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Title: A Telemetric, Gravimetric Platform for Real-Time Physiological Phenotyping of Plant–Environment Interactions

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Author Questionnaire

1. **Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all set**

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 44
Number of Shots: 58, 33 of them SCREEN that have already been uploaded by authors. Only 25 for videographer
Videographer: No need to film the SCREEN shots.

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Menachem Moshelion:** Plants respond to the environment in very complex ways. Physiological trait measurements are among the fastest to respond, usually much earlier than morphological differences can be detected. This high-resolution functional phenotyping method makes it possible to quantify the plants' environment interactions continuously and un-destructively on many treatments.

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

1.2. **Ahan Dalal:** This system can be used for real time measurements of a wide range of physiological traits on many plants simultaneously without any image-analysis while controlling the conditions for each plant in the array.

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

OPTIONAL:

1.3. **Yael Grunwald:** Here we demonstrate the most detailed protocol. However, we recommend that first-time users begin with the simplified protocol, which makes it easier to become acquainted with the most important experimental procedures, hardware and software.

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

Introduction of Demonstrator on Camera

1.4. **Menachem Moshelion:** Demonstrating the procedure will be **Itamar Shenhar**, a master student from my laboratory.

1.4.1. INTERVIEW: Author saying the above.

1.4.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Protocol

2. Pot Preparation and Plant Growth

2.1. Begin by inserting the soil filter into the pot **[1]**. Spread the nylon mesh on top of the whole pot and place the net-holder on top of the net **[2]**. Then, slowly push the net holder half-way down the inside of the whole pot, making sure that the net remains uniformly spread as it is pushed down between the two pots **[3]**.

2.1.1. WIDE: Establishing shot of talent beginning to insert the soil filter.

2.1.2. Talent spreading the mesh on top of the whole pot.

2.1.3. Talent pushing the net holder down the inside of the pot.

2.2. Insert the fiberglass stick between the two pots and push it all the way down to the bottom of the whole pot, making sure that it is on the outer side of the net and does not push the net **[1]**.

2.2.1. Talent pushing the stick down the pot.

2.3. Before pushing the net holder all the way down, push the net down by hand from inside the pot and adjust it so that it is spread uniformly and tightly over the bottom, which will ensure proper drainage **[1]**. *Videographer: This step is important!*

2.3.1. Talent adjusting the net from inside the pot.

2.4. Slide the gasket ring from the bottom of the pot set-up a third of the way up the side of the pot, making sure that the slits of the ring open toward the bottom of the pot **[1]**. Label the pots according to their locations in the array inside the greenhouse **[2]**.

2.4.1. Talent sliding the gasket ring up the side of the pot.

2.4.2. Talent labeling the pot.

2.5. To grow the plants, germinate the seeds in cavity trays with the desired potting medium **[1]**. If possible, do this inside the same greenhouse to be used for the main part of the experiment in order to acclimate the plants to the environmental conditions **[2]**. Start the experiment when the plant transpires approximately 10% of the maximum pot water capacity **[3]**.

2.5.1. Talent sowing the seeds.

2.5.2. Talent showing the grown seedlings with roots those are dense enough to take the shape of the cavity (root-soil plug).

2.5.3. Talent showing the cavities filled with soil without seedlings (soil plug).

3. Experiment Setup

3.1. Before starting a new experiment, label and calibrate the lysimeters using a spirit level and standard weights. Perform the calibration while the green container, including all plugs, is on the load cell, following the steps from the text manuscript.

3.1.1. Talent labeling the lysimeter using spirit level

3.1.2. Talent showing the lysimeter cover, green container and the standard weights for calibration.

3.2. To start a new experiment, open the operating software, then open the **Experiments** tab in the menu on the left side of the screen, click on **Create New**, name the experiment, and click **OK [1]**.

Video Editor: For all screen captures, authors request that we include everything within the indicated time codes but feel free to speed the footage up as necessary.

3.2.1. SCREEN: 61280_screenshot_1 (4.2-4.4).mp4. 0:07 – 0:23.

3.3. Open the new experiment, then open **Plants** and click on **Create New**. In the window, change the **Plant name**, **Position**, **Tare weight** and **Soil type**, then click **Apply [1]**.

3.3.1. SCREEN: 61280_screenshot_1 (4.2-4.4).mp4. 0:24 – 1:08.

3.4. Click on **Plants** again and a table will appear on the screen. Export the table by clicking on **Export Plants** on the bottom left corner of the table. Then, open the saved Excel file, change the parameters as required, and save **[1]**.

3.4.1. SCREEN: 61280_screenshot_1 (4.2-4.4).mp4. 1:09 – 1:48.

3.5. Click **Import plants** at the bottom left corner of the table. When a small window pops up saying **Plants imported successfully**, click **OK** and check that the changes you made in the Excel file have been imported successfully **[1]**.

3.5.1. SCREEN: 61280_screenshot_1 (4.2-4.4).mp4. 1:49 – 2:09.

3.6. Click on the new experiment and on **Start experiment** in the window that appears. Finally, click **OK** to start recording data from this experiment **[1]**.

3.6.1. SCREEN: 61280_screenshot_1 (4.2-4.4).mp4. 2:10 – 2:21.

3.7. Weigh the pre-prepared empty pots. If using parts that are similar to one another, the average weight of 10 of them will be sufficient **[1]**. Mix the potting medium thoroughly with plenty of water unless sand or other homogenous soil media is used, and uniformly fill all of the pre-prepared empty pots **[2]**.

3.7.1. Talent weighing a pot.

3.7.2. Talent filling a pot.

3.8. Tap the bottom of the pot against the floor a few times to make sure that the potting medium is well distributed **[1]**. Insert a cast of a cavity mold that is similar in shape and size to the root-soil plug of the seedlings into the middle of the potting medium and push it in completely **[2]**.

3.8.1. Talent tapping the pot against the floor.

3.8.2. Talent inserting the cast of a cavity mold into the potting medium.

3.9. Water the pots well and rinse off the outside of the pots, then allow them to drain for 30 minutes before continuing on to the next step **[1]**.

3.9.1. Talent watering and rinsing the pots.

3.10. Place all of the filled pots on the lysimeter array in the green containers according to the experimental design **[1]**, making sure that the plugs of the green containers are in the right place and that the green containers are positioned properly in the groove **[2]**. *Videographer: This step is difficult and important!*

3.10.1. Talent showing that the plugs of the green container are in the right place.

3.10.2. Talent placing a pot on the lysimeter, with a good view of the full table with 72 lysimeters (in time-lapse, 1/3rd at a time).

3.10.3. Time-lapse 1 NOTE: These time-lapses may be some added shots, but authors did not add any VO for them and I'm not sure why they're here.

3.10.4. Time-lapse 2

3.10.5. Time-lapse 3

3.10.6. Time-lapse 4

3.11. In the operating software, open the Experiment tab and click on the **Measure Components** tab under the new experiment. Confirm the new measurement from the time given on the top of the screen as **Previous Sample was on**. Click on **Measure Object [1]**.

3.11.1. SCREEN: 61280_screenshot_2 (4.12-4.16) modified.mp4. 0:04 – 00:30.

3.12. Click **Next** when the **Record weights** and **Delta Tag** windows pop up. In the **Tag record** window, name the **New meta-tag** as **first measurement** and click **Finish.** When the **Weights recorded successfully** window pops up, click **OK [1]**. Place the multi-outlet irrigation drippers **[2]** and pot covers on each pot on the lysimeter **[3-added]**.

3.12.1. SCREEN: 61280_screenshot_2 (4.12-4.16) modified.mp4. 0:31 – 01:19.

3.12.2. Talent placing a multi-outlet irrigation dripper and a pot cover on each pot.

3.12.3. Added: View of full table with multi-outlet irrigation drippers and pot covers placed on all pots.

3.13. Confirm the new measurement and click on **Measure Object** again. Click **Next** when the **Record weights** pops up. In the **Delta Tag** window, select **1st measurement** in the **Meta-tag to subtract** section and click **Next [1]**.

3.13.1. SCREEN: 61280_screenshot_2 (4.12-4.16) modified.mp4. 01:20 – 02:12.

3.14. In the **Tag record** window, name the **New meta-tag** as **Static components** and click **Finish**. When the **Weights recorded successfully** window pops up, click **OK [1]**.

3.14.1. SCREEN: 61280_screenshot_2 (4.12-4.16) modified.mp4. 02:12 – 02:40.

3.15. Click on **Plants** on the left side and make sure that the values are updated **[1]**. Then, under the new experiment, open the **Treatment Scenarios** tab and click **Create New [2]**.

3.15.1. SCREEN: 61280_screenshot_2 (4.12-4.16) modified.mp4. 02:41 – 03:10.

3.15.2. SCREEN: 61280_screenshot_3 (4.19-4.21).mp4. 0:04 – 0:13.

3.16. Under Plan, click on the new step. In the center of the screen, choose any Test name for **Treatment** and confirm by clicking **OK**. Choose **Never** for Termination, then click **Apply [1]**.

3.16.1. SCREEN: 61280_screenshot_3 (4.19-4.21).mp4. 0:14 – 0:31.

3.17. Open the **Irrigation treatments** on the left side and the chosen test name. Click on the new step. In the center of the screen, change the **valve opening time** a few minutes ahead of the current time along with other parameters as required by the experiment and click **Apply [1]**.

3.17.1. SCREEN: 61280_screenshot_3 (4.19-4.21).mp4. 0:32 – 0:56.

3.18. Open the **Plants** under the new experiment and click on the new plant name. In the center of the screen, change the **Treatment Plan** to **Plan**. A window will pop up, click **OK** to confirm. Check that the **step** has the same name as the irrigation test name, then click **Apply [1]**.

3.18.1. SCREEN: 61280_screenshot_3 (4.19-4.21).mp4. 1:08 – 1:32.

4. Experiment

4.1. Check visually that all of the pots are irrigated and that excess irrigation liquid is dripping out of the perforated drain plug of the green container **[1]**. Remove the, unperforated plug from the green container and let the water drain out completely **[2]**. Then, put the plug back in its place **[3]**.

4.1.1. Talent checking a pot to make sure that it is irrigated.

4.1.2. Talent removing the plug from the green container.

4.1.3. Talent putting the plug back in.

4.2. Open the Experiment tab and click on **Measure Components** under the new experiment. After confirming the measurement, click on **Measure Object** under **Measure Plant Parameters [1]**.

4.2.1. SCREEN: 61280_screenshot_4 (5.4).mp4. 0:04 – 0:20.

4.3. After clicking through the **Record weights** and the **Delta Tag** windows, name the **New meta-tag** as **Cast-pre** and click **Finish**. Click **OK** when the **Weights recorded successfully** window pops up **[1]**. Gently take the casts **[2]** out from the pots **[3-added]**.

4.3.1. SCREEN: 61280_screenshot_4 (5.4).mp4. 0:21 – 0:55.

4.3.2. Talent taking the cast out from the pot.

4.3.3. Added: View of full table without cast.

4.4. Click on **Measure Components**, then confirm measurement as before. Under **Measure Plant Parameters** click **Measure Object**. Click **Next** when the **Record weights** window pops up. Select **Cast-pre** in the **Meta-tag to subtract** section and click **Next**. In the Tag record window, name the **New meta-tag** as **Cast-post** and click **Finish [1]**.

4.4.1. SCREEN: 61280_screenshot_5 (5.5-5.6).mp4. 0:04 – 0:58.

4.5. Click on Plants" on the left side and make sure that the values are updated. In the Plants table, manually update the "Tare weight" with the weight of the pre-prepared empty pots taken manually. **[1]**.

4.5.1. SCREEN: 61280_screenshot_5 (5.5-5.6).mp4. 0:59 – 1:12.

4.6. Click **Measure Components** and confirm the measurement. Under **Measure Plant Parameters**, click on **Measure Soil Wet Weight**. Click **OK** when a small window pops up for confirmation. Click on **Plants** and check that the values are updated **[1]**.

4.6.1. SCREEN: 61280_screenshot_6 (5.7).mp4. 0:06 – 0:40.

4.7. Manually measure the average weight of 5 to 10 cavity-filled soil **[1]** that are well irrigated and well drained, without seedlings **[2-added]**.

4.7.1. Talent measuring manually the weight of soil plug using weighing balance.

4.7.2. Added: Close-up to the weight of 40 g in the balance.

4.8. Again, click **Measure Components** and confirm the measurement. Click on **Set Seedlings Bulk-Soil Weight**. When a small window pops up, enter the average weight of soil plug and click OK. Click on **Plants** and check that the values are updated **[1]**.

4.8.1. SCREEN: 61280_screenshot_7 (5.9).mp4. 0:12 – 0:51.

4.9. Return to **Measure Plant Parameters** and click **Measure Plant Initial Net Weight**. In the Tag record window, select **Set New Record** as **Plant Net Weight Reference Point** and click **Finish**. Click OK when the **Weights recorded successfully** window pops up. Click on **Plants** and check that the values are updated **[1]**.

4.9.1. SCREEN: 61280_screenshot_8 (5.10).mp4. 0:04 – 0:58.

4.10. Make sure that the seedlings in the cavity trays are well irrigated. Gently pull the seedlings with their root-soil plug from the cavities, taking care not to injure them, and place them carefully into the cavities made by casts in the pots **[1]**. Repeat the **Plant Net Weight** measurement as previously described in **Meta-tag** measurement **[5]**.

4.10.1. Talent transferring one seedling, with a good view of the full table with 72 lysimeters (in time-lapse, 1/3rd at a time).

4.10.2. Time-lapse 1 NOTE: More added time lapses, no VO.

4.10.3. Time-lapse 2

4.10.4. Time-lapse 3

4.10.5. SCREEN: 61280_screenshot_9 (5.12).mp4. 0:05 – 1:10.

4.11. To consider reserve water in the green container, follow directions in the text manuscript to ensure that the green container is full again **[1]**. Return to **Measure Plant Parameters** and click **Measure Reserved Water Weight**. Click **OK** when the **Reserve Water Weight** and **Success** windows pop up **[2]**.

4.11.1. Added: Use 4.1.1.

4.11.2. SCREEN: 61280_screenshot_10 (5.16-5.17).mp4. 0:10 – 0:34.

4.12. Click on **Plants** and export the table by clicking **Export Plants** on the bottom left corner of the table. Open the Excel file and subtract the measured Plant Net Weight and Seedling Bulk-Soil Weight from the reserved water weight measurement, and save **[1]**.

4.12.1. SCREEN: 61280_screenshot_10 (5.16-5.17).mp4. 0:35 – 1:53. *Video Editor: Speed this up or cut it as needed.*

4.13. Click on **Update plants** on the bottom left corner of the table, select the Excel file and click **Open** to update. When **Update plants** window pops up, click **OK**. Check that the values are updated **[1]**.

4.13.1. SCREEN: 61280_screenshot_10 (5.16-5.17).mp4. 1:54 – 2:15.

4.14. Under **Measure Plant Parameters**, click on **Calculate Soil Dry Weight**. When the window pops up, insert **Soil Wet Weight** and **Soil Dry Weight**, or insert manually-calculated **Soil Water Content.** Click **Apply** and then click **Finish.** When a window pops up, click **OK** to confirm. Follow the text manuscript to calculate the **Soil Water Content** value **[1]**.

4.14.1. SCREEN: SCREEN: 61280_screenshot_20 (7.1-7.2).mp4. 0:16 - 1:27

4.15. Open **Irrigation Treatments** and click **Create New**. Input the name and click **OK**. Open the new irrigation name and click on **Create New**, then open the default treatment

plan. Set the **Valve opening time** and other parameters as required and click **Apply [1-TXT]**.

4.15.1. SCREEN: 61280_screenshot_11 (7.4-7.4.2).mp4. 0:04 – 01:47. *Video Editor: Speed this up or cut it as needed.* **TEXT: Repeat the steps to create new irrigation treatments.**

4.16. This demonstrates a dynamic and modulating irrigation treatment scenario for the entire experimental period, based on user preferences. Open **Treatment Scenarios**, open **Plan** and click on the default plan name. Choose a treatment name from the list and click **OK** to confirm. Choose appropriate condition for **Termination** and click **Apply [1]**.

4.16.1. SCREEN: 61280_screenshot_12 (7.4.3-7.4.4).mp4. 0:02 – 0:37.

4.17. All possibilities include a Time Out option that will close the tap even if the set conditions were not reached. Finally, follow manuscript directions to complete the Plants table **[1]**.

4.17.1. SCREEN: 61280_screenshot_12 (7.4.3-7.4.4).mp4. 0:38 – 0:59. *Video Editor: Speed this up.*

5. Data Analysis

5.1. Open the data analytics software and log in with your username and password **[1-TXT]**. Click on **select experiment** and then on **select control system** and **select experiment [2]**.

5.1.1. SCREEN: 61280_screenshot_13 (8.1).mp4. 0:01 – 0:02. **TEXT: https://spac.plant-ditech.com**

5.1.2. SCREEN: 61280_screenshot_13 (8.1).mp4. 0:03 – 0:10.

5.2. In the left-hand side column of the screen, click on **Experiments** and type the name of the experiment in the **Name** bar under the **Search** section. The name of the experiment will then appear below the Search section, in the **Experiments** section. Click on the experiment name to open the **Info** and **Plants** sections **[1]**.

5.2.1. SCREEN: 61280_screenshot_13 (8.1).mp4. 0:11 – 0:25.

5.3. In the **Info** section, edit the **WUE start** and **WUE end** dates for a period of at least 3 days before the start of the drought treatment, then click **Update**. The WUE and the *R* squared value for every pot will appear or update in the **Plants** section **[1]**.

5.3.1. SCREEN: 61280_screenshot_14 (8.2).mp4. 0:02 – 0:20.

5.4. In the **Plants** section, choose to exclude any plant or pot with a negative WUE value or an R-squared value of less than 0.5 by clicking on the eye symbol under the Active

column, which will then turn red. The WUE data can be exported as an Excel file by clicking on **Export Data** in the **Plants** section **[1-TXT]**.

5.4.1. SCREEN: 61280_screenshot_14 (8.2).mp4. 0:21 – 0:49. **TEXT: See Supplementary Material for the screenshots of data analysis of Graph viewer, Histogram, T test, Anova, and Piecewise linear curve**

Results

6. Results: HTP-telemetric Measurements of Changes in Atmospheric Conditions and the Physiology of Plants

6.1. Environmental conditions were monitored throughout the experiment by an atmospheric probe. The collected data indicate that the photosynthetically active radiation and vapor pressure deficit remained similar over the different days and throughout the course of the day **[1]**.

6.1.1. LAB MEDIA: Figure 4.

6.2. The volumetric water content of the drought-treated pots was measured by soil probes throughout the experimental period. Data collected from one drought-treated Indica plant is shown here **[1]**.

6.2.1. LAB MEDIA: Figure 5.

6.3. The mean calculated plant weight increased consistently among both the Karla-control and the Karla-drought treatments during the first stage of the experiment, when all of the plants received similar irrigation **[1]**.

6.3.1. LAB MEDIA: Figure 6. *Video Editor: Emphasize the area of the graph up until August 10th.*

6.4. When the drought treatment was applied to the Karla plants, those plants stopped gaining weight and did not resume gaining weight until the recovery stage. At that point, there was an increase in weight that proceeded more slowly than what was observed for the control **[1]**.

6.4.1. LAB MEDIA: Figure 6. *Video Editor: Emphasize the yellow line (drought).*

6.5. In contrast, the weights of the Karla-control plants increased continuously throughout the experimental period **[1]**.

6.5.1. LAB MEDIA: Figure 6. *Video Editor: Emphasize the blue line (control).*

Conclusion

7. Conclusion Interview Statements

7.1. **Itamar Shenhar:** Other methods like sample collection for gene expression, mineral content, and photosynthetic activity can be done during these experiments with a precise reference to the plant's behavior and environmental conditions.

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

7.2. **Ahan Dalal:** Functional phenotyping is a practical plant–environment-interactions characterization method, which gives the user a good tool for comparing different plant response-profiles under different ambient conditions. Each specific profile could be selected for deeper study, or as a source for beneficial physiological traits for breeding programs.

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