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Title: Assembly and Use of a Plant Gnotobiotic Growth System for Plant–Microbe Interaction Studies in Cereals

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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? **No**

- 3. Filming location:** Will the filming need to take place in multiple locations? **No**

- 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. **Yes**

Current Protocol Length

Number of Steps: 24

Number of Shots: 49

Interviews

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

INTRODUCTION:

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

- 1.1. **Manuel Kleiner**: Our research investigates how microorganisms colonize plant roots and influence plant resilience to stress.
 - 1.1.1. 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.~~

- 1.2. **Manuel Kleiner**: This protocol can be applied to studying plant-microbe interactions in a wide range of plant species, extending beyond traditional small model plants.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Manuel Kleiner**: This protocol allows researchers to study plant-microbe interactions under controlled conditions and establish causality between microbial effectors and plant responses.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

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

- 1.4. **Manuel Kleiner**: The most challenging aspect of this protocol is the careful handling of the watering lines to maintain sterility.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Suggested B roll: 4.4.3*

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

- 1.5. **Manuel Kleiner:** Future studies can combine this system with synthetic microbial communities to identify plant growth–promoting effectors and investigate mechanisms of microbial community assembly.
 - 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.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

~~What is the key takeaway you hope viewers remember after watching your video article?~~

- 1.6. **Manuel Kleiner, Associate Professor, NC State University:** (authors will present their testimonial statements live)
 - 1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~How does the video format of your article help you to share your work with the broader scientific community?~~

- 1.7. **Manuel Kleiner, Associate Professor, NC State University:** (authors will present their testimonial statements live)
 - 1.7.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Protocol

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

- Assemble the gnotobiotic system
- Prepare and sterilize materials and solutions
- Surface-sterilize maize and wheat Seeds

Video Editor: Use a title card to show this information

2. Planting Sterile Seeds in the GNOVA Gnotobiotic System

Demonstrator: Clara Tang

2.1. To begin, take the pots prepared for the experiment [1] and remove the plastic cap from them [2]. Pour 378 milliliters of Murashige and Skoog medium into the pot. For clay and sand substrates, use 360 and 358 milliliters, respectively, to achieve 70% water-filled pore space [3].

2.1.1. WIDE: Talent placing the pots on the working area. ***Videographer:** Please obtain CU shot as well for this shot.*

2.1.2. Talent removing the plastic cap from the pot. **Author's Notes: 2.1.2 and 2.1.3 were combined into a single shot.**

2.1.3. Talent dispensing medium into the pot.

2.2. Using sterile tweezers, plant the sterilized seeds to the correct depth. Sow wheat at a depth of 0.5 inches and maize at a depth of 1 inch [1]. Replace the plastic cap on top of the pot [2] and then remove it [3]. Alternatively, sow sterile pre-germinated seedlings into GNOVA (Nova) [4]. **Notes: VO is added for the additional shot, and the sentence numbers are adjusted to accommodate the added shots.**

2.2.1. Talent sowing wheat or maize at the appropriate depth. **Author's Notes: 2.2.1, 2.3.1 and the added shot were combined in a single shot.**

Videographer's Notes: 2.2.1, 2.3.1, added shot and 2.2.2 were combined in a single shot. Notes: The author's and videographer's notes do not match. Check the footage

2.3.1 Talent replacing the plastic cap on top of the pot. **Notes: This shot has been moved here.**

Added shot: Talent removing the plastic cap from the pot

2.2.2. Talent sowing sterile pre-germinated seedlings into GNOVA.

~~2.3. Replace the plastic cap on top of the pot [1].~~

- 2.3.1. ~~Talent replacing the plastic cap on top of the pot.~~ Notes: Moved this after 2.2.1 as per the author's request.

3. Final Assembly of the Growth System

- 3.1. Transfer the pot and glass cylinder to a growth chamber [1]. Hold the glass cylinder with the bottom facing down close to the pot [2].
- 3.1.1. Talent transferring the pot and glass cylinder to a growth chamber or greenhouse.
- 3.1.2. Talent holding the glass cylinder with the bottom facing down close to the pot.
- 3.2. Hold the sterilization wrap and the plastic cap [1]. In a single movement, remove the plastic cap quickly from the pot and the sterilization wrap from the glass cylinder [2]. Lower the glass cylinder onto the top and seat it on the ledge of the 3D printed base [3]. Notes: The VO has been updated per the revised shot.
- 3.2.1. Talent ~~holding the sterilization wrap and plastic cap with a single hand quickly removing the plastic cap from the pot.~~ Author's Notes: 3.2.1 – 3.2.3 were combined in a single shot.
- 3.2.2. Talent removing the sterilization wrap ~~from the glass cylinder~~ and plastic cap with a single motion.
- 3.2.3. Talent lowering the glass cylinder onto the pot and seating it on the ledge of the 3D printed base.
- 3.3. Grow wheat at 22 degrees Celsius during the day and 16 degrees Celsius at night under fluorescent lights with a 12-hour photoperiod [1]. For maize, maintain 27 degrees Celsius during the day and 23 degrees Celsius at night under the same conditions. [2].
- 3.3.1. Shot of wheat plants growing under fluorescent lights in the growth chamber.
- 3.3.2. Shot of maize plants growing under fluorescent lights in the growth chamber.
- Notes: The additional shot provided by the author has not been added, as it is not required.

4. Irrigation Using a Portable Flame

- 4.1. Attach a long silicon tubing piece to the bottle top dispenser [1].
- 4.1.1. WIDE: Talent attaching a long silicon tubing piece to the bottle top dispenser.
- ~~4.2. Maintain sterility during watering using a portable flame. If a portable flame is unavailable, perform irrigation with 10 percent bleach to maintain sterility [1].~~
- ~~4.2.1. Talent assembling the required material near a portable flame.~~ Author's Notes: Remove it.
- 4.3. Place the flame near the irrigation line [1]. Remove the syringe barrel from the irrigation

line and the filter holder [2]. Keep all tubing ends within the flame zone [3].

4.3.1. Talent placing the flame near the irrigation line.

4.3.2. Talent removing the syringe barrel from the irrigation line and the filter holder.

4.3.3. Talent holding all tubing ends within the flame zone.

4.4. Attach the filter holder to the irrigation line [1]. Connect the two syringe barrels [2]. Then connect the other long piece of silicon tubing to the bottle-top dispenser to the bottom port of the filter holder [3].

4.4.1. Talent attaching the filter holder to the irrigation line.

4.4.2. Talent connecting the two syringe barrels together.

4.4.3. Talent connecting the long silicon tubing piece to the bottom port of the filter holder.

4.5. Dispense 150 milliliters of 0.5x times Murashige and Skoog media [1].

4.5.1. Talent dispensing 150 milliliters of 0.5 times Murashige and Skoog media.

4.6. Separate the two syringe barrels [1]. Detach the silicon tubing from the filter holder and disconnect the filter holder from the irrigation line [2]. Replace it with one syringe barrel [3]. Notes: The VO is revised as per the updated shot.

4.6.1. Talent separating the two syringe barrels.

4.6.2. Talent detaching the silicon tubing from the filter holder and detaching the filter holder from the irrigation line.

4.6.3. Talent replacing the silicon tubing filter holder with one syringe barrel.

5. Irrigation Using 10% bleach solution

5.1. Use a 10 percent bleach irrigation strategy in large experiments with more than 50 samples to increase watering throughput [1].

5.1.1. Shot of experimental units prepared for high-throughput watering.

5.2. Dispense 10 percent volume per volume bleach solution into two small plastic beakers [1]. Place the beakers near the irrigation line [2] and remove the syringe barrel from the irrigation line and the filter holder [3]. Submerge the tubing ends in a 10 percent bleach solution until needed [4]. Notes: VO is added for the additional shot, and the sentence numbers are adjusted to accommodate the added shots.

5.2.1. Talent dispensing 10 percent volume per volume bleach solution into two small plastic beakers.

5.2.2. Talent placing the beakers near the irrigation line.

5.2.3. Talent removing the syringe barrel from the irrigation line and the filter holder.

Author's Notes: 5.2.3 and the added shot were combined in a single shot.
Videographer's Notes: 5.2.3 and 5.3.1 were combined in a single shot.

Notes: The author's and videographer's notes do not match. Check the footage

Added shot: Talent submerging all tubing ends in the bleach solution.

- 5.3. Remove the syringe barrel from the filter holder [1]. Submerge all tubing ends in the 10 percent bleach solution until needed [2]. Shake off the excess bleach solution [3]. Attach the filter holder to the pot's irrigation line [4]. Notes: VO is added for the additional shot, and the sentence numbers are adjusted to accommodate the added shots.

Added shot: Talent removing the syringe barrel from the filter holder.

- 5.3.1. Talent submerging all tubing ends in the 10 percent bleach solution.
5.3.2. Talent shaking excess bleach solution from the tubing ends.
5.3.3. Talent attaching the filter holder to the irrigation line of the pot.
- 5.4. Connect the other long silicon tubing piece of the bottle top dispenser to the bottom port of the filter holder [1].
- 5.4.1. Talent connecting the long silicon tubing piece of the bottle top dispenser to the bottom port of the filter holder.
- 5.5. Dispense 150 milliliters of 0.5x times Murashige and Skoog media [1]. Detach the silicon tubing from the filter holder [2].
- 5.5.1. Talent dispensing 0.5 times Murashige and Skoog media.
5.5.2. Talent detaching the silicon tubing from the filter holder.
- 5.6. Replace the filter in the irrigation line with a syringe barrel after removing excess bleach. If the irrigation volume changes, use about 20 milliliters of media to prime the line [1].
- 5.6.1. Talent replacing the filter in the irrigation line with a syringe barrel.

6. Harvesting and Post-Growth Analysis of Gnotobiotic Plant Cultures

- 6.1. Modify the harvest protocol to align with the specific research goals and the volume of samples being processed [1].
- 6.1.1. Shot of samples ready for harvesting. Author's Notes: Use the shot taken after 3.3.2
- ~~6.2. Transfer the gnotobiotic systems from the growth chamber to the laboratory [1]. Place the systems close to the laminar flow hood [2].~~
- ~~6.2.1. Talent transferring the gnotobiotic systems from the growth chamber to the laboratory.~~

- 6.2.2. Talent placing the gnotobiotic systems next to the laminar flow hood.
- 6.3. Remove the glass cylinder from the pot [1]. Quickly place the pot in the laminar-flow hood [2] and remove it from the 3D-printed base [3].
 - 6.3.1. Talent removing the glass cylinder from the pot. Author's Notes: 6.3.1 and 6.3.2 were combined in a single shot.
 - 6.3.2. Talent placing the pot in the laminar flow hood.
 - 6.3.3. Talent removing the pot from the 3D printed base.
- 6.4. Hold the plant at the base and dump the soil substrate into a container [1]. Gently remove large clumps of soil from the roots. If necessary, collect soil samples in accordance with the specific research objectives [2].
 - 6.4.1. Talent holding the plant at the base and dumping the soil substrate into a container.
 - 6.4.2. Talent gently shaking large clumps of soil substrate from the roots.
- 6.5. Using sterile scissors, separate the shoots from the roots [1]. Record the fresh shoot weight [2].
 - 6.5.1. Talent separating the shoots from the roots with sterile scissors.
 - 6.5.2. Talent recording the fresh shoot weight.
- 6.6. Clean the roots by gently shaking them in a beaker containing deionized water [1]. Blot dry the roots [2] and record the fresh root weight [3].
 - 6.6.1. Talent gently shaking the roots in a beaker containing deionized water.
 - 6.6.2. Talent blotting the roots dry.
 - 6.6.3. Talent recording the fresh root weight.

Results

7. Results

- 7.1. Wheat and maize reached 100% germination within GNOVA [1], whereas in the bag system, wheat achieved 67% germination [2] and maize achieved 75% germination [3].
 - 7.1.1. LAB MEDIA: Figure 6. *Video editor: Highlight the purple bars labeled 100%*
 - 7.1.2. LAB MEDIA: Figure 6. *Video editor: Highlight the green bar labeled 67%*
 - 7.1.3. LAB MEDIA: Figure 6. *Video editor: Highlight the green bar labeled 75% .*
- 7.2. At 2 weeks, wheat grown within GNOVA reached Zadock's growth stage 13, whereas wheat grown in the bag system remained at Zadock's growth stage 11 [1].
 - 7.2.1. LAB MEDIA: Figure 7A.
- 7.3. At week 5, wheat within GNOVA reached Zadock's growth stage 21, as indicated by the appearance of tillers [1], whereas tillers were absent in the bag system and plants remained at Zadock's growth stage 13 [2].
 - 7.3.1. LAB MEDIA: Figure 7B. *Video editor: Zoom in on the wheat plants on the right side, showing additional side shoots.*
 - 7.3.2. LAB MEDIA: Figure 7B. *Video editor: Highlight the plant in the bag (left side) container where side shoots are not visible.*
- 7.4. After 12 weeks of growth in GNOVA, the wheat plants displayed more chlorotic leaves, and the glass cylinder was filled with plant biomass [1]. When the wheat plants were grown for an additional two weeks in GNOVA, there was no noticeable increase in biomass [2].
 - 7.4.1. LAB MEDIA: Figure 7D.
 - 7.4.2. LAB MEDIA: Figure 7E,F.
- 7.5. The growth of maize was assessed within GNOVA for four weeks and compared to maize grown in bags [1].
 - 7.5.1. LAB MEDIA: Figure 8A and B.
- 7.6. The fresh biomass of maize cultivated in GNOVA increased by 593% relative to that grown in the bag system. [1].
 - 7.6.1. LAB MEDIA: Figure 8C and D *Video editor: Highlight the taller purple bar.*
- 7.7. Maize grown within GNOVA had significantly greater shoot and root length than maize grown in the bag system [1].

7.7.1. LAB MEDIA: Figure 8E and F *Video editor: Highlight the taller purple bar.*

Pronunciation Guides:

1. Murashige
Pronunciation link: <https://www.howtopronounce.com/murashige>
IPA: /ˌmʊrəˈʃiːɡeɪ/
Phonetic Spelling: moo·ruh·SHEE·gay
2. Skoog
Pronunciation link: <https://www.howtopronounce.com/skoog>
IPA: /skuːɡ/
Phonetic Spelling: skoog
3. Sterilized
Pronunciation link: <https://www.merriam-webster.com/dictionary/sterilize>
IPA: /ˈsterəˌlīz/ (verb base: sterilize)
Phonetic Spelling: STEHR·uh·lyze
4. Sterile
Pronunciation link: <https://www.merriam-webster.com/dictionary/sterile>
IPA: /ˈsterəl/
Phonetic Spelling: STEHR·uhl
5. Germinated
Pronunciation link: <https://www.merriam-webster.com/dictionary/germinate>
IPA: /ˈjərməˌnāt/ (verb base: germinate)
Phonetic Spelling: JER·muh·nayt
6. Seedlings
Pronunciation link: <https://www.merriam-webster.com/dictionary/seedling>
IPA: /ˈsēd-lɪŋ/
Phonetic Spelling: SEED·ling
7. GNOVA
Pronunciation link: No confirmed link found
IPA: No verified American English IPA available
Phonetic Spelling: guh·NOH·vuh
8. Photoperiod
Pronunciation link: <https://www.merriam-webster.com/dictionary/photoperiod>
IPA: /ˈfō-tōˌpɪr-ē-əd/
Phonetic Spelling: FOH·toh·PEER·ee·uhd
9. Fluorescent
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescent>
IPA: /flōˈre-sənt/
Phonetic Spelling: flor·ESS·uhnt

10. Silicon
Pronunciation link: <https://www.merriam-webster.com/dictionary/silicon>
IPA: /ˈsɪləˌkæn/
Phonetic Spelling: SIL·uh·kon
11. Syringe
Pronunciation link: <https://www.merriam-webster.com/dictionary/syringe>
IPA: /səˈrɪnj/
Phonetic Spelling: suh·RINJ
12. Irrigation
Pronunciation link: <https://www.merriam-webster.com/dictionary/irrigation>
IPA: /ˌɪrəˈɡæʃən/
Phonetic Spelling: ir·uh·GAY·shuhn
13. Bleach
Pronunciation link: <https://www.merriam-webster.com/dictionary/bleach>
IPA: /ˈblēch/
Phonetic Spelling: bleech
14. Gnotobiotic
Pronunciation link: <https://www.howtopronounce.com/gnotobiotic>
IPA: /ˌnoʊtəʊbaɪˈɑːtɪk/
Phonetic Spelling: NOH·toh·bye·AHT·ik
15. Laminar
Pronunciation link: <https://www.merriam-webster.com/dictionary/laminar>
IPA: /ˈlɑmənər/
Phonetic Spelling: LAM·uh·ner
16. Deionized
Pronunciation link: <https://www.merriam-webster.com/dictionary/deionize>
IPA: /diˈiəˌnīz/ (verb base: deionize)
Phonetic Spelling: dee·EYE·uh·nyze
17. Biomass
Pronunciation link: <https://www.merriam-webster.com/dictionary/biomass>
IPA: /ˈbiˌoʊˌmas/
Phonetic Spelling: BYE·oh·mass
18. Germination
Pronunciation link: <https://www.merriam-webster.com/dictionary/germination>
IPA: /ˌjərməˈnæʃən/
Phonetic Spelling: JER·muh·NAY·shuhn
19. Zadock's
Pronunciation link: No confirmed link found
IPA: No confirmed American English IPA found
Phonetic Spelling: ZAY·docks
20. Tillers
Pronunciation link: <https://www.merriam-webster.com/dictionary/tiller>

IPA: /'tɪlər/

Phonetic Spelling: TIL·er

21. Chlorotic

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

IPA: /klə'rəʃtɪk/

Phonetic Spelling: kluh·ROT·ik

22. Maize

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

IPA: /'māz/

Phonetic Spelling: mayz

23. Substrate

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

IPA: /'səb,ʃtrāt/

Phonetic Spelling: SUB·strayt

24. Tweezers

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

IPA: /'twē-zərz/

Phonetic Spelling: TWEE·zerz