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Project Page Link: <https://review.jove.com/account/file-uploader?src=21298808>

Title: Visualization of Leaf and Bracteal Nectaries of Cotton using Digital Microscopy to Improve Scoring Accuracy and Data Preservation

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NOTE: Authors changed the name Jodi Scheffler to Jodi A Scheffler

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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**

If **Yes**, can you record movies/images using your own microscope camera? **No**

Digital Microscope, VHX 600

SCOPE: 2.7.1-2.7.3, 2.8.1-2.8.2, 1.9.1, 2.10.1-2.10.3, 2.13.1, 2.14.1-2.14.2

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? **No, it is in one location but needs to shoot in the green house and in the lab. The green houses are in the surroundings of lab building. All greenhouse shots can be combined while all lab shots can be combined.**

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: 14

Number of Shots: 32

Introduction

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. **Lavanya Mendu:** ~~The current study aims on the method that accurately scores the expression of the nectary trait. To illustrate this, cotton leaf and bracteal nectaries were used to demonstrate the fine expression differences of the nectary trait.~~
The current study aims on the method that accurately scores the expression of the nectary trait. To illustrate the nectary trait expression, both cotton leaf and bracteal nectaries were used as model tissues.

NOTE: Author's edits to 1.1 have been added.

- 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. **Lavanya Mendu:** ~~Overcomes the limitation of traditional scoring that is inaccurate and unreliable for nectary trait expression.~~
This method overcomes the limitation of traditional scoring that is inaccurate and unreliable for nectary trait expression.

NOTE: Author's edits to 1.2 have been added.

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

CONCLUSION:

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

- 1.3. **Lavanya Mendu:** ~~This method identifies the right populations that express nectariless traits with downstream applications in Marker Assisted Selection.~~
Accurate scoring of nectariless plants from the segregated populations of nectaried and nectariless crosses is essential for the selection of nectariless plants that can reduce plant insect damage as well as reduce yield losses. So, selection of this trait is important for downstream applications like DNA markers associated with nectariless

traits that are valuable tools for Marker Assisted Selection.

NOTE: Author's edits to 1.2 have been added.

- 1.3.1. 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

2. ~~Field Collection and Microscopic Phenotyping~~ Greenhouse Sampling of Cotton Leaves and Floral Nectaries

NOTE: The title was changed at the postshoot stage

Demonstrator: Lavanya Mendu

- 2.1. To begin, transport a cooler and labeled zip lock bags to the greenhouse for sample collection [1]. Collect young leaf tissue from 2- to 3-month-old greenhouse grown cotton plants [2] and place it into the labeled sample bags [3].

Videographer's NOTE: Shots 2.1.1 and 2.1.2 are mislabeled in slate. The files have proper slate numbers.

- 2.1.1. WIDE: Talent carrying a cooler and labeled zip lock bags into the greenhouse area.
- 2.1.2. Talent collecting young leaf tissue from a cotton plant.
- 2.1.3. Talent placing it into a labeled zip lock bag.

- 2.2. Place at least 2 leaves per plant sample into the respective labeled sample bag [1]. Then seal each sample bag securely after collection [2].

Videographer's NOTE: Shots 2.2.1 and 2.2.2 are mislabeled in slate. The files have proper slate numbers.

- 2.2.1. Talent placing multiple leaves into a labeled sample bag.
- 2.2.2. Close up of the talent sealing the zip lock bag.

- 2.3. Place each sealed sample bag containing leaf tissue into the cooler [1].

- 2.3.1. Talent placing sealed sample bags into a cooler. ~~TXT: Transport the cooler into the lab~~

NOTE: The text overlay has been removed because it is mentioned in the next step.

- 2.4. Collect healthy flowers from the top branches when the plants are at the mid flowering stage [1]. Place at least 2 flowers into a labeled sample bag and seal the bag [2]. Transfer the sealed sample bags containing flowers into the cooler for transport to the laboratory [3].

Videographer's NOTE: For shots 2.4.1 and 2.4.2: Combined, see file not slate.

- 2.4.1. Talent selecting healthy flowers from the top branches of a cotton plant.
- 2.4.2. Talent placing flowers into a labeled zip lock bag and sealing it.
- 2.4.3. Talent placing the sealed flower sample bags into the cooler.

Microscope-Based Imaging and Scoring of Leaf Nectaries

NOTE: Treat this as a title card for a separate section (added at postshoot stage)

- 2.5. Next, turn on a microscope and complete the initial startup steps [1]. Place a half folded A4 (A-4) white sheet, cut to match the size of the microscope stage, onto the stage [2].
 - 2.5.1. Talent switching on the microscope and preparing the system.
 - 2.5.2. Talent placing a half folded A4 white sheet neatly onto the microscope stage.
- 2.6. Use the small light knobs on the console controller to adjust the light on the microscope stage [1]. Turn the small light switch knob on the console to the maximum position [2] and rotate the large brightness knob to medium high by turning it three quarters of the way [3].
 - 2.6.1. Talent adjusting the small light knobs on the console controller.
 - 2.6.2. Talent turning the small light switch knob to maximum.
 - 2.6.3. Talent rotating the large brightness knob three quarters of the way.
- 2.7. On the computer, select 10 times magnification in the software [1]. Then transfer the leaf tissue onto the precut white sheet placed on the microscope stage [2]. ~~Flip the~~ Place the leaf tissue and position it at the center of the stage with the abaxial side facing up [3].

Videographer's NOTE: In [3] edit the text to remove flip.

Author's NOTE: In [3], Replace ' Flip the' with 'Place the'

Videographer: Please capture the shots labeled SCOPE with a SCOPE kit.

Alternatively, capture the screen of the instrument

- 2.7.1. SCOPE/SCREEN: 10X magnification is being selected in the microscope software interface.
- 2.7.2. SCOPE/SCREEN: Talent placing the leaf tissue onto the white sheet on the microscope stage.

- 2.7.3. SCOPE/SCREEN: Shot of ~~the leaf being flipped~~ talent placing the flipped leaf so that the abaxial side faces up and centering it on the stage.
- 2.8. Now gently rotate the coarse and fine adjustment knobs to focus on the midrib of the leaf [1]. Continue adjusting until the image appears clear and without blur [2].
- 2.8.1. SCOPE/SCREEN: The midrib is being focused.
- 2.8.2. SCOPE/SCREEN: Show the live image of the leaf midrib coming into sharp focus without blur.
- 2.9. ~~Position the lower end of the midrib, where the nectary is located, at the center of the field of view, including the diverging lateral veins~~ Position the lower end of the midrib, where the nectary is located, at the center of the field of view while ensuring clear visibility of the diverging lateral veins [1]. Using the coarse and fine adjustment knobs, further refine the focus to improve digital image clarity [2].
- NOTE: VO in [1] slightly modified to make the action clearer.**
- 2.9.1. SCOPE/SCREEN: Show the nectary region centered on the monitor, including the midrib and diverging lateral veins.
- 2.9.2. Talent adjusting the coarse and fine knobs while the image sharpens on the screen.
- Videographer's NOTE: 2.9.2 was mis-shot as scope racking focus.**
- 2.10. Capture the nectary image [1]. When prompted by the program window, save the image with the sample identification number at the required location [2]. Score leaf nectary digital image from 1 to 4 based on the nectary phenotype ~~based on the acquired digital image~~ [3]. **NOTE: VO in [1] slightly modified at the postshoot stage**
- 2.10.1. SCOPE/SCREEN: The nectary image is being captured.
- 2.10.2. SCOPE/SCREEN: Display the save dialog window with location options and demonstrate saving the image with the sample identification number.
- 2.10.3. SCOPE/SCREEN: A phenotype score is being assigned.

Flower Tissue Preparation and Microscopic Imaging of Bracteal Nectaries

NOTE: Treat this as a title card for a separate section (added at postshoot stage)

- 2.11. Use forceps or ~~manually~~ hand to remove the bracts from the flower [1]. Place the flower onto a clean cutting board [2].
- 2.11.1. Talent removing bracts from the flower using forceps or by hand.
- 2.11.2. Talent placing the flower onto a clean cutting board.

2.12. Using a sterile blade, cut the stalk of the flower [1]. Then make a straight incision along the edge of the removed bracts [2].

2.12.1. Talent cutting the flower stalk with a sterile blade.

2.12.2. Talent making a straight incision along the edge where the bracts were removed.

2.13. Place the prepared flower tissue upside down onto the precut white sheet on the microscope stage [1]. Use the coarse and fine adjustment knobs to ~~adjust the focus to~~ improve the clarity of the image displayed on the screen [2].

2.13.1. Talent positioning the flower tissue upside down on the white sheet on the microscope stage.

2.13.2. SCOPE/SCREEN: Microscopic image of the flower is being seen.

2.14. ~~Save the image with the appropriate sample identification number [1]. Score the leaf nectary and bracteal nectaries between 1 to 4 based on the observed phenotype in the acquired digital images [2].~~

Videographer's NOTE: Author said omit shot 2.14.1 and refer to lab media.

Author's NOTE: The authors have deleted [2] and the corresponding shot 2.14.2

NOTE: The LAB MEDIA was not included because the supplementary figure 2, cited in the manuscript does not match the text in the VO.

2.14.1. SCOPE/SCREEN: Display the save dialog window and demonstrate saving the image with the sample identification number.

2.14.2. ~~SCOPE/SCREEN: Show the saved digital images of the nectaries while the talent assigns phenotype scores from 1 to 4.~~

Results

3. Results

3.1. Digital image scoring of the leaf abaxial surface on the lower side of the mid rib showed two phenotypes with score 1, with no nectaries on the mid rib [1], and score 4, with a fully developed nectary with nectar [2].

3.1.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the dashed circle*

3.1.2. LAB MEDIA: Figure 4A. *Video editor: Highlight the dashed circle labeled*

3.2. When flower samples were analyzed for bracteal nectaries, two phenotypes were observed, with score 1 showing no nectary [1] and score 4 showing a fully formed nectary producing nectar [2].

3.2.1. LAB MEDIA: Figure 4D *Video editor: Highlight the dashed circle labeled “ No bracteal nectary” in D*

3.2.2. LAB MEDIA: Figure 4C *Video editor: Highlight the dashed circles*

3.3. Digital images of leaf nectaries of nectaried plants clearly visualized nectary traits at 10 times magnification [1], 20 times magnification [2], and 40 times magnification [3].

3.3.1. LAB MEDIA: Figure 5B. *Video editor: Highlight the circled leaf nectary area labeled “10X”.*

3.3.2. LAB MEDIA: Figure 5C. *Video editor: Highlight the circled leaf nectary area labeled “20X”.*

3.3.3. LAB MEDIA: Figure 5D. *Video editor: Highlight the circled leaf nectary area labeled “40X”.*

3.4. Digital images of bracteal nectaries of nectaried plants were also clearly visualized nectary traits at 10 times magnification [1], 20 times magnification [2], and 40 times magnification [3].

3.4.1. LAB MEDIA: Figure 5F. *Video editor: Highlight the circled bracteal nectary area labeled “10X”.*

3.4.2. LAB MEDIA: Figure 5G. *Video editor: Highlight the circled bracteal nectary area*

labeled "20X".

3.4.3. LAB MEDIA: Figure 5H. *Video editor: Highlight the circled bracteal nectary area labeled "40X".*

3.5. Selected leaf digital images corresponding to the standard scoring format showed score 1 with absence of nectary [1], score 2 with an underdeveloped nectary [2], score 3 with a smaller nectary [3], and score 4 with a fully developed nectary [4].

3.5.1. LAB MEDIA: Figure 6. *Video editor: Highlight the dashed circle labeled "Score 1" in A*

3.5.2. LAB MEDIA: Figure 6. *Video editor: Highlight the dashed circle labeled "Score 2" in B*

3.5.3. LAB MEDIA: Figure 6. *Video editor: Highlight the dashed circle labeled "Score 3" in C*

3.5.4. LAB MEDIA: Figure 6. *Video editor: Highlight the dashed circle labeled "Score 4" in D*

Pronunciation Guides

1. Phenotyping

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

IPA: /'fi:nɒʊˌtaɪpɪŋ/

Phonetic Spelling: fee·noh·tai·ping

2. Greenhouse

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

IPA: /'ɡriːnˌhaʊs/

Phonetic Spelling: green·hows

3. Nectaries

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

IPA: /'nekˌteri/

Phonetic Spelling: नेक·tair·ee

4. Videographer

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

IPA: /ˌvɪdiˈɑːɡrəfər/

Phonetic Spelling: vih·dee·aa·gruh·fer

5. Magnification

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

IPA: /ˌmæɡnəfəˈkeɪʃən/

Phonetic Spelling: mag·nuh·fuh·kay·shuhn

6. Abaxial

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

IPA: /æbˈæks.i.əl/

Phonetic Spelling: ab·ak·see·uhl

7. Midrib

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

IPA: /ˈmɪdˌrɪb/

Phonetic Spelling: mid·rib

8. Nectary

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

IPA: /ˈnekˌteri/

Phonetic Spelling: नेक·tair·ee

9. Phenotype

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

IPA: /ˈfiːnəˌtaɪp/

Phonetic Spelling: fee·nuh·taiyp

10. Bracteal

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

IPA: /ˈbræk.ti.əl/

Phonetic Spelling: brak·tee·uhl

11. Forceps

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

IPA: /ˈfɔːrˌseps/

Phonetic Spelling: for·seps

12. Sterile

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

IPA: /ˈstɛrəl/, /ˈstɪrəl/

Phonetic Spelling: ster·uhl

13. Incision

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

IPA: /ɪnˈsɪʒən/

Phonetic Spelling: in·sizh·uhn

14. Microscopic

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

IPA: /ˌmaɪkrəˈskɑːpɪk/

Phonetic Spelling: mai·kruh·skop·ik

15. Laboratory

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

IPA: /ˈləbrəˌtɔːri/, /ˈləbəˌtɔːri/

Phonetic Spelling: lab·ruh·tor·ee

