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Title: Multipronged Phenotyping Approaches to Characterize Sugarcane Root Systems

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Author Questionnaire

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

2. **Software:** Does the part of your protocol being filmed demonstrate software usage? **YES**

3. **Interview statements: Please select one**.

   ✓ ☒ Interviewees self-record interview statements.

4. **Proposed filming date:** To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **08/29/2022**
When you are ready to submit your video files, please contact our Content Engineer, Devon Halley.

Protocol Length
Number of Steps: 23
Number of Shots: 45

Introduction

REQUIRED:

1.1. **Krishnapriya V.**: As a primary conductor of water and nutrients, roots are indispensable for growth and development. A thorough understanding of the root system is important for sugarcane, as it takes a year to complete its life cycle **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.4.1. and 4.3.1.*

1.2. **Vasantha S.**: The holistic approach here integrates the available platforms for root phenotyping. This will benefit in advancing the knowledge of sugarcane root systems and their responses to adverse growth conditions **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.5.2. and 4.6.1.*

1.3. **Krishnapriya V.**: This methodology is specific to the sugarcane crop, as it has a unique root system as a long crop. However, with minor modifications, this method may be adapted to other crops **[1]**.

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

1.4. **Arun Kumar R.**: We need to exercise caution while interpreting the results obtained from individual protocol. An in-situ study of root systems is ideal for sugarcane, but controlled platforms may be useful for specialized purposes **[1]**.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.2.1., 4.1.1. and 5.1.1.*

1.5. **Arun Kumar R.**: Understanding various aspects of a root phenotype is essential to re-design the sugarcane varieties. Automating the techniques and integrating big data analytics or artificial intelligence to interpret the results would broaden our expertise in the field **[1]**.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.4.3.*

1.6. **Vasantha S.**: Our efforts in characterizing root system traits under normal and abiotic stress situations have paved the way for exploratory research areas, like root plasticity under varying nutrient regimes and biotic stresses **[1]**.

1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.6.1.*

Protocol

2. Field Excavation by Trench Sampling

2.1. To begin, raise the commercial sugarcane hybrids or varieties in the field using two-budded setts planted at a row spacing of 120 centimeters and 90 centimeters within the rows. Follow the recommended package of practices to ensure good crop establishment and growth **[1]**. NOTE: Step2.1to2.3_trench are images used as slide shows in video form.

2.1.1. WIDE: Sugarcane field with varieties planted at a row spacing of 120 cm, with 90 cm within rows being seen.

2.2. At the end of the maturity phase, employ an excavator to dig a trench of 1.5 meters deep and 1.0 meters wide in the field **[1]**. Clear the soil from the root zones without damaging the roots with continuous water jetting **[2]**. NOTE: Step2.1to2.3_trench are images used as slide shows in video form.

2.2.1. A trench in the field near the sugarcane crop being seen.

2.2.2. Water jetting being seen clearing the soil from the root zone.

2.3. Once the adhering soil loosens due to jetting, uproot the cane along with the root system **[1]** and take it to the laboratory for manual measurement of the growth parameters, namely, the number of roots, root length, volume, and weight **[2]**. NOTE: Step2.1to2.3_trench are images used as slide shows in video form.

2.3.1. Talent uprooting the cane along with the root system.

2.3.2. Talent taking measurements of the sugarcane root inside the laboratory.

3. Root Core Sampler to Reduce Sampling Errors

3.1. Use a cylindrical root core sampler of 61 centimeters in height, 16 centimeters in diameter, weighing 8 kilograms, and fabricated using mild steel or MS material for sugarcane root sampling in the field **[1]**.

3.1.1. WIDE: Cylindrical root core sampler being seen.

3.2. It is provided with a sharp edge at the bottom for easy penetration **[1]** and has collars of 3 centimeters in diameter at the top to lift the sampler **[2]**.

3.2.1. Talent showing the sharp edge present at the bottom of the root core sampler.

3.2.2. Talent showing top with collars present at the top.

3.3. At the maturity phase of the commercial sugarcane crop, fasten the top edge of the sampler to the primary shoot or cane **[1]** and hammer continuously to reach the desired soil depth of 45 centimeters **[2]**. Then, lift the entire soil mass into the sampler **[3]** and wash it carefully under running water to separate the adhering roots **[4]**. NOTE: Step3.3_rootcoring are images used as slide shows in video form.

3.3.1. Talent fastening the top edge of the sampler to the cane.

3.3.2. Talent hammering the sampler.

3.3.3. Talent lifting the sampler along with soil from the root zone.

3.3.4. Talent washing the roots.

3.4. After thoroughly washing the roots, manually record the physical parameters, namely volume, surface area, length, and weight **[1]**. Spread the roots on transparent trays **[2]** for scanning and analyzing the corresponding digitized images using the software **[3-TXT]**.

3.4.1. Talent recording the physical parameters of the plant.

3.4.2. Talent spreading the roots on transparent trays.

3.4.3. SCREEN: Digitized images of the root being seen.

4. Root Phenotyping Structure to Facilitate Sampling at Different Phenophases

4.1. Construct a root phenotyping structure comprising three adjacent compartments of 4.5 meters by 10.1 meters for sampling the sugarcane roots **[1]**. Make provision for manually dismantling the side walls to reveal the underground root system up to a depth of 80 to 100 centimeters **[2]**.

4.1.1. WIDE: The root phenotyping structure being seen.

4.1.2. Talent pointing at the provisions made for manually dismantling the side walls

4.2. Fill and compact the structure with field soil, leaving a headspace of around 20 centimeters and having adequate drainage holes to facilitate soil aeration **[1]**.

4.2.1. Drainage holes of phenotyping structure being seen.

4.3. Sow the bud chips of germplasm clones comprising *Saccharum officinarum*, *Saccharum spontaneum*, *Saccharum barberi*, *Saccharum sinense*, and *Saccharum robustu* **[1]**. Allow them to germinate for 30 days in portrays comprising rooting media **[2-TXT]**.

4.3.1. Talent sowing the bud chips in a root phenotyping structure.

4.3.2. 30 days old, germinated sugarcane species being seen. **TXT: Rooting Media:; Red soil: Farmyard manure: Sand = 2:2:1** *Video Editor: The second line is a ratio.*

4.4. After 30 days of germination, transplant the uniform and healthy settlings to the structure in the rows with a spacing of 90 centimeters and 60 centimeters distance within rows. Follow the recommended POP to ensure good crop establishment and growth **[1]**.

4.4.1. Talent transplanting settling to the structure. Uniform and healthy transplanted sugarcane present in the structure being seen.

4.5. During the formative phase of 60 to 120 days after planting and the grand growth phase of 120 to 150 days after planting, manually remove the side walls made of the precast slabs **[1]** before continuously spraying the water jet to expose the roots **[2]**.

4.5.1. Talent manually removing the side walls.

4.5.2. Continuous spraying of the water jet resulting in exposed roots.

4.6. Uproot the entire root system and transfer it to the laboratory to measure the physical parameters. Then, spread the roots on transparent trays for scanning and analyzing the corresponding digitized images in software **[1]**.

4.6.1. Entire uprooted root system being seen.

~~4.7. Withhold the irrigation in one of the compartments to impose the drought stress and plug the drainage holes in the second compartment to maintain soil saturation and simulate waterlogging stress **[1]**. In the third compartment, irrigate the soil according to the recommended POP to maintain the field capacity and serve as a control **[2]**.~~ NOTE: The authors didn't provide footage for this step.

~~4.7.1. Talent plugging the drainage hole.~~

~~4.7.2. Talent irrigating the third compartment. The properly irrigated soil from the third compartment being seen.~~

5. Hydroponic Culture of Plants to Study Rhizosphere Biology

5.1. To study the rhizosphere biology and the finer details of root biology, fabricate an in-house hydroponic system in an environment-controlled glasshouse conducive to sugarcane growth **[1]**. NOTE: Step5.1and5.2_hydroponics are images used as slide shows in video form.

5.1.1. WIDE: An in-house hydroponic system being seen.

5.2. In the 20 by 20 by 50 centimeters dimensions tanks with aquarium pumps for aeration **[1]**, add around 15 liters of modified Hoagland's nutrient solution **[2]**. NOTE: Step5.1and5.2_hydroponics are images used as slide shows in video form.

5.2.1. The glass tanks with aquarium pump being seen.

5.2.2. Talent adding 15L of modified Hoagland's nutrient solution to the glass tanks.

5.3. Sow the bud chips of sugarcane varieties and *Saccharum* species clones and allow them to germinate for 30 days in the portrays comprising composted coir pith **[1]** before transplanting the uniform and healthy settlings to hydroponic tanks at the frequency of three settlings per tank **[2-TXT]**.

5.3.1. Talent sowing the bud chips in the portrays containing composted coir pith.

5.3.2. Talent transferring the healthy settlings to hydroponic tanks. **TXT: Cover tanks with black cloth to block light from reaching the roots; Attach a plastic mesh at the glass tanks' brim to support the plants**

~~5.4. Ensure the light does not reach the roots by covering the tanks with black cloth **[1]**. Also, attach a plastic mesh at the glass tanks' brim to support the plants **[2]**.~~NOTE: The authors didn't provide footage for this step. The information has been added as onscreen text in 5.3.

~~5.4.1. Talent covering the tank with black cloth.~~

~~5.4.2. Talent attaching a plastic mesh at the brim of the glass tanks for support.~~

5.5. After 60 days, when the germination phase ends, immerse the roots of intact plants in 50 milliliters of sterile double-distilled water or trap solution for 4 hours **[1]** and collect the root exudates during the peak photosynthetic activity **[2-TXT]**.

5.5.1. Talent immersing the roots of the intact plant in 50 mL trap solution.

5.5.2. Talent collecting root exudates **TXT: Peak Photosynthetic Activity: 0800h to 1200h**

~~5.6. Filter the collected solution through Whatman filter paper **[1]** and pass it through glass columns filled with anion exchange **[2]**, followed by cation exchange resins **[3]**. Evaporate the eluted fractions to dryness **[4]** before storing them at minus 20 degrees Celsius until further processing **[5]**.~~NOTE: The authors didn't provide footage for this step.

~~5.6.1. Talent filtering the collected solution through Whatman filter paper.~~

~~5.6.2. Talent passing the filtered solution through the anion exchange column.~~

~~5.6.3. Talent passing the solution through the cation-exchange resins.~~

~~5.6.4. Talent placing the eluted fractions for drying.~~

~~5.6.5. Talent placing the dried fractions at −20 °C.~~

~~5.7. Analyze the collected root exudate samples using HPLC to determine organic acids **[1]** and a spectrophotometer to estimate the total phenolics, proteins, sugars, and amino acids according to standard protocol **[2]**.~~NOTE: The authors didn't provide footage for this step.

~~5.7.1. Talent injecting a sample in HPLC.~~

~~5.7.2. Talent placing the sample in a spectrophotometer.~~

5.8. Assess the activity of the enzymes, peroxidase and superoxide dismutase, and total phenolic content in the third month according to standard protocol **[1]**. NOTE: The VO has been edited.

~~5.8.1. Talent monitoring the roots.~~NOTE: The authors didn't provide footage for this step.

5.8.2. Talent performing an addition for the biochemical test.

5.9. Finally, assess the response to root injury by inflicting a longitudinal slice in the primary root up to the root tip using a sterile surgical blade and monitor the changes periodically **[1]**.

5.9.1. Talent inflicting a longitudinal slice in the primary root using a surgical blade.

Results

6. Results

6.1. In the root phenotyping **[1]**, the longer roots of Co 62175 *(C-O-6-2-1-7-5)* demonstrated a superior root system for cumulative root length **[2]**, root surface area **[3]**, root volume **[4]**, and average root diameter **[5]**.

6.1.1. LAB MEDIA: Figure 5.

6.1.2. LAB MEDIA: Figure 5. *Video Editor: Please emphasize the tallest bar belonging to Co 62175 in 5C.*

6.1.3. LAB MEDIA: Figure 5. *Video Editor: Please emphasize the tallest bar belonging to Co 62175 in 5D.*

6.1.4. LAB MEDIA: Figure 5. *Video Editor: Please emphasize the tallest bar belonging to Co 62175 in 5E.*

6.1.5. LAB MEDIA: Figure 5. *Video Editor: Please emphasize the tallest bar belonging to Co 62175 in 5F*

6.2. Saccharum species showed significant variations in morphological traits during the formative phase **[1]**. *Saccharum spontaneum* had the highest cumulative root length per clump **[2]**, while *Saccharum sinense* showed the lowest **[3]**.

6.2.1. LAB MEDIA: Figure 6.

6.2.2. LAB MEDIA: Figure 6. *Video Editor: Please emphasize the tallest bar belonging to S. spontaneum in 6A.*

6.2.3. LAB MEDIA: Figure 6. *Video Editor: Please emphasize the shortest bar belonging to S. sinense in 6A.*

6.3. *Saccharum spontaneum* showed **[1]** the highest root surface area **[2]**, root dry weight **[3]**, root volume **[4]**, and the root-to-shoot ratio **[5]**.

6.3.1. LAB MEDIA: Figure 6.

6.3.2. LAB MEDIA: Figure 6. *Video Editor: Please emphasize the tallest bar belonging to S. spontaneum in 6B.*

6.3.3. LAB MEDIA: Figure 6. *Video Editor: Please emphasize the shortest bar belonging to S. sinense in 6F.*

6.3.4. LAB MEDIA: Figure 6. *Video Editor: Please emphasize the shortest bar belonging to S. sinense in 6G.*

6.3.5. LAB MEDIA: Figure 6. *Video Editor: Please emphasize the shortest bar belonging to S. sinense in 6H.*

6.4. *Saccharum robustum* **[1]** showed the highest root volume **[2]** and root diameter **[3]**.

6.4.1. LAB MEDIA: Figure 6.

6.4.2. LAB MEDIA: Figure 6. *Video Editor: Please emphasize the tallest bar belonging to S. spontaneum in 6C.*

6.4.3. LAB MEDIA: Figure 6. *Video Editor: Please emphasize the shortest bar belonging to S. sinense in 6D.*

6.5. Anatomical studies revealed that **[1]** long root hairs **[2]**, sclerenchymatous exodermis **[3]**, reduced cortical cell layers, increased cortical aerenchyma **[4]**, and increased stele area, and xylem vessel number with large diameter were beneficial under drought conditions **[5]**.

6.5.1. LAB MEDIA: Figure 7.

6.5.2. LAB MEDIA: Figure 7. *Video Editor: Please emphasize the root hairs denoted by 'rh' in 7B.*

6.5.3. LAB MEDIA: Figure 7. *Video Editor: Please emphasize the exodermis denoted by 'ex' in 7B.*

6.5.4. LAB MEDIA: Figure 7. *Video Editor: Please emphasize the empty areas of aerenchyma denoted by 'ae' in 7B.*

6.5.5. LAB MEDIA: Figure 7. *Video Editor: Please emphasize the small round holes of xylem denoted by 'mx' in 7B.*

6.6. Whereas under waterlogging stress **[1]**, increased root diameter **[2]** and higher cortical cell area with increased aerenchymatous cells were observed **[3]**.

6.6.1. LAB MEDIA: Figure 7. *Video Editor: Please emphasize 7C.*

6.6.2. LAB MEDIA: Figure 7. *Video Editor: Please emphasize the circular area enclosing small circles in 7C.*

6.6.3. LAB MEDIA: Figure 7. *Video Editor: Please emphasize the empty area of aerenchyma denoted by 'ae' in 7C.*

6.7. *Saccharum robustum* **[1]** showed the highest total carboxylate exudation **[2]**, while the lowest exudation was recorded in *Saccharum spontaneum* **[3]**.

6.7.1. LAB MEDIA: Figure 8.

6.7.2. LAB MEDIA: Figure 8. *Video Editor: Please emphasize the last black dot belonging to S. robustum 8A.*

6.7.3. LAB MEDIA: Figure 8. *Video Editor: Please emphasize the black dot belonging to S. spontaneum in 8A and all the bars belonging to S. spontaneum in 8 B, C, D, E.*

6.8. In Sugarcane varieties **[1]**, a significant difference in the root hair density was observed **[2]**, with variation in shape and pigmentation of the root cap **[3]**.

6.8.1. LAB MEDIA: Figures 9 A-C.

6.8.2. LAB MEDIA: Figures 9 A-C. *Video Editor: Please emphasize small hairs on roots in 9A and 9B.*

6.8.3. LAB MEDIA: Figures 9 A-C. *Video Editor: Please emphasize the colored part present at the end of the roots.*

6.9. The highest peroxidase activity and total phenolic content **[1]** were present in Co 86032 *(C-O-8-6-0-3-2)* **[2]** and the lowest in Co 62175 *(C-O-6-2-1-7-5)* **[3]**.

6.9.1. LAB MEDIA: Figures 9 D-F.

6.9.2. LAB MEDIA: Figures 9 D-F. *Video Editor: Please emphasize the last tallest bar belonging to Co86032 in 9D and 9F.*

6.9.3. LAB MEDIA: Figures 9 D-F. *Video Editor: Please emphasize the middle shortest bar belonging to Co 2175 in 9D and 9F.*

6.10. During rejuvenation studies **[1]**, CoC 671 showed the appearance of secondary roots in 3 days, **[2]** and Co 06022 took 10 days, with symptoms of senescence on the third day **[3]**.

6.10.1. LAB MEDIA: Figures 10.

6.10.2. LAB MEDIA: Figures 10. *Video Editor: Please emphasize 10 A-E.*

6.10.3. LAB MEDIA: Figures 10. *Video Editor: Please emphasize 10 F-I.*

Pronunciation Guides:

1. **Exodermis**
   Pronunciation link: https://www.merriam-webster.com/dictionary/exodermis
   IPA: /ˌɛksəˈdɜrmɪs/
   Phonetic Spelling: ek-suh-DUR-mis
2. **Aerenchyma**
   Pronunciation link: https://www.merriam-webster.com/dictionary/aerenchyma

IPA: /ˌɛriˈŋkɪmə/
Phonetic Spelling: air-RINK-ih-muh

3. **Peroxidase**
   Pronunciation link: https://www.merriam-webster.com/dictionary/peroxidase
   IPA: /ˌpɛrəˈɒksɪˌdeɪz/
   Phonetic Spelling: peh-ruh-OK-sih-dayz
4. **Superoxide dismutase**

- Superoxide
  Pronunciation link: https://www.merriam-webster.com/dictionary/superoxide
  IPA: /ˌsuːpəˈɒksaɪd/
  Phonetic Spelling: soo-puh-OK-side
- Dismutase
  Pronunciation link: No confirmed link found
  IPA: /ˌdɪsˈmjuːteɪs/
  Phonetic Spelling: dis-MYOO-tays

5. **Rhizosphere**
   Pronunciation link: https://www.merriam-webster.com/dictionary/rhizosphere
   IPA: /ˈraɪzəˌsfɪər/
   Phonetic Spelling: RY-zoh-sfeer
6. **Hoagland**
   Pronunciation link: https://www.merriam-webster.com/dictionary/Hoagland
   IPA: /ˈhoʊgˌlænd/
   Phonetic Spelling: HOHG-land
7. **Sclerenchymatous**
   Pronunciation link: No confirmed link found
   IPA: /sklɛrəŋˈkaɪmətəs/
   Phonetic Spelling: skleh-ren-KIE-ma-tus
8. **Phenotyping**
   Pronunciation link: https://www.merriam-webster.com/dictionary/phenotype
   IPA: /ˈfinoʊˌtaɪpɪŋ/
   Phonetic Spelling: FEE-no-type-ing
9. **Exudates**
   Pronunciation link: https://www.merriam-webster.com/dictionary/exudate
   IPA: /ˈɛgzjəˌdeɪt/
   Phonetic Spelling: EG-zue-dayt
10. **Uproot**
    Pronunciation link: https://www.merriam-webster.com/dictionary/uproot
    IPA: /ˌʌpˈrut/
    Phonetic Spelling: up-ROOT