/respiration>

IPA: /,res.pə'reɪ.fən/

Phonetic Spelling: res·puh·ray·shuhn

19. Transpiration

Pronunciation link: <https://www.merriam-webster.com/dictionary/transpiration>

IPA: /,træn.spə'reɪ.fən/

Phonetic Spelling: tran·spuh·ray·shuhn

20. Condensate

Pronunciation link: <https://www.merriam-webster.com/dictionary/condensate>

IPA: /'kɑ:n.den.seɪt/

Phonetic Spelling: kaan·den·sayt