FINAL SCRIPT: APPROVED FOR FILMING

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Title: In Vivo and In Vitro Infection of Potato Roots with Plant Parasitic Nematodes for the Assessment of Induced Structural Changes

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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? **Yes**

   SCOPE:5.1.2

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? **Yes, 150 km apart**

Current Protocol Length
Number of Steps: 32
Number of Shots: 56

Introduction

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

REQUIRED:

1.1. **Jorge M. S. Faria:** Research on parasitic nematode infections in crops is influenced by the variability of environmental conditions, which can significantly impact experimental outcomes and data reproducibility.

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

What technologies are currently used to advance research in your field?

1.2. **Ana Cristina Figueiredo:** We have developed in vitro co-cultures of potato transgenic roots with plant parasitic nematodes as a reliable alternative that occupies less space, requires less time to obtain, and is free from contamination or from host genetic variability.

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

What are the current experimental challenges?

1.3. **Manuel Mota:** Tracking the temporal progression of nematode infection in crop roots remains challenging. Differential staining techniques provide a reliable method for identifying infection sites and distinguishing nematode life stages with precision.

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

What advantage does your protocol offer compared to other techniques?

1.4. **Pedro Barbosa:** Compared to greenhouse trials, potato hairy roots support a continuous and controlled system for providing all stages of nematode development, independent of seasonal or climatic variations.

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

What research questions will your laboratory focus on in the future?

1.5. **Claudia Vicente:** The protocol described offers several promising future applications. For example, it enables detailed studies into the molecular and cellular mechanisms of

parasitism, providing insights into how plant parasitic nematodes infect and manipulate hosts.

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:

What motivated you to choose JoVE for publishing your research?

1.6. **Jorge M. S. Faria:** I have been familiar with JoVE's educational and scientific videos since my university years, finding them to be a valuable visual resource for understanding and implementing new methodologies.

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

Videographer: Please capture the testimonials in both French and English

Protocol

2. Maintenance of Root Lesion Nematodes (RLN) for Infection of Potato Plants

Demonstrator: Pedro Barbosa

2.1. To begin, wash a carrot under running tap water and then with a common detergent solution to remove debris **[1].** Once dried with paper towel, insert a sterilized metal skewer into the top of the carrot, about 1 to 2 centimeters inwards **[2]**. With a wash bottle fitted with a nozzle, wet the carrot with 96% ethanol **[3].**

2.1.1. WIDE: Talent washing the carrot under running tap water and then detergent.

2.1.2. Talent inserting the metal skewer into the carrot, in a flow hood.

2.1.3. Talent using a wash bottle to wet the carrot with ethanol.

2.2. Blot the bottom tip of the carrot on a sterilized filter paper, then carefully pass it through a flame **[1]**.

2.2.1. Shot of the carrot bottom being blotted on a sterilized filter paper, then it is passed over a flame.

2.3. With a sterile peeler, peel the carrot from the top down and repeat flaming **[1].** After discarding the top and bottom sections, place the middle section in a sterile Petri dish **[2]**.

2.3.1. Talent peeling the carrot with a sterile peeler.

2.3.2. Shot the middle section being placed in a sterile Petri dish.

2.4. Using a sterile blade and tweezers, cut 1-centimeter-thick sections **[1].** Transfer the sections into sterile Petri dishes **[2].**

2.4.1. Shot of the carrot sections being cut with a sterile blade and tweezers.

2.4.2. Shot of the carrot sections into sterile Petri dishes.

2.5. After sealing the plate, keep it under UV light for 60 minutes on each side for sterilizing the carrot disks **[1].** Incubate the carrot disks at 25 degrees Celsius in darkness for 1 to 2 weeks **[2-TXT].**

2.5.1. Shot of carrot disks being sterilized under UV light.

2.5.2. Talent places the sterilized carrot discs at 25 °C. **TXT: Discard discs that show signs of microbial contamination**

2.6. Next, with a sterile blade, make an X-shaped incision at the center of the carrot disk, cutting only halfway deep **[1].** Pipette 50 microliters of root lesion nematode suspension containing at least 50 mixed life stages into the incision **[2].**

2.6.1. Shot of an X-shaped incision being made at the center of the carrot disk.

2.6.2. Talent pipetting RLN suspension into the incision.

2.7. After sealing the Petri dish, incubate it at 25 degrees Celsius in darkness for up to 3 months **[1-TXT].**
  2.7.1. Talent places the inoculated carrot disks at 25 °C. **TXT: Monitor weekly for signs of nematode growth**

2.8. To extract the root lesion nematodes, transfer the carrot disks with visible necrosis to a 75-micrometer mesh sieve with 8-centimeter diameter, placed in a sterile glass bowl **[1-TXT]**. Next, pour antibiotic solution over the sieve until disks are covered **[2].**
  2.8.1. Talent transfers necrotic carrot disk to a 75 µm mesh sieve set in a sterile glass bowl. **TXT: Leave 1 cm gap between the sieve bottom and bowl concavity**
  2.8.2. Talent pouring antibiotic solution over carrot disks in the sieve.

2.9. After overnight incubation in dark, use a sterilized pipette to transfer the nematodes from the bottom of the bowl to a sterilized glass staining block **[1].** Pipette 1 milliliter of antibiotic solution into the staining block and let the nematodes settle for 30 to 40 minutes **[2].** Pipette out the used antibiotic solution and repeat the washing process 4 to 5 times **[3]**.
  2.9.1. Talent pipetting RLNs into a staining block.
  2.9.2. Talent pipettes 1 mL of antibiotic solution into the staining block.
  Videographer: Please try to capture the nematodes settling down in the block
  2.9.3. Talent pipettes out the used antibiotic solution

2.10. Use the root lesion nematode suspension immediately or store at 11 degrees Celsius **[1].**
  2.10.1. Shot of the RLN suspension

3. In Vivo Infection of Potato Plants with Plant Parasitic Nematodes

Demonstrator: Pedro Barbosa

3.1. Select potato tubers of the same size and discard any with holes, bruises, or soft sections **[1-TXT].**
  3.1.1. Talent sorting potato tubers, discarding damaged ones. **TXT: Remove sprouts of upto 1 mm before sowing to synchronize sprouting**

3.2. Fill 5-liter pots with a 1 to 1 mixture of autoclaved soil and sand and mix in 22.5 grams of slow-release NPK fertilizer **[1].** Then sow the potatoes at a depth of 9 centimeters below the soil surface **[2].**
  3.2.1. Talent filling pots with soil mixture then adding 22.5g of slow-release NPK fertilizer.

3.2.2. Talent placing a potato tuber at a depth of 9 centimeters in the soil.

3.3. Place the pots in a greenhouse under 50 to 70% humidity **[1].** Water frequently to maintain soil moisture at 70% of maximum water-holding capacity, avoiding temperature extremes **[2-TXT].**
3.3.1. Talent placing pots inside the greenhouse.
3.3.2. Talent waters the pots. **TXT: Continue conditions until potato shoots emerge at soil surface**

3.4. Once potato plants have emerged, create evenly distributed 4 to 6 holes around each plant to seed depth **[1].** Pipette 8 milliliters of suspension containing 30,000 living mixed life-stage Root Lesion Nematodes into the holes then cover the holes with soil mixture **[2-TXT].**
3.4.1. Talent making small holes around the plant.
3.4.2. Talent pipetting RLN suspension into the holes and covering holes with soil mixture. **TXT: Ensure inoculum ratio of 4:1 live RLN per g of soil**

3.5. For control pots and pots containing Root Lesion Nematodes, withhold watering on the day of inoculation **[1].** After 2 months of culture, uproot the potato plants and separate the shoots and roots for weighing **[2].**
3.5.1. Shot of labeled RLN pots without water.
3.5.2. Talent uprooting a potato plant and weighing the shoots and roots separately.

3.6. Carefully wash the root system **[1].** Examine the roots for RLN attack sites using appropriate staining techniques **[2].**
3.6.1. Talent washing the root system under running water.
3.6.2. Shot stained roots being placed under a microscope.

4. Establishment of In Vitro Co-Cultures of Potato Transgenic Roots for Infection with Plant Parasitic Nematodes

Demonstrator: Jorge M. S. Faria

4.1. Place washed and sterilized potato tubers in a container **[1].** Cover the tubers with a 25 % commercial bleach solution, close the container and mix for 15 minutes **[2].** Once the bleach is disposed**,** rinse the tubers three times with sterilized tap water **[3].**
4.1.1. Talent placing tubers in a container.
4.1.2. Shot of the container being filled with commercial bleach solution, closed and mixed.
4.1.3. Talent removing the bleach solution and adding sterilized tap water to the tubers.

4.2. Next, in a flow hood, immerse the tubers in 80% ethanol solution for 15 minutes with vigorous agitation **[1].** After pipetting out the ethanol, rinse three times with sterilized tap water **[2].**
  4.2.1. Shot of tubers in ethanol under the flow hood vigorously shaking.
  4.2.2. Talent adds water to the tubers, after removing the tubers.

4.3. With a sterile scalpel, remove the peripheral portions of the tubers **[1].** Section the inner central piece into 0.5-centimeter-thick segments **[2].**
  4.3.1. Talent removing the peripheral portions of the tubers.
  4.3.2. Talent cutting tuber sections with a sterile scalpel.

4.4. To inoculate the sections, first mix 1 milliliter of *Rhizobium rhizogenes* suspension with 9 milliliters of SH *(S-H)* medium **[1-TXT].** Dip the tip of a sterile scalpel into the diluted suspension and wound the surface of the potato segments five times **[2]**.
  4.4.1. Talent adds 1 mL bacterial suspension to a tube with 9 mL SH medium. **TXT: Schenk and Hildebrandt (SH) medium with 30 g/L sucrose, pH: 5.6**
  4.4.2. Talent dipping the tip in bacterial suspension and then poking it in the potato segment.

4.5. Once the segments are dried, place them on semi-solid SH medium **[1]** and incubate at 25 degrees Celsius for 3 days in the dark to facilitate plasmid transfection **[2].**
  4.5.1. Talent placing tuber segments on SH medium plates.
  4.5.2. Talent placing the plate in the incubator.

4.6. At the end of the third day, transfer the infected segments to plates containing semi-solid SH medium supplemented with antibiotics **[1-TXT].**
  4.6.1. Talent transferring infected tuber segments to antibiotic-supplemented SH medium. **TXT: Renew medium weekly for 3 months; Antibiotics: Cefotaxime and Carbenicillin (150 µg/mL)**

4.7. After 3 months, use sterile tweezers to gather a 1-gram cluster of transgenic roots **[1].** Transfer the roots to the center of plate with fresh, semi-solid, antibiotic-free SH medium **[2-TXT].**
  4.7.1. Shot of the transgenic root clusters being pulled with sterile tweezers.
  4.7.2. Talent transferring transgenic root clumps to the center of plate with fresh, semi-solid, antibiotic-free SH medium. **TXT: To maintain culture stability, maintain roots under monthly sub-culture at 25 °C for 1 year**

5. Infection of Potato Transgenic Roots with Plant Parasitic Nematodes for Assessment of Structural Changes

Demonstrator: Jorge M. S. Faria

5.1. To collect the nematode egg masses, obtain the root galls **[1]**. Use a pair of sterile ultra-fine point tweezers to carefully extract egg masses under a binocular stereomicroscope set to 20X magnification **[2].**

5.1.1. Talent placing the root gall on slide or in plate.

5.1.2. SCOPE: SCOPE_5_1_2_option2.mp4 00:00-00:20

5.2. Place the egg masses in a covered Petri dish containing 5 milliliters of sterile tap water and let them hatch for 48 hours **[1].** Next, in a flow hood, pipette 5 milliliters of the J2 suspension containing 100 nematodes per milliliter onto a 20-micrometer mesh sieve **[2].**

5.2.1. Talent transfers the egg masses to a covered Petri dish with 5 mL sterile tap water.

5.2.2. Talent pipetting nematode suspension onto a sieve in a flow hood.

5.3. After washing with sterile tap water, immerse the bottom half of the sieve containing J2 nematodes in a 20% hydrogen peroxide solution and mix in circular motion for 15 minutes **[1].**

5.3.1. Shot of the bottom half of the sieve being immersed in 20% H_2O_2 solution and rotated to mix.

5.4. Dispense sterile tap water through the sieve, over the nematodes and repeat the washing process 2 times **[1].** Tilt the sieve so that the nematodes collect at the border during the final wash **[2].** Then pipette 1 milliliter of sterile ultrapure water from the sieve border to recover the nematodes **[3-TXT].**

5.4.1. Talent pipettes sterile water over the sieve.

5.4.2. Talent tilting the sieve and the nematodes collecting at the sieve border.

5.4.3. Shot of 1 mL sterile ultrapure water being pipetted from the sieve border. **TXT: Store at 11 °C or use immediately**

5.5. In a flow hood, subculture a 1-gram clump of potato transgenic roots onto SH plates with 100 sterile nematodes **[1-TXT].**

5.5.1. Talent placing transgenic root clumps and nematodes onto SH medium. **TXT: Small galls will appear after 2 - 3 weeks**

5.6. Monitor the co-culture regularly under an inverted microscope at 100X magnification **[1].** When egg masses become visible, subculture the roots to a new SH medium plate, **[2-TXT].**

5.6.1. Talent viewing the co-culture under an inverted microscope

5.6.2. Talent transferring roots with galls to a new SH medium plate. **TXT: Keep co-cultures under a monthly sub-culture routine at 25 °C in darkness**

Results

6. Results

6.1. The use of carrot disks resulted in an average 100 times increase in nematode populations, within 3 months **[1].**

6.1.1. LAB MEDIA: Figure 1 A-C *Video Editor: Please emphasize 1 B and C*

6.2. Potato plants showed no visible symptoms under low root lesion nematode population numbers **[1].**

6.2.1. LAB MEDIA: Figure 2.

6.3. Several life stages of *Pratylenchus penetrans* were observed in the root cortex after staining with acid fuchsin, indicating penetration of the tissue **[1],** and the associated necrotic lesions were clearly visible in infected areas **[2].**

6.3.1. LAB MEDIA: Figure 5A. *Video editor: Highlight the thin, elongated worms inside the root structure*

6.3.2. LAB MEDIA: Figure 5B.

6.4. Development of potato transgenic roots showed initial cell mass growth along scalpel-induced wounds in the potato tuber section **[1],** followed by the emergence of transgenic roots **[2].** Sustained growth of the roots was observed in the culture medium **[3],** and root clumps were successfully transferred to fresh culture medium for continued growth **[4].**

6.4.1. LAB MEDIA: Figure 3A. *Video editor: Highlight the area pointed at by the red arrow*

6.4.2. LAB MEDIA: Figure 3B. *Video editor: Please zoom in on the inset image*

6.4.3. LAB MEDIA: Figure 3C

6.4.4. LAB MEDIA: Figure 3D

6.5. Potato transgenic root cultures were successfully infected with *Meloidogyne chitwoodi* second-stage juveniles to establish plant-nematode co-cultures **[1].** Root galls containing adult females and egg masses were observed in the infected cultures **[2].**

6.5.1. LAB MEDIA: Figure 4A.

6.5.2. LAB MEDIA: Figure 4B and C. *Video editor: Please emphasize the inset 4 C*

6.6. Gall tissues formed by *Meloidogyne chitwoodi* **[1]** were visible after infection of transgenic potato roots, with distinct stages of nematode development **[2]** and reproduction identifiable, including the formation of egg masses and eggs **[3]**.

6.6.1. LAB MEDIA: Figure 6A. *Video editor: Focus on the large swollen structure*
6.6.2. LAB MEDIA: Figure 6B.
6.6.3. LAB MEDIA: Figure 6C and D

Pronunciation Guides:

ethanol
Pronunciation link: https://www.merriam-webster.com/dictionary/ethanol
IPA: /ˈɛθəˌnɑl/
Phonetic Spelling: eth-uh-nahl

nematode
Pronunciation link: No confirmed link found
IPA: /ˈnɛməˌtoʊd/
Phonetic Spelling: nem-uh-tohd

necrosis
Pronunciation link: No confirmed link found
IPA: /nɪˈkroʊsɪs/
Phonetic Spelling: nih-kroh-sis

inoculation
Pronunciation link: No confirmed link found
IPA: /ɪˌnɑkjəˈleɪʃən/
Phonetic Spelling: ih-nok-yuh-lay-shun

transgenic
Pronunciation link: No confirmed link found
IPA: /ˌtrænzˈdʒɛnɪk/
Phonetic Spelling: tranz-JEN-ik

plasmid
Pronunciation link: No confirmed link found
IPA: /ˈplæzmɪd/
Phonetic Spelling: plaz-mid

Rhizobium rhizogenes
Pronunciation link: No confirmed link found
IPA: /raɪˈzoʊbiəm raɪˈzoʊdʒəˌniːz/
Phonetic Spelling: ry-ZOH-bee-um ry-ZOH-jeh-neez

Meloidogyne chitwoodi
Pronunciation link: https://www.howtopronounce.com/meloidogyne-chitwoodi
IPA: /ˌmɛloʊˌɪˈdɔdʒəni ˌkɪtˈwʊdi/
Phonetic Spelling: meh-loh-id-OJ-uh-nee kit-WOO-dee

acid fuchsin
Pronunciation link: No confirmed link found

IPA: /ˈæsɪd ˈfʊksɪn/
Phonetic Spelling: as-id fooks-in