FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 68541
Scriptwriter Name: Poornima G
Project Page Link: https://review.jove.com/account/file-uploader?src=20905053

Title: Asymbiotic Germination and Leaf Explant-Based Regeneration of the Endangered Medicinal Orchid *Hemipilia cucullata* from Mature Seeds

Authors and Affiliations:
Yage Tu[1], Jianming Wang[2], Zhechen Qi[1], Weimei Jiang[2]

[1]Zhejiang Province Key Laboratory of Plant Secondary Metabolism and Regulation, College of Life Sciences and Medicine, Zhejiang Sci-Tech University
[2]Laboratory of Systematic & Evolutionary Botany and Biodiversity, College of Life Sciences, Zhejiang University

Corresponding Authors:
Zhechen Qi zqi@zstu.edu.cn
Weimei Jiang wmjiang@zju.edu.cn

Email Addresses for All Authors:
Yage Tu 17816113750@163.com
Jianming Wang 17398015536@163.com
Zhechen Qi zqi@zstu.edu.cn
Weimei Jiang wmjiang@zju.edu.cn

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**

Current Protocol Length
Number of Steps: 22
Number of Shots: 29

Introduction

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

1.1. **Weimei Jiang:** My research focuses on optimizing tissue culture protocols for endangered orchids to enhance conservation, large-scale propagation, and sustainable utilization of these valuable medicinal plants.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

What are the current experimental challenges?

1.2. **Weimei Jiang:** A major challenge is achieving consistent germination and regeneration rates across orchid species, as each requires species-specific media optimization and precise control of growth conditions.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.5.1*

What significant findings have you established in your field?

1.3. **Weimei Jiang:** We have developed efficient tissue culture protocols for multiple endangered plants, enabling species recovery and supporting reintroduction into natural habitats.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.1*

What research gap are you addressing with your protocol?

1.4. **Weimei Jiang:** No efficient, reproducible tissue culture system exists for *Hemipilia cucullata*, hindering propagation, restoration, and conservation. Terrestrial orchids often need specific symbiotic fungi, whose isolation and identification are complex; asymbiotic methods simplify propagation and bypass these steps.

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*

What advantage does your protocol offer compared to other techniques?

1.5. **Yage Tu:** Our protocol enables high germination and rapid plantlet regeneration from mature seeds, eliminating fungal dependency and offering a faster, more reliable approach for Hemipilia cucullata propagation.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 5.2.1*

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

Protocol

2. Capsule Sterilization and Seed Extraction

Demonstrator: Yage Tu

2.1. To begin, place the capsule in a beaker **[1]**. Add 1 to 2 drops of detergent into the beaker for rinsing **[2]**.

2.1.1. WIDE: Talent placing the capsule into a clean glass beaker.

2.1.2. Talent adding 1 to 2 drops of detergent.

2.2. Wash the capsule under running tap water for 5 minutes **[1]**. Then, using sterile absorbent paper, remove the remaining surface water from the capsule **[2]**. Now, immerse the capsule in 75 percent ethanol for 30 seconds **[3]**.

2.2.1. Talent holding the beaker under a running tap for an extended wash.

2.2.2. Talent gently patting the capsule dry using sterile absorbent paper.

2.2.3. Talent submerging the capsule into a container filled with 75 percent ethanol.

2.3. Sterilize the capsule by immersing it in 50 milliliters of 20 percent sodium hypochlorite solution containing approximately 5 percent available chlorine **[1]**. Then, add 2 drops of undiluted household dishwashing detergent to the solution and incubate the capsule for 10 to 12 minutes **[2]**.

2.3.1. Talent placing the capsule into a beaker containing 50 milliliters of 20 percent sodium hypochlorite solution.

2.3.2. Talent adding 2 drops of undiluted dishwashing detergent and setting a timer for 12 minutes.

2.4. Rinse the capsule five times with sterile distilled water to remove any residual disinfectant **[1]**. Then, using a sterile scalpel, remove approximately 1 millimeter from both the apex and the pedicel ends of the capsule **[1]**.

2.4.1. Talent pouring sterile distilled water over the capsule.

2.4.2. Talent trimming the top and base of the capsule using a sterile scalpel on a sterile surface.

2.5. Make a small incision at the upper end of the capsule to expose the seeds, and collect them in a sterile Petri dish **[1-TXT]**.

2.5.1. Talent making a small cut at the top of the capsule to reveal seeds inside. **TXT: Prepare MS liquid medium with appropriate supplements**

~~2.5.2. Talent transferring the opened capsule into a sterile Petri dish.~~ **NOTE:** Not filmed, VO merged

3. *Hemipilia cucullata* Seed Germination

Demonstrator: Yage Tu

3.1. Using sterile forceps, evenly disperse approximately 500 to 600 seeds into each culture bottle containing the prepared medium **[1]**. Allow the seeds to absorb water and settle at the bottom of the liquid medium **[2]**.

3.1.1. Talent using sterile forceps to distribute seeds into culture bottles on a sterile bench.

3.1.2. Close-up shot of seeds slowly settling at the bottom of the medium.

3.2. Maintain the cultures at 25 degrees Celsius with a 12-hour light and dark cycle and a light intensity of 36 micromoles per square meter per second **[1]**.

3.2.1. Shot of bottles being placed on the shelves inside a controlled culture room.

3.3. Observe seed germination weekly **[1]**. Record any visible morphological changes and photograph the cultures for documentation **[2]**.

3.3.1. Talent examining bottles on a sterile bench.

3.3.2. Talent taking photographs of culture bottles using a mobile phone.

4. Shoot Elongation and Induction of Protocorm-Like Bodies (PLBs) from Leaf Explants

Demonstrator: Yage Tu

4.1. Select healthy plantlets, 1 to 2 centimeters in size, without visible blackening or complete yellowing, for shoot elongation **[1]**. Using sterilized and cooled forceps, aseptically transfer 8 to 12 plantlets into each bottle containing freshly prepared shoot elongation medium **[2]**. Evenly distribute the plantlets within each bottle **[3-TXT]**.

4.1.1. Shot of pointing to a healthy plantlet.

4.1.2. Talent using sterilized forceps to transfer the plantlets into bottles.

4.1.3. Talent adjusting the position of each plantlet inside the bottle for uniform spacing. **TXT: Incubation: 25 °C; 12 h light/dark cycle; Light intensity: 36 µmol·m^{-2}·s^{-1}**

4.2. After 5 weeks of shoot elongation, select plantlets with fully developed shoots and healthy leaves for leaf explant sampling **[1-TXT]**.

4.2.1. Talent pointing to the plantlets with well-formed shoots and vibrant leaves. **TXT: Prepare the protocorm-like body (PLB) induction medium**

~~4.3. Next, prepare the protocorm-like body induction medium and adjust the pH to 5.8 **[1-TXT]**.~~

4.3.1. ~~Talent placing the bottles containing medium on the bench. **TXT: Prepare the protocorm-like body (PLB) induction medium**~~**NOTE:** Not filmed, VO moved as on-screen text

4.4. Excise fully expanded sterile leaves from a healthy plantlet using a sterile scalpel **[1]**. Remove the apical region and leaf margins **[2]**, then cut each leaf into approximately 0.5 by 0.5-centimeter segments using a sterile scalpel under aseptic conditions **[3]**.

4.4.1. Talent trimming off fully expanded leaves with sterile scalpel.

4.4.2. Talent cutting away the tips and margins of the leaves.

4.4.3. Talent slicing the trimmed leaves into uniform square segments with a scalpel.

4.5. Place 10 leaf segments onto the PLB induction medium with the adaxial surface facing upward **[1]**. Make sure each segment touches the medium firmly. Then culture them for 5 weeks. **[2]**.

4.5.1. Talent placing the leaf segments carefully onto the medium inside the bottle with the adaxial surface up.

4.5.2. Close-up shot of the segments placed on the medium.

4.6. Finally, assess the number of successfully induced PLBs and calculate the induction rate as a percentage **[1]**. Record the average bud height in centimeters and count the number of buds formed per explant **[2]**.

4.6.1. Talent examining the PLBs and recording data in a lab notebook.

4.6.2. Talent measuring bud height with a ruler.

Results

5. Results

5.1. Seed germination in liquid medium was visibly characterized by the emergence of green embryos from the seed coat at around 60 days **[1]**, which subsequently formed spherical protocorms by 80 to 85 days **[2]**.

5.1.1. LAB MEDIA: Figure 1C.

5.1.2. LAB MEDIA: Figure 1E.

5.2. The highest germination rate of 72% was achieved in liquid medium supplemented with 0.5 milligrams per liter NAA **[1]**.

5.2.1. LAB MEDIA: Table 1. *Video editor: Highlight the row for "Liquid Medium – NAA (0.5)" showing 72.0 ± 6.0*

5.3. B5 medium containing 0.5 milligrams per liter benzyladenine and 0.2 milligrams per liter NAA yielded the highest protocorm proliferation rate, averaging 5.3 newly formed protocorms per explant **[1]**.

5.3.1. LAB MEDIA: Table 2. *Video editor: Highlight the row for "B5 + BA (0.5) + NAA (0.2)" showing 5.3 ± 0.5*

5.4. Protocorms cultured in B5 medium with the optimized growth regulator combination displayed vigorous growth and healthy green pigmentation **[1]**, outperforming those grown on MS medium under the same conditions **[2]**.

5.4.1. LAB MEDIA: Figure 2A.

5.4.2. LAB MEDIA: Figure 2B.

5.5. The highest number of branches per plantlet, averaging 4, was observed with 1 milligram per liter of each benzyladenine and NAA **[1]**, followed closely by NAA alone at 0.5 milligram per liter **[2]**.

5.5.1. LAB MEDIA: Table 3. *Video editor: Highlight the row for "BA (1.0) + NAA (1.0)" showing 4.0 ± 0.7*

5.5.2. LAB MEDIA: Table 3. *Video editor: Highlight the row for "NAA (0.5)" showing 3.9*

± 0.7

5.6. Shoot elongation was most enhanced with the combination of 0.5 milligrams per liter benzyladenine and 1 milligram per liter NAA, resulting in an average plantlet height of 4.8 centimeters **[1]**.

5.6.1. LAB MEDIA: Figure 2D.

5.7. The highest induction rate of protocorm-like bodies, 44.3%, was achieved using 3 milligrams per liter benzyladenine with 0.2 milligrams per liter NAA **[1]**.

5.7.1. LAB MEDIA: Table 4. *Video editor: Highlight the row for "BA (3.0) + NAA (0.2)" showing "44.3 ± 5.1" in the induction rate column*

1. Protocorm

- **Pronunciation link**: Merriam-Webster (or American OED pronunciation) Merriam-WebsterOxford English Dictionary
- **IPA**: /ˈproʊdoʊˌkɔrm/
- **Phonetic Spelling**: PROH-doh-korm

2. Benzyladenine

- **Pronunciation link**: HowToPronounce audio How To Pronounce
- **IPA** (estimated): /ˌbenzɪlˈædəˌniːn/
- **Phonetic Spelling**: ben-ZIL-uh-DEE-neen

3. Cytokinin (as in type of plant hormone)

(No direct link found)

- **IPA**: /ˌsaɪtəˈkɪnɪn/
- **Phonetic Spelling**: sy-tuh-KIN-in

4. Hypochlorite (from sodium hypochlorite)

(No direct link found)

- **IPA**: /ˌhaɪpoʊˈklɔːraɪt/
- **Phonetic Spelling**: hy-poh-KLOR-ite

5. Pedicel (part of capsule)

(No direct link found)

- **IPA**: /ˈpɛdəˌsɛl/
- **Phonetic Spelling**: PED-ih-sel

6. Ethanol (75 percent ethanol)

(No direct link found)

- **IPA**: /ˈɛθəˌnɔl/
- **Phonetic Spelling**: ETH-uh-nol

7. Detergent (though common, may cause hesitation)

(No link)

- **IPA**: /dɪˈtɜrʤənt/
- **Phonetic Spelling**: dih-TUR-jent

8. Hemipilia cucullata (species name)

(No link found)

- **IPA**: /ˌhɛmɪˈpɪliə kəˈkʌlətə/
- **Phonetic Spelling**: heh-mih-PIH-lee-uh kuh-KUL-uh-tuh

9. Protocorm-like bodies (PLBs)

(Protocorm already given)

- **IPA**: /ˈproʊdoʊˌkɔrm laɪk ˈbɒdiz/
- **Phonetic Spelling**: PROH-doh-korm like BOD-eez

10. Adaxial (surface orientation)

(No link found)

- **IPA**: /əˈdæksiəl/
- **Phonetic Spelling**: uh-DAK-see-uhl

11. NAA (Naphthaleneacetic acid)

(Abbreviation used commonly)

- **IPA**: /næfθəˌliːn əsɛtɪk ˈæsɪd/
- **Phonetic Spelling**: naff-thuh-LEEN ah-SEH-tik AS-id

12. BA (Benzyladenine, previously given but acronym)

- Refer to **Benzyladenine** above

13. Micromoles (µmol·m^{-2}·s^{-1})

(No link)

- **IPA**: /ˌmaɪkroʊˈmoʊlz/
- **Phonetic Spelling**: MY-kroh-mohlz

14. Germination (as in seed germination)

(Common, but in scientific context)

- **IPA**: /ˌdʒɜrməˈneɪʃən/
- **Phonetic Spelling**: jur-muh-NAY-shun

15. Protocorm-like body induction medium

(Composed mostly of known parts)

- **IPA**: /ˈproʊdoʊˌkɔrm laɪk ˈbɒdi ɪnˈdʌkʃən ˈmidiəm/
- **Phonetic Spelling**: PROH-doh-korm like BOD-ee in-DUK-shun MEE-dee-um