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A Plate Growth Assay to Quantify Embryonic Root Development of *Zea mays*

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Abstract

Murashige-Skoog medium solutions have been used in a variety of plant plate growth assays, yet most research uses *Arabidopsis thaliana* as the study organism. For larger seeds such as maize (*Zea mays)*, most protocols employ a paper towel roll method for experiments, which often involves wrapping maize seedlings in wet, sterile germination paper. What the paper towel roll method lacks, however, is the ability to image the roots over time without risk of contamination. Here, we describe a sterile plate growth assay that contains Murashige-Skoog medium to grow seedlings starting two days after germination. This protocol uses a section of a paper towel roll method to achieve uniform germination of maize seedlings, which are sterilely transferred onto large acrylic plates for the duration of the experiment. The media can undergo modification to include an assortment of plant hormones, exogenous sugars, and other chemicals. The acrylic plates allow researchers to freely image the plate without disturbing the seedlings and control the environment in which the seedlings are grown, such as modifications in temperature and light. Additionally, the protocol is widely adaptable for use with other cereal crops.

Key features

- Builds upon plate growth methods routinely used for *Arabidopsis* seedlings but that are inadequate for maize.
- Real-time photographic analysis of seedlings up to two weeks following germination.
- Allows for testing of various growth conditions involving an assortment of additives and/or modification of environmental conditions.
- Samples are able to be collected for genotype screening.

Keywords: Plate assay, Murashige-Skoog, Maize, Imaging, Embryonic root system, Embryonic shoot system

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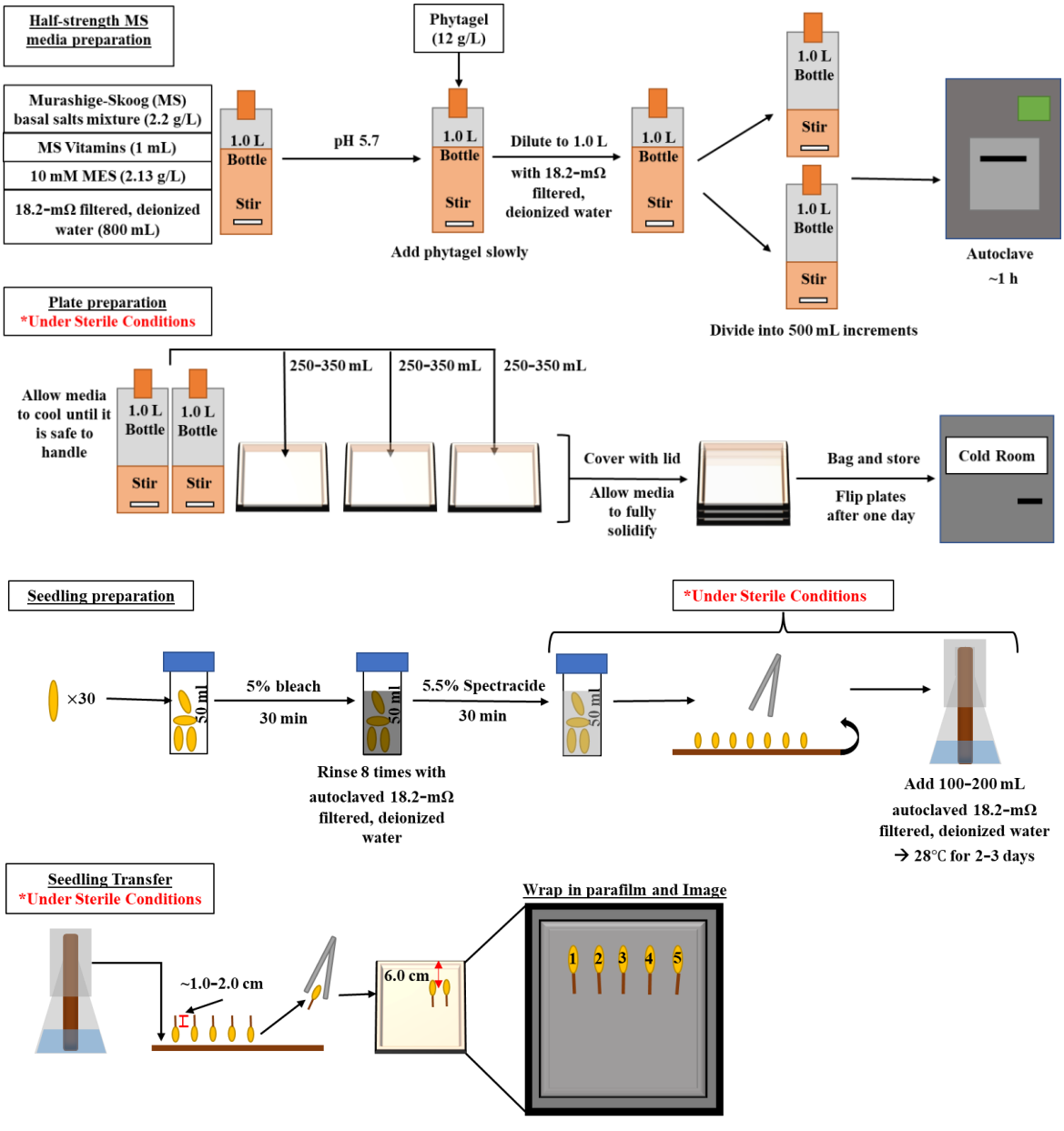
Graphical overview



Background

Murashige-Skoog (MS) medium solutions have been used to provide the essential nutrients for plant development in root growth assays and can readily undergo alterations to incorporate a broad range of nutrients, chemicals, exogenous sugars, and plant hormones. A variety of these assays are routinely used in growing *Arabidopsis thaliana* (LaMontagne et al., 2016; Collins et al., 2020; Deslandes-Hérold et al., 2022; Mathew et al., 2023; Montpetit et al., 2023) and have included additives such as sodium chloride (Zhou et al., 2018), polyethylene glycol (PEG) (Zheng et al., 2016), and gibberellins (Unterholzner et al., 2015). For crops that produce larger seeds, such as maize, few protocols have been described employing MS media for seedling development. Most protocols utilize paper towel rolls to germinate and grow seedlings (Abdel-Ghani et al., 2016; Draves et al., 2022). Similarly, other additives can

be incorporated into paper towel roll methods. A disadvantage to this method, however, includes imaging. To image the roots consistently, the rolls need to be removed from the incubator and laid out on a surface long enough to collect data before re-rolling and placing them back in the incubator, risking contamination if not carefully done. Imaging the progress of root growth over designated time intervals becomes restricted (Dowd et al., 2019).

Here, we introduce a sterile plate growth assay consisting of a half-strength MS medium used to grow maize seedlings starting two days after germination (DAG). This procedure is based on a protocol in a previously published paper (Julius et al., 2021) and was modified extensively to produce an assay functional to a wide range of experiments. Like paper towel roll methods, which can control environmental conditions such as temperature and light (Hochholdinger, 2009), this procedure also grants researchers control over the environment in which the seedlings develop. Moreover, the plates can be easily removed from an incubator and imaged at any time without disturbing the seedlings. The procedure can grow seedlings under a variety of conditions and treatments in a sterile environment, allowing researchers to investigate numerous effects in the embryonic root and shoot systems of maize and other cereal crops.

Materials and reagents

Materials

1. Three 24.5 cm × 24.5 cm acrylic square plates (Fischer Scientific, catalog number: 12-565-224)
2. Four 1.0 L bottles (w/caps)
3. Two stir bars
4. One spatula
5. One piece of 4" × 4" weigh paper (Fisherbrand, catalog number: 09-898-12B)
6. One piece of 6" × 6" weigh paper (Fisherbrand, catalog number: 09-898-12C)
7. One 1.0 L graduated cylinder
8. One 2-gallon zip-loc bag
9. One black marker
10. One 1.0 L Erlenmeyer flask
11. One 1.0 L beaker
12. One piece of 38# Regular weight seed germination paper, 10" × 15" (Anchor Paper Company, catalog number: SD3815L)
13. Three sets of flathead forceps
14. One 50 mL conical tube (Fisher Scientific, catalog number: 14-955-239)
15. Parafilm; 15 square (or 75 cm) strips
16. Aluminum foil
17. Plastic 6" ruler

Biological materials

This protocol was initially optimized to successfully grow maize seedlings of the B73 cultivar. Subsequent studies using other chemicals, maize mutants, and cereal seeds were performed (Slewinski and Braun, 2010; Tran et al., 2019; Huang et al., 2020; Babst et al., 2021), and examples will be provided later in this protocol. However, we recommend practicing this protocol with B73 seedlings.

1. Maize seedlings (B73 background)

Reagents

1. Bleach (liquid)
2. Ethanol
3. Spectracide (Immunox multi-purpose fungicide spray concentrate; any hardware store)

4. Murashige-Skoog basal medium (MP Biomedicals LLC, catalog number: 2623122)
5. MS vitamin solution (MP Biomedicals LLC, catalog number: 2625149)
6. MES (Millipore Sigma, catalog number: 6110-OP)
7. Phytagel (Sigma-Aldrich, catalog number: P8169)
8. Sodium hydroxide (NaOH) (Fisher Scientific, catalog number: S318-1)

Solutions

1. 5% bleach (see Recipes)
2. 5.5% spectracide (see Recipes)
3. 70% ethanol (see Recipes)
4. Half-strength MS media (see Recipes)

Recipes

1. 5% bleach

Note: Use 18.2 mΩ filtered, deionized water.

Reagent	Final concentration	Quantity (1.0 L)
Bleach	5%	50 mL
H_2O	n/a	950 mL
Total	n/a	1,000 mL

2. 5.5% spectracide

Note: Use 18.2 mΩ filtered, deionized water.

Reagent	Final concentration	Quantity (1.0 L)
Spectracide	5.5%	55 mL
H_2O	n/a	945 mL
Total	n/a	1,000 mL

3. 70% ethanol

Note: Use 18.2 mΩ filtered, deionized water. The solution will be mixed in a spray bottle; pour volumes as needed.

Reagent	Final concentration	Quantity (1.0 L)
Ethanol (absolute)	70%	700 mL
H_2O	n/a	300 mL
Total	n/a	1,000 mL

4. Half-strength MS media

Note: Use 18.2 mΩ filtered, deionized water. Preparation of 1.0 L of media makes approximately three plates; see Procedure C for preparation instructions. Any additional ingredients must be calculated and experimented with independently.

Murashige-Skoog (MS) basal salts mixture (2.2 g/L)
MS vitamins at 1× (1 mL of vitamins per 1.0 L of media)
10 mM MES (2.13 g/L)
Phytagel (12 g/L)
18.2 mΩ filtered, deionized water (autoclaved)
Optional: Other additives can be incorporated, but the volumes need to be calculated for a 1.0 L solution.

Equipment

1. Incubator (Lindberg/Blue M, model: GI200C) set at 28 °C
2. Laminar flow hood
3. pH meter
4. 200–1,000 pipettor
5. 200–1,000 pipette tips (sterile aerosol barrier tips)
6. Orbital shaker (VWR, model: DS-500)
7. Stir plates
8. Weigh scale
9. Flatbed scanner (Ricoh, model: MP C6503)

Software

1. ImageJ (version 1.53k) (https://imagej.nih.gov/ij/)
2. SmartRoot (version 4.21) (https://smartroot.github.io/)
3. PDF to JPG converter (https://pdftoimage.com/pdf-to-jpg)
4. Smartphone app to convert images to PDFs, such as Microsoft Lens-PDF Scanner

Procedure

A. Sterilization techniques

It is crucial to work under sterile conditions when handling autoclaved media, equipment, and sterilized seedlings. We will indicate where sterile technique is applied in the procedure. This will be noted by "*Caution: Under sterile conditions*" at the beginning of the step.

1. Tie back long hair or wear a hat to prevent hair follicles from landing in any sterilized area and wear a face mask.
2. Wash your hands and forearms thoroughly with soap and warm water.
3. Put on a new pair of nitrile gloves, spray them with 70% ethanol, and rub your hands together.
   Note: We recommend spraying your gloves with 70% ethanol periodically while working.

B. Autoclaving materials

1. Autoclaving glassware and equipment
   a. Wrap three sets of forceps and one piece of germination paper (individually) with aluminum foil and cover the openings of one 1.0 L Erlenmeyer flask and one 1.0 L beaker with aluminum foil.
      Note: If needed, you can autoclave more glassware and equipment depending on the number of experiments.
   b. Apply a piece of autoclave tape on each piece of equipment and autoclave them.
2. Autoclaving water
   a. Fill two 1.0 L bottles with 18.2 mΩ filtered, deionized water and autoclave both bottles.
      Note: Loosen the cap on the bottles prior to autoclaving. You can autoclave more water if necessary.
   b. Allow the water to cool before use.
      Note: This water is used in seed rinsing (step D4), wetting the germination paper (step D9), and for the incubation process (step D12).

C. Half-strength MS media plate preparation

1. Rinse two stir bars in distilled water. Add a stir bar into two 1.0 L bottles and place on the stir plates.
   Note: The media will be sterilized in step C9. Steps prior to this do not require sterile conditions or equipment. One of the bottles can be set aside until after preparation of 1.0 L of media.
2. Weigh out the following and transfer into one bottle: 2.13 g of MES and 2.2 g of MS basal medium.
   *Note: This is the step at which other additives (*e.g.*, glucose) may be incorporated into the media, but the quantities need to be calculated prior.*
3. Using a 200–1,000 µL pipettor, add 1.0 mL (1,000 µL) of MS vitamins.
4. Add 800 mL of 18.2 mΩ filtered, deionized water and turn on the stir plate.
   Note: You do not need to autoclave the 1.0 L graduated cylinder prior to adding the water.
5. Adjust the pH to 5.7 using NaOH and a pH meter.
6. Weigh out and add 12 g of phytagel to the media.
   Note: It is critical to add the phytagel gradually while the media is stirring rapidly. If the phytagel clumps in the media, it will improperly melt during autoclaving. We recommend having the phytagel powder on 6" × 6" weigh paper folded in half. Carefully lift the weigh paper above the mouth of the bottle and slowly add a spatula's worth at a time. It should take several minutes to add the phytagel.
7. Dilute the media to 1.0 L with 18.2 mΩ filtered, deionized water; keep the media on the stir plate for 5 min.
8. Place the second 1.0 L bottle onto the second stir plate. Pour a 500 mL aliquot of the media into the second bottle.
   Note: Aliquoting the media to 500 mL prevents the media from boiling over during autoclaving.
9. Leave both bottles on the stir plates with the media stirring rapidly for 5 min; autoclave the media.
10. While the media is in the autoclave, turn the UV lights and the fan on in the laminar flow hood to sterilize the work area.
    Note: The UV light should be kept on for 15 min at minimum. However, you can keep the UV light on throughout the duration of the autoclave cycle.
11. After autoclaving, place the media back on the stir plates stirring rapidly; allow the media to cool until it can be handled.
    Note: If you cannot pour the media at the current time, you can store it in a 60 °C oven. The media cannot be remelted after it has cooled.
12. *Caution: Under sterile conditions.* While the media is cooling, turn off the UV lamp and wipe down the surface of the laminar flow hood with 70% ethanol.
13. *Caution: Under sterile conditions.* Pour 250–350 mL aliquots of media into three acrylic square plates. Immediately place the lids on after pouring and allow to cool for at least 1 h.
    Note: We recommend keeping the laminar flow hood fan on while the media cools.
14. *Caution: Under sterile conditions.* With nitrile gloves on, date and label each plate with a black marker and place them in a two-gallon zip-loc bag.
15. *Caution: Under sterile conditions.* Store lid-side up in a walk-in 4 °C cold room (or store at 4 °C). After a day, flip the plates upside down until use.
    Note: Plates are good for up to a month.

D. Seedling germination and transplantation

1. Count out the number of seeds that will be used for the experiment.
   Note: Keep in mind that some of the seeds will not germinate, so you may need to include more seeds in this section. A maximum of 10 seeds can grow on a plate at a time.
2. Place the seeds in a 50 mL conical tube.
3. Sterilize the seeds with 5% bleach and leave on the orbital shaker set at a speed of 150 rpm for 30 min.
4. Pour out the bleach. Rinse the seeds by pouring 50 mL of autoclaved 18.2 mΩ filtered, deionized water into the conical tube, sealing it with the cap, and agitating the seedlings for three seconds. Pour out the water and repeat seven more times.

5. Treat the seeds with 5.5% spectracide and leave on the orbital shaker set at a speed of 150 for 30 min.
6. Pour out the spectracide into an appropriate waste container.
7. Turn on the fan and the UV lights in the laminar flow hood for 15 min.
   Note: The UV light should be kept on for 15 min at minimum. However, you can keep the UV light on throughout the duration of the sterilization process.
8. *Caution: Under sterile conditions.* Turn off the UV light and spray the surface with 70% ethanol.
9. *Caution: Under sterile conditions.* Unwrap and spread a sheet of sterilized germination paper across the sterile laminar flow hood surface and wet it with autoclaved 18.2 mΩ filtered, deionized water.
   Note: We recommend keeping the germination paper on the aluminum foil it was autoclaved in so that it can be handled more easily if it is not in a position for rolling (see step D11).
10. *Caution: Under sterile conditions.* With sterilized forceps, arrange the sterilized seeds onto the paper spaced approximately 1.0–2.0 cm apart.
    Note: If more than 15 seedlings are used, create a second row of seedlings 6–10 cm below the first row to allow space for root development. We recommend having at most 30 seedlings per piece of germination paper. Any number above 30 may make it difficult to insert the germination paper roll into the Erlenmeyer flask.
11. *Caution: Under sterile conditions.* Carefully roll the germination paper up tightly.
12. *Caution: Under sterile conditions.* Unwrap an autoclaved 1.0 L Erlenmeyer flask and add 100–200 mL of autoclaved 18.2 mΩ filtered, deionized water.
13. *Caution: Under sterile conditions.* Fold approximately three inches of the bottom of the rolled-up germination paper and place it inside the 1.0 L Erlenmeyer flask.
14. *Caution: Under sterile conditions.* Cover the flask and the paper with a sterilized 1.0 L beaker.
15. *Caution: Under sterile conditions.* Move the apparatus into a 28 °C incubator and allow the seeds 2–3 days to germinate (Figure 1).
    Note: You may need to unwrap the paper to see if the seeds have germinated. Do this in the laminar flow hood with sterilized surfaces, clean hands, and a new pair of nitrile gloves sprayed with 70% ethanol.

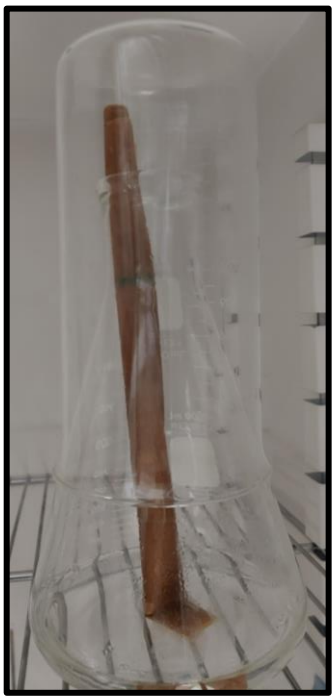


Figure 1. Example of a paper towel roll inside an Erlenmeyer flask residing within an incubator set at 28 °C. Seedlings within the germination paper have their roots growing aligned with the gravitropic vector. The germination paper will wick up water to keep the seeds saturated.

16. Before transplantation, turn on the laminar flow hood fan and UV lights for 15 min.
    Note: The UV light should be kept on for 15 min at minimum. However, you can keep the UV light on for a longer period of time.
17. *Caution: Under sterile conditions.* Turn off the UV light and wipe down the surface with 70% ethanol.
18. *Caution: Under sterile conditions.* Bring three growth media plates from the cold room and the apparatus containing the germinated seedlings. Re-sterilize your nitrile gloves with 70% ethanol.
19. *Caution: Under sterile conditions.* Carefully remove, unroll the germination paper, and check for a primary root length of approximately 1.0–2.0 cm.
20. *Caution: Under sterile conditions.* Take out and uncover one media plate. With sterilized forceps, gently transfer the viable seedlings to the plate and re-cover it with the lid. Repeat for the remaining plates.
    Note: At most, ten seedlings can fit per plate.
21. *Caution: Under sterile conditions.* Embed each seedling into the media approximately 6.0 cm from the top of the plate. Make sure that the primary roots are facing down towards the bottom of the plate (aligned with the gravity vector) during incubation.
    Note: We recommend using two sets of sterilized forceps to transfer the seedlings. Using an extra set of forceps will help keep the seedling from sliding in the media. You can dig out a seed-sized section of the media to help embed the seedling into the media, but you will have to gently push the seedling to insert it. Align the primary root to be flush with the surface of the media. Be careful not to break the developing primary root and/or the root tip. The seedling will need to be disposed of if that occurs.
22. *Caution: Under sterile conditions.* Cover the completed plate with the lid and set it off to the side (on the sterilized laminar flow hood surface). If you are transplanting more seeds, repeat steps D21–D22 until all seedlings are transplanted.
    Note: We recommend wiping the laminar flow hood surface with 70% ethanol and using two new sets of sterilized tweezers between plates.
23. *Caution: Under sterile conditions.* Lift each plate up and hold it vertically to make sure none of the seedlings move or fall.
24. *Caution: Under sterile conditions.* Seal the sides of the plate(s) up with two full wraps of parafilm.
    Note: You can also substitute parafilm with medipore tape.
25. *Caution: Under sterile conditions.* Label the plate with experimental details and number the seedlings on the back of the plate with a black sharpie.
26. *Caution: Under sterile conditions.* Take the plate(s) out to the flatbed scanner and scan the plate. Use color settings, convert the image to jpeg, set the dpi to 300, input a custom scan size of 10.0 in. × 10.0 in., and transfer the scanned image to a computer file.
    Note: The purpose of the custom scan size is to get the scan as close to the plate's lid edge as possible. If necessary, input different custom scan sizes to achieve this.
27. *Caution: Under sterile conditions.* Store the plate(s) in the 28 °C incubator at a near vertical angle with the primary roots facing downward.
28. Image the plate(s) every two days until six days after transferring the seedlings onto plates like in step D26.

Data analysis

Primary root lengths are measured using a plugin in ImageJ, SmartRoot (version 4.21), on JPG images of whole plates over the course of six days; shoots are measured using ImageJ (version 1.53k) on individual JPG images taken before analysis using a black backdrop and a downloadable PDF scanner (see Software #4) of each root system following day six. Both use the Windows operating system. Here, we provide a step-by-step guide on how to use each software to measure root and shoot growth. For ImageJ, we measured a shoot as an example; for SmartRoot, we measured a primary root. We recommend confirming the results on at least one more replicate (at least an extra ten seedlings). All images need to be converted to JPG or TIF formats before opening in ImageJ. We have listed two programs to aid in this process (see Software #3 and #4), but ImageJ should work with other software.

A. Using ImageJ

1. Open your image(s) in ImageJ.
   Note: There are three ways to open an image. (1) Click on File *in the toolbar,* Open, *and search. (2) Press CTRL + O and search. (3) Save your image to your desktop and drag the file onto the ImageJ window.*
2. The program defaults to measure images in pixels; to set the scale, click on the line tool, click, hold on the starting point of the scale, drag the cursor through the length of the scale distance (have a ruler present in the image), release, and press CTRL + M (Figure 2).

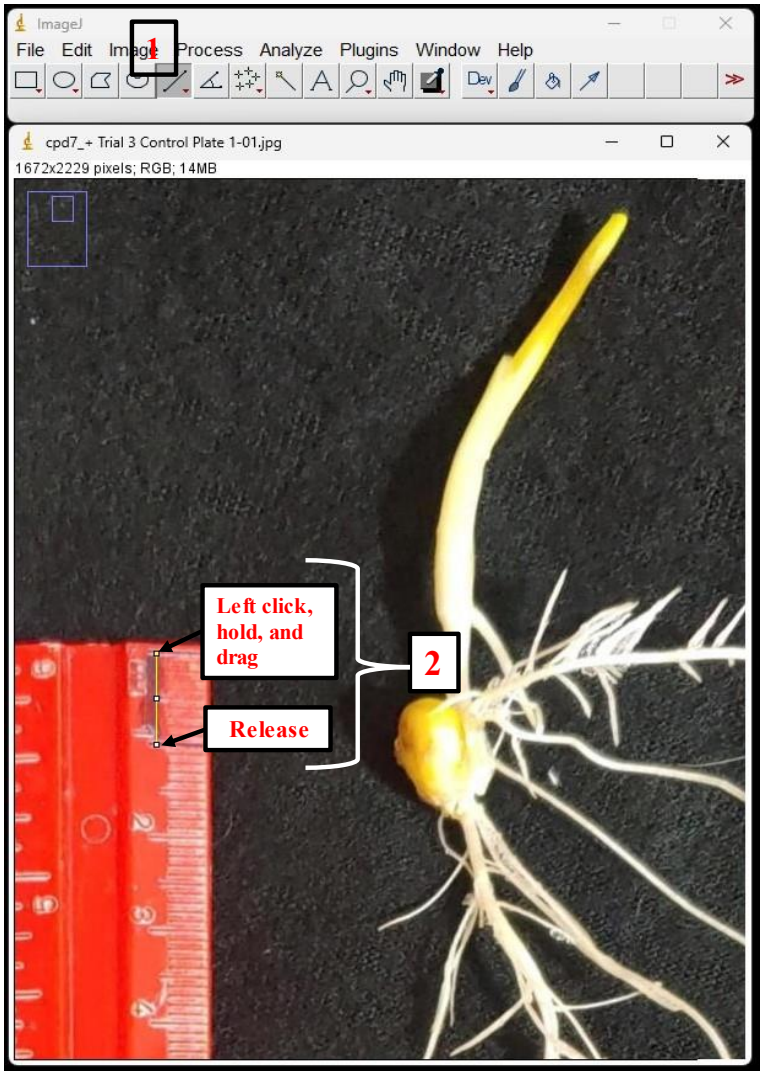


Figure 2. Obtaining the pixel value used in setting the scale. The numbers indicate the steps at which to measure. Select the straight-line tool found in the ImageJ window (step one). Left-click the area where the scale begins (in this case, the start of the 1.0 cm mark), hold down the left mouse button, drag it until the end of the desired scale, and release (step two). Press CTRL + M to get the measurement in pixels.

3. A new window labeled *Results* will open; use the pixel value under *Length* for the scale. Next, click on *Analyze* (in the toolbar) and then *Set Scale* (Figure 3).

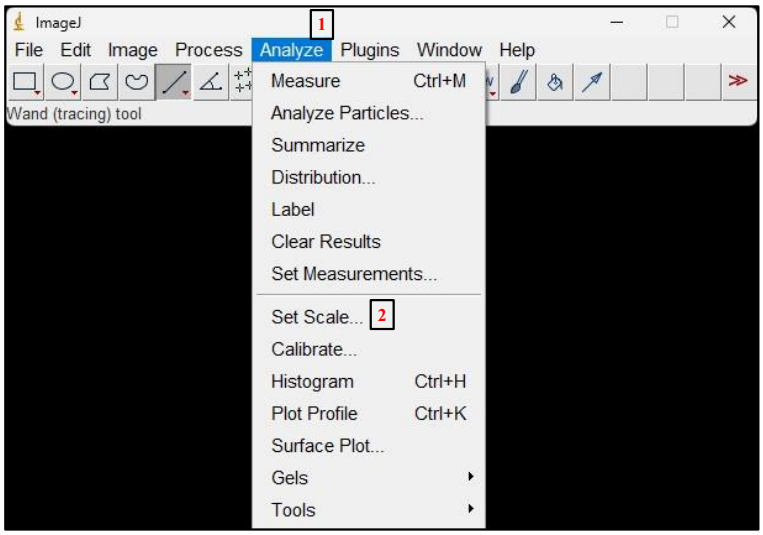


Figure 3. Opening the window to set the scale. The numbers indicate the steps to open the *Set Scale* window. Click on *Analyze* in the ImageJ toolbar (step one) and then click on *Set Scale* (step two).

4. Enter the pixel value in the *Distance in pixels* box, enter the scale distance (for example, 1.0) in *Known distance*, keep the *Pixel aspect ratio* at 1.0, set the *Unit of length* (for example, cm), and click *OK* (Figure 4).

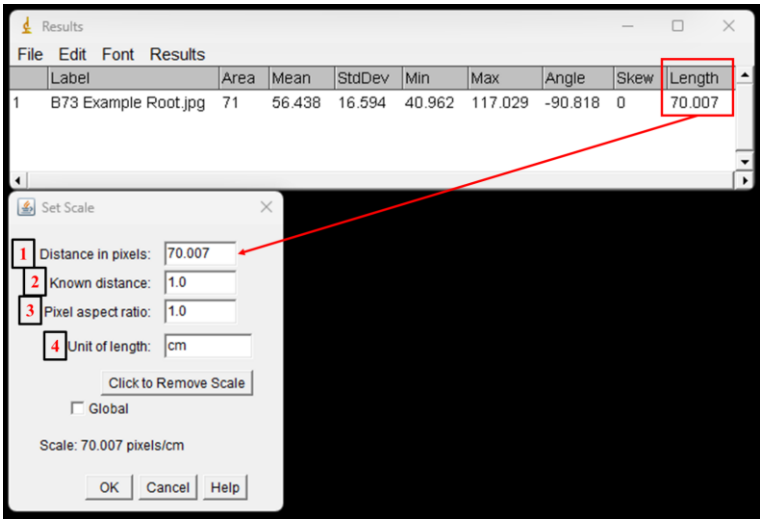


Figure 4. Setting the scale using the collected pixel value. The numbers indicate the steps to enter in the scale of the image in centimeters. Enter the length (denoted by the red box) into the *Distance in pixels* box (step one), enter the known distance of the scale you are using (1.0 if you measured 1.0 cm segment of a ruler; step two), keep the *Pixel aspect ratio* box 1.0 (step three), and change the *Unit of length* box to "cm" (step four).

5. To measure, right-click on the straight-line tool, choose between the segmented line and freehand line tool, and trace out the shoot (Figure 5).
Note: If using the segmented line tool, left-click on the starting point, move the cursor down the root and left-click again. Proceed to trace the root until the root tip is reached. Either double left-click or right-click to end the trace and press CTRL + M to measure. If using the freehand line tool, hold down left-click and drag the mouse until the root tip is reached.

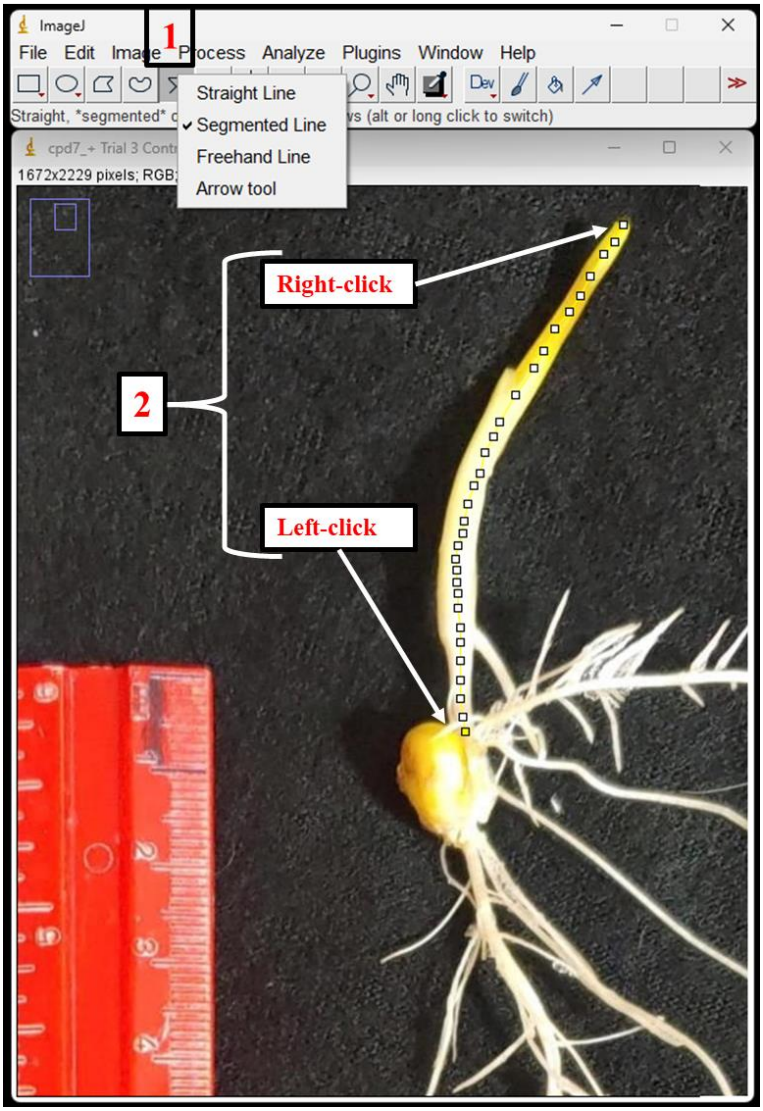


Figure 5. Measuring the shoot. Numbers indicate the steps to measure the shoot with the segmented line tool. Right-click the straight-line tool in the ImageJ window and select *Segmented Line* (step one). Start by left-clicking the spot where the shoot begins, left-click to trace the shoot up to its tip, and right-click to finish the measurement (step two). Press CTRL + M to measure.

6. Release the left-click and press CTRL + M to measure.
7. Repeat steps A5–A6 (see Data analysis) until all shoots are measured.
8. To save the data table (i.e., "results"), go to *File* on the *Results* window, click on *Save As*, and save to a location.

B. Using SmartRoot

1. Open your image(s) on ImageJ.
2. For each image, click on *Image* (in the toolbar), *Type*, and change the image to *8-bit* (Figure 6).

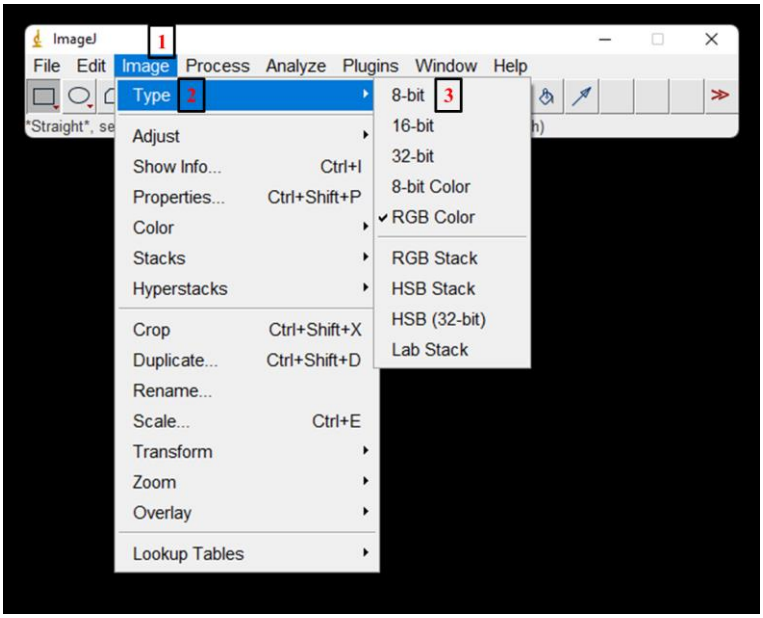


Figure 6. Preparing images for analysis in SmartRoot. The numbers indicate the order to convert an image to *8-bit*. Click on *Image* in the toolbar (step one), then *Type* (step two), and click on *8-bit* (step three) to convert the image.

3. Re-save the images as jpegs onto the desktop or folder of choice.
4. To open SmartRoot, click on *Plugins* (in the toolbar), *SmartRoot*, then *SR Explorer* (Figure 7).
   Note: Three new windows will open on your desktop.

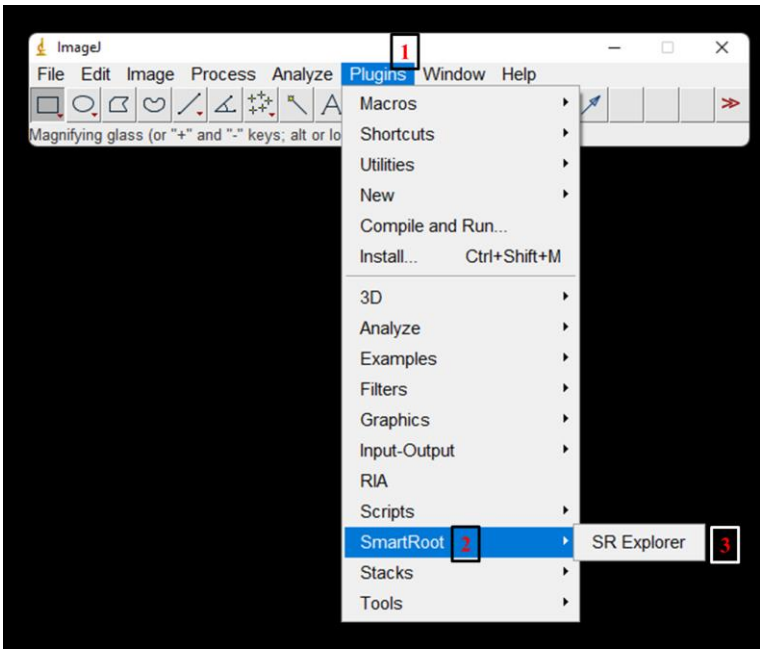


Figure 7. Opening the SmartRoot plugin. The numbers indicate the order to open SmartRoot. Click on *Plugins* in the toolbar (step one), then click on *SmartRoot* (step two) and on *SR Explorer* (step three).

5. In the *SmartRoot* window, click on *Display Axis* and *Display Nodes* under *Layers* (the window defaults to this tab; Figure 8).

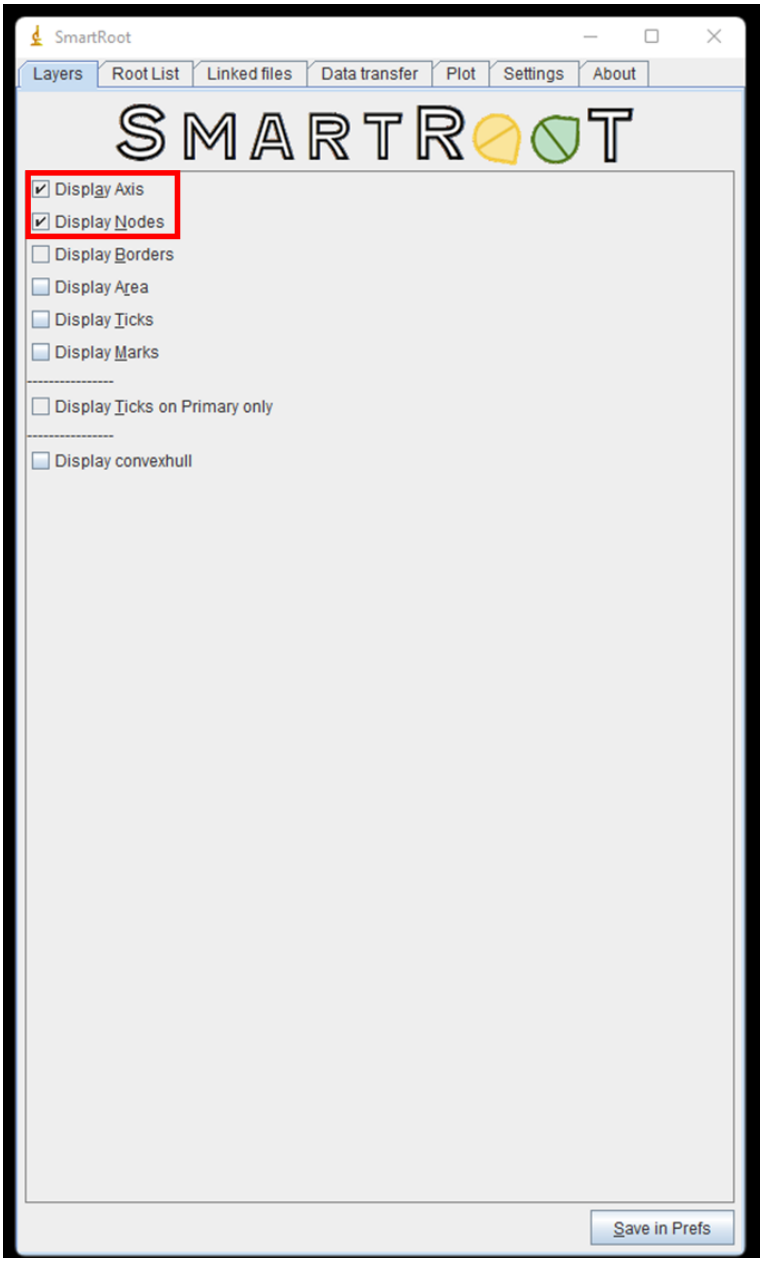


Figure 8. Setting up SmartRoot. The red box indicates the two items that require checkmarks in their boxes: *Display Axis* and *Display Nodes*.

6. Under the *Data Transfer* tab in the *SmartRoot* window, uncheck *Send to SQL database* and check *Send to CSV file*. Click on *Choose* and input your desired location and name for the date table; click on *Save* (Figure 9).

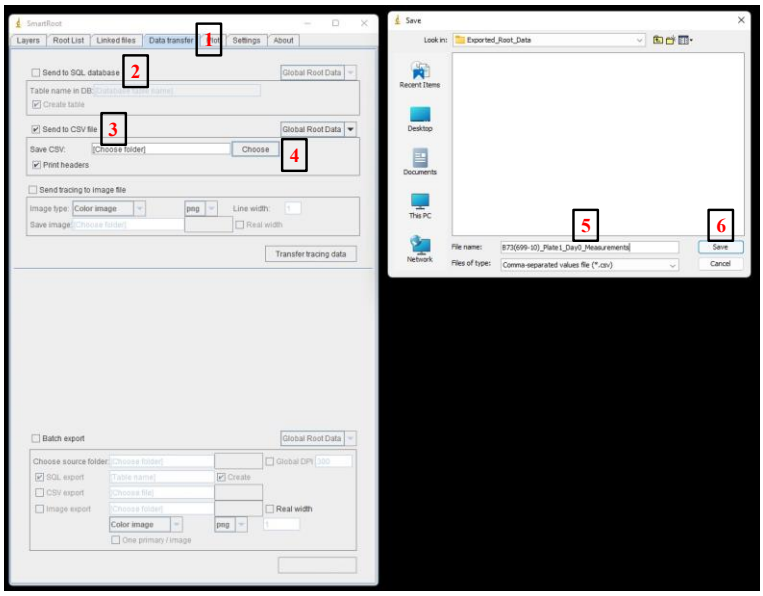


Figure 9. Setting up the file path for saving the root measurements. The numbers indicate the order at which this process occurs. Click on *Data transfer* (step one), uncheck *Send to SQL database* (step two), check *Send to CSV file* (step three), click on *Choose* and pick a folder to send the data to (step four), name the file (step five), and click *Save* (step six).

7. To open an image, click on the SmartRoot Explorer window, find the image, and double-click on it.
8. To measure, invert the image (CTRL + Shift + I), click on the *Trace Root* tool (orange circle with an orange crosshair found on ImageJ's toolbar), left-click on the bottom of the kernel, and trace the root by left-clicking until the root tip is reached. Right-click your final point, and a new window will open; name the root and click *OK* (Figure 10).
   Note: To see the measurement, open the SmartRoot window, click on Root List *in the tabs, click on* Refresh *in the bottom-right corner, and click on the desired root.*

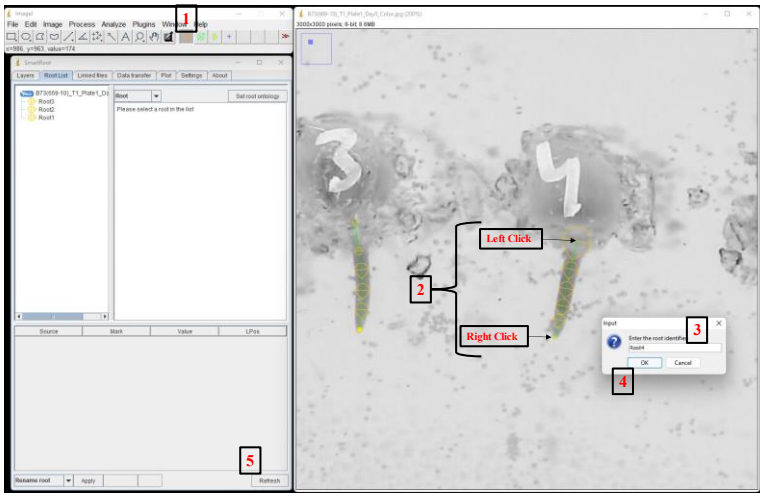


Figure 10. Measuring and logging the primary root length into the data table. The numbers indicate the order at which this process occurs. Click on the trace root icon in the ImageJ window (step one). Trace the root by left-clicking on the bottom edge of the kernel, then left-clicking on the root until the root tip is reached, and right-clicking to end the measurement (step two). Enter a name for the root (step three) and

click *OK* to log the data (step four). Click *Refresh* while in the *Root List* tab in the *SmartRoot* window to see the measurement appear (step five).

9. Measure the rest of the roots, click on *Data transfer* in the tabs, and click *Transfer tracing data*; an Excel table will appear in the location inputted earlier (Figure 11).
Note: The length measurement will appear under column D in the Excel table (labeled "length").

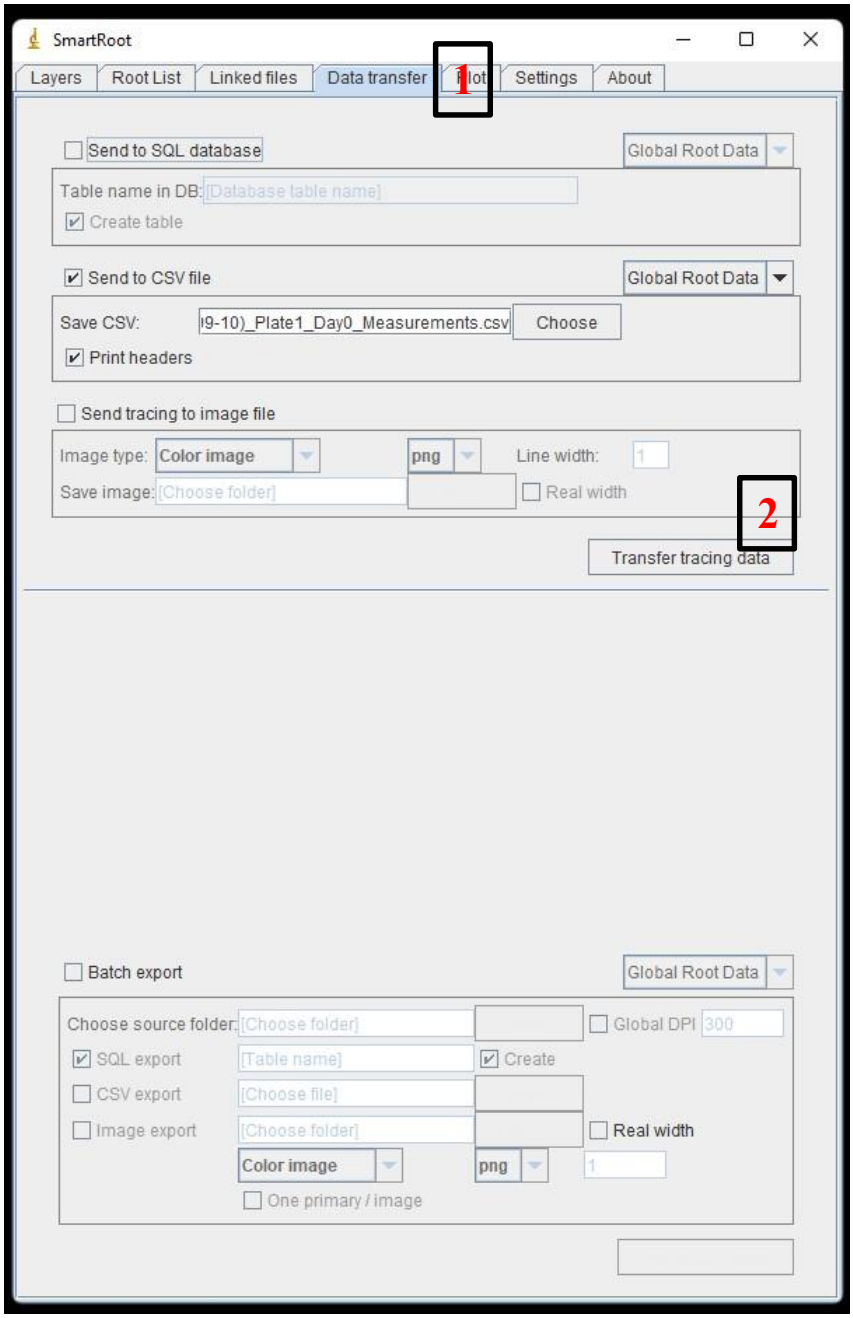


Figure 11. Saving the root measurements in the designated file. In the *SmartRoot* tab, click on *Data Transfer* (step one), then click on *Transfer tracing data*. The measurements will appear as an Excel file in the designated location.

Validation of protocol

Results from the B73 growth experiments were validated with 43 seedlings. The lengths of primary roots and shoots are represented by standard box-and-whisker plots. Primary roots were measured four days after seedling transfer onto media plates (approximately 6 DAG; Figure 12A), and shoots measured were six days after transfer (approximately 8 DAG; Figure 12B). Three B73 plates imaged at days 0, 2, and 4 are provided for illustration of how a plate could appear (Figure 13).

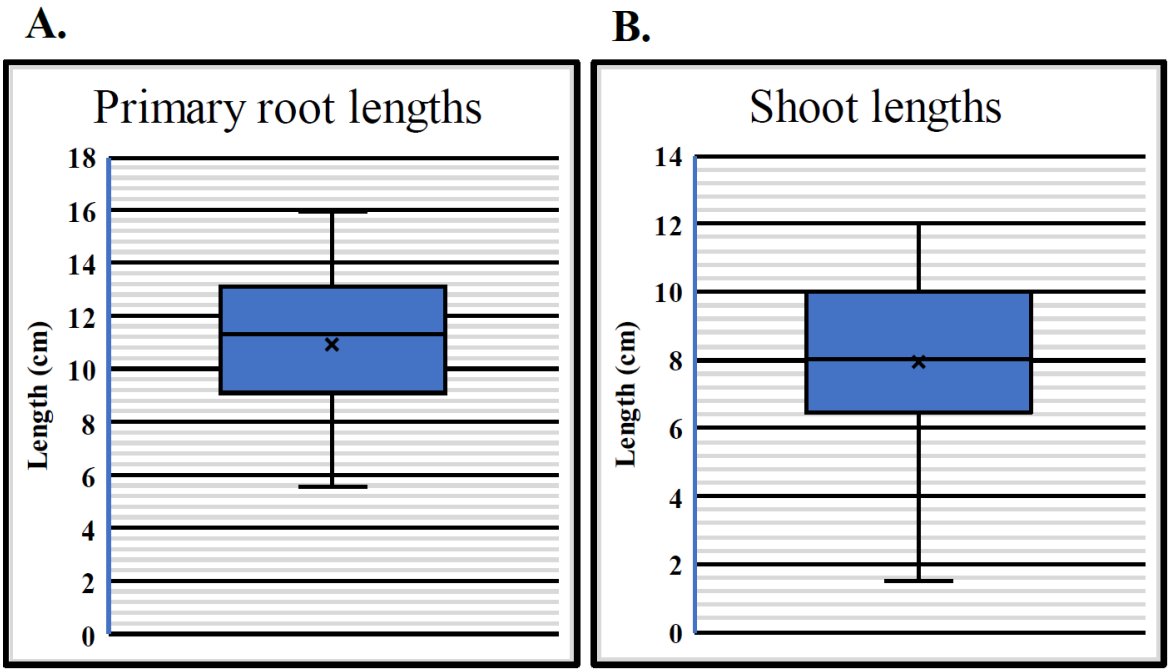


Figure 12. Average primary root and shoot lengths of B73 embryonic systems. (A) Primary root lengths were measured 6 days after germination (DAG) (four days after being transferred to a plate). (B) Shoot lengths were measured 8 DAG (six days after being transferred to a plate). n = 43 replicates. Averages are represented by the X. Error bars are represented by a 95% confidence interval.

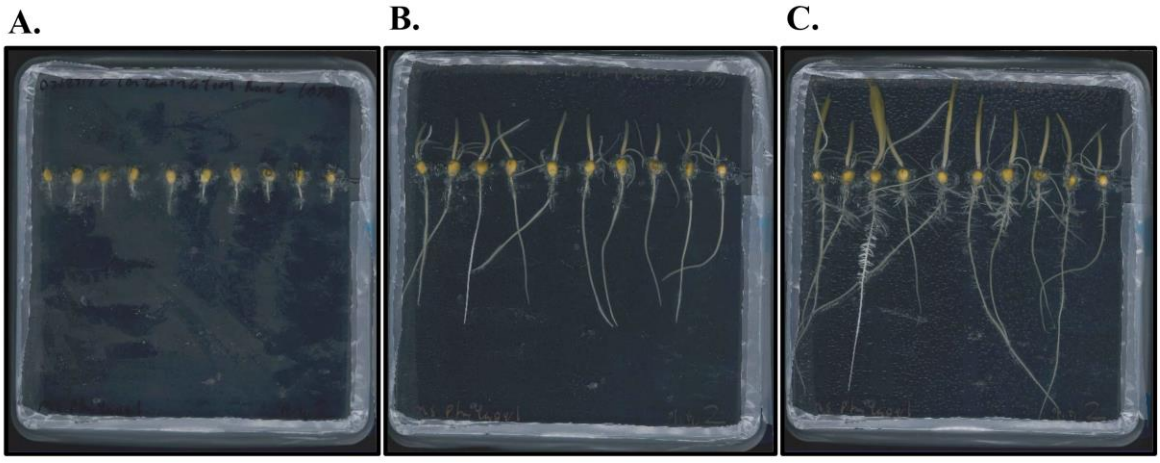


Figure 13. B73 seedling growth on MS media plates. (A) B73 seedlings are imaged after transfer onto plates [2 days after germination (DAG)], (B) two days after transfer (4 DAG), and (C) four days after transfer (6 DAG).

General notes and troubleshooting

General notes

1. The media should be modifiable to include a broad range of treatments. Two experiments are provided to exhibit the types of material that can be added. (1) As described by Julius et al. (2021), sucrose was supplemented into the MS media to test whether decreased root growth caused by the mutant *carbohydrate partitioning defective28* (*cpd28*) is due to a lack of carbon mobilization. (2) We incorporated PEG (Van Der Weele et al., 2000; Verslues and Bray, 2004) to test if the MS media plates can integrate chemicals and grew B73 seedlings for comparison to plates without PEG. As expected, by infusing PEG into the MS plates, B73 seedlings grown in PEG displayed shorter primary roots 6 DAG (Figure 14A) and shoots 8 DAG (Figure 14B) than B73 seedlings on basic MS plates lacking PEG.

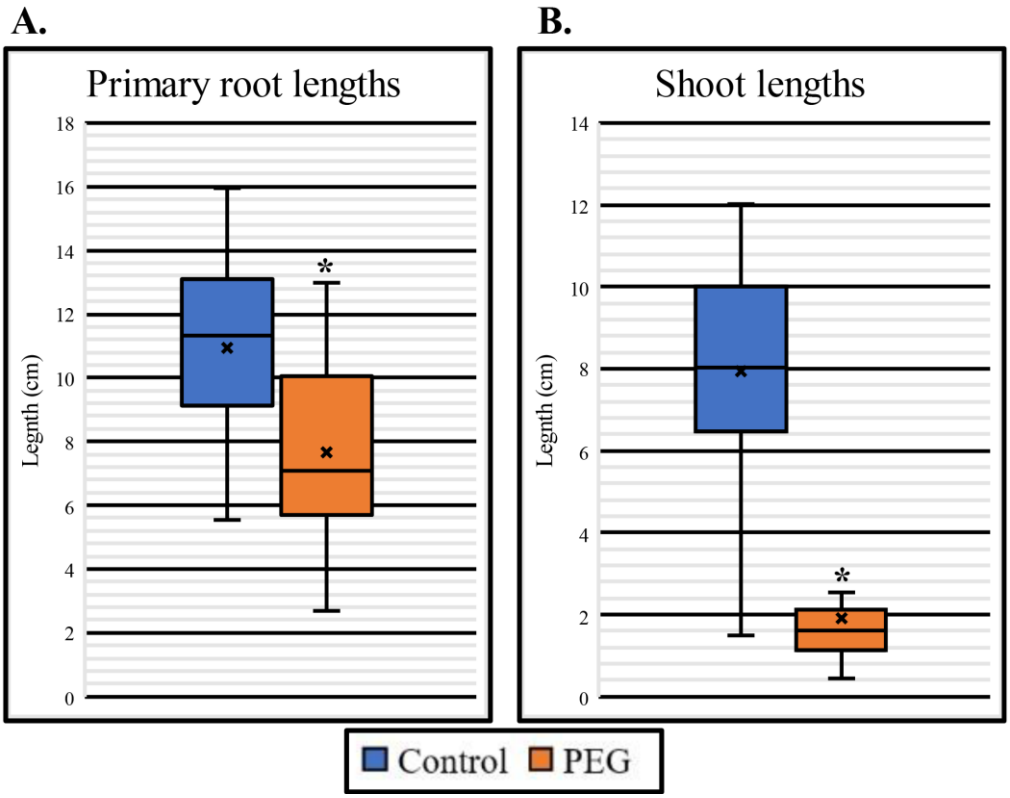


Figure 14. Polyethylene glycol (PEG)-treated B73 seedlings display shorter primary roots and shoots. (A) Primary root lengths are measured 6 days after germination (DAG) (four days after plate transfer). (B) Shoot lengths are measured 8 DAG (six days after plate transfer). N = 43 replicates for control; N = 20 replicates for PEG-treated. Averages represented by X, * represents $P < 0.001$ using a Student's *t*-test. Error bars are represented by a 95% confidence interval.

2. Tissue samples of the roots can be collected for genotyping. For example, roots of seedlings of a previously characterized mutant, *Carbohydrate partitioning defective1* (*Cpd1)* (Julius et al., 2018) and their wild-type siblings underwent DNA extraction (Leach et al., 2016), followed by PCR and gel electrophoresis (GE). Here, we demonstrate that we can genotype *Cpd1* vs. wild-type seedlings through GE using an agarose-based gel after tissue collection, DNA extraction, and PCR (Figure 15).

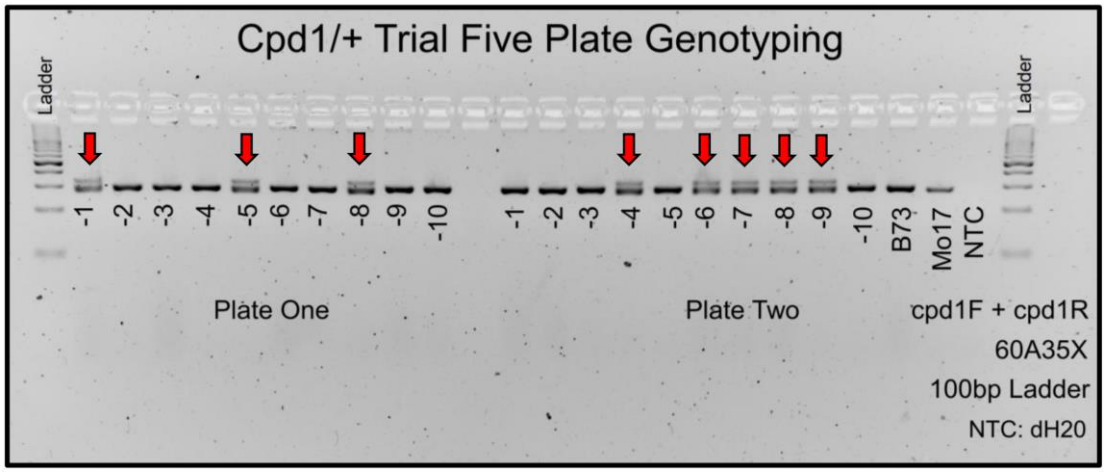


Figure 15. Genotyping results of a set of *Cpd1/+* and wild-type seeds. A gel image of two families (N = 10 each) segregating *Cpd1/+* or wild-type individuals, with B73 and Mo17 genomic DNA backgrounds as controls with 18.2 mΩ filtered, deionized water as a no template control (NTC). The solid black bands indicate the homozygous recessive wild types, and the double bands (red arrows) indicate the heterozygous mutants.

3. This protocol can be applied to other crop plants, such as wheat, millet, sorghum, rice, soybean, common bean, etc. (Figure 16).

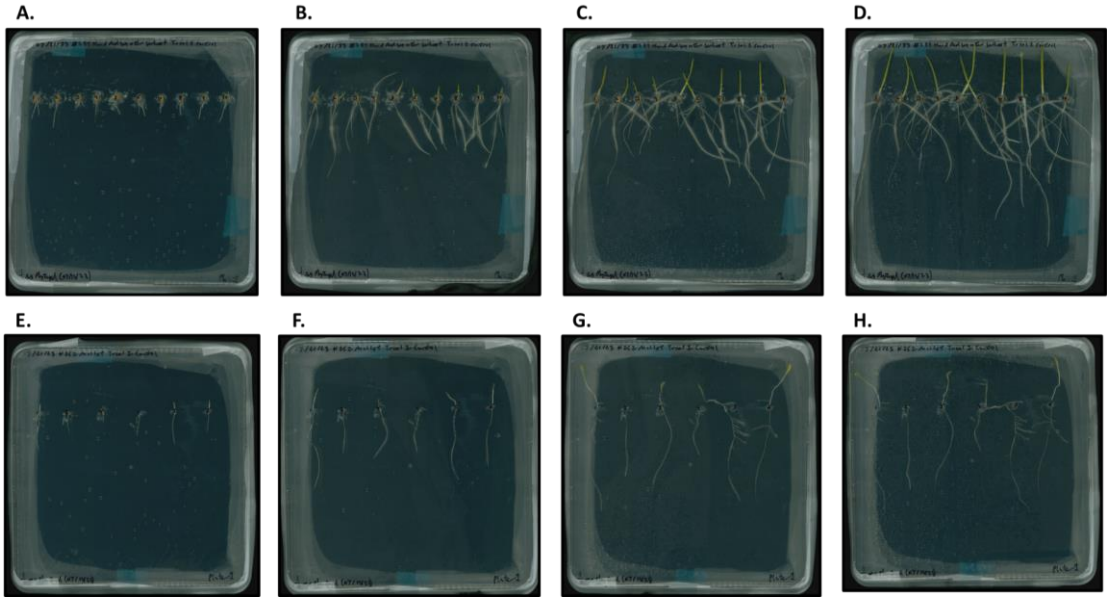


Figure 16. Wheat and Millet growth on MS media plates. (A) Wheat seedlings are imaged after transfer onto plates at 2 days after germination (DAG), (B) two days after transfer (4 DAG), (C) four days after transfer (6 DAG), and (D) six days after transfer (8 DAG). (E) Millet seedlings are imaged after transfer onto plates at 2 DAG, (F) two days after transfer (4 DAG), (G) four days after transfer (6 DAG), and (H) six days after transfer (8 DAG).

Troubleshooting

1. If the seed stock yields a lower rate of germination, substitute the old stock out for a new, fresh stock of the desired seeds.

2. If fungal contamination appears on the seeds, two practices can be done to prevent future contamination. (1) Examine your seed stock and check for signs of mold or other signs of decomposition. Exchange seed stock if necessary. If it is not the seed stock, (2) make new solutions of 5% bleach and 5.5% spectracide (see Recipes).
3. If fungal or bacterial contamination appears on the plates during an experiment, take extra caution when pouring a new set of plates and transferring seedlings. Two practices can be implemented to prevent future contamination. (1) Reduce the time the media is exposed to the environment when working under the laminar flow hood. (2) Minimize how far your arms hang over the plate. This can be done by having the side of the plate where the seedlings are aligned closest to you, with the primary roots facing away when inserting them into the media.

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Competing interests

No competing interests to declare.

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