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TITLE:

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

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KEYWORDS:

Critical soil water availability (θ), drought stress standardization, functional traits, genotype × environment interactions, gravimetric system, minimization of pot effect, *Oryza sativa* L., physiological phenotyping, whole-plant transpiration kinetics

SUMMARY:

This high-throughput, telemetric, whole-plant water relations gravimetric phenotyping method enables direct and simultaneous real-time measurements, as well as the analysis of multiple yield-related physiological traits involved in dynamic plant–environment interactions.

ABSTRACT:

Food security for the growing global population is a major concern. The data provided by genomic tools far exceeds the supply of phenotypic data, creating a knowledge gap. To meet the challenge of improving crops to feed the growing global population, this gap must be bridged.

Physiological traits are considered key functional traits in the context of responsiveness or sensitivity to environmental conditions. Many recently introduced high-throughput (HTP) phenotyping techniques are based on remote sensing or imaging and are capable of directly measuring morphological traits, but measure physiological parameters mainly indirectly.

This paper describes a method for direct physiological phenotyping that has several advantages for the functional phenotyping of plant–environment interactions. It helps users overcome the many challenges encountered in the use of load-cell gravimetric systems and pot experiments. The suggested techniques will enable users to distinguish between soil weight, plant weight and soil water content, providing a method for the continuous and simultaneous measurement of dynamic soil, plant and atmosphere conditions, alongside the measurement of key physiological traits. This method allows researchers to closely mimic field stress scenarios while taking into consideration the environment's effects on the plants' physiology. This method also minimizes pot effects, which are one of the major problems in pre-field phenotyping. It includes a feed-back fertigation system that enables a truly randomized experimental design at a field-like plant density. This system detects the soil-water-content limiting threshold (θ) and allows for the translation of data into knowledge through the use of a real-time analytic tool and an online statistical resource. This method for the rapid and direct measurement of the physiological responses of multiple plants to a dynamic environment has great potential for use in screening for beneficial traits associated with responses to abiotic stress, in the context of pre-field breeding and crop improvement.

INTRODUCTION

Ensuring food security for a growing global population under deteriorating environmental conditions is currently one of the major goals of agriculture research[1,2,3]. The availability of new molecular tools has greatly enhanced crop-improvement programs. However, while genomic tools provide a massive amount of data, the limited understanding of actual phenotypic traits creates a significant knowledge gap. Bridging this gap is one of the greatest challenges facing modern plant science[4,5,6]. To meet the challenges that arise in the process of crop improvement and minimize the genotype–phenotype knowledge gap, we must balance the genotypic approach with a phenocentric one[7,8].

Recently, various high-throughput phenotyping (HTP) platforms have made possible the nondestructive phenotyping of large plant populations over time and these platforms may help us to reduce the genotype–phenotype knowledge gap[6,8,9,10]. HTP screening techniques allow the measurement of traits in massive numbers of plants within a relatively short period of time, thanks to robotics and conveyor belts or gantries used to move the plants or sensors (respectively), as opposed to hand-operated techniques based on gas exchange or photography. Nevertheless, the massive amounts of data produced by HTP systems present additional data-handling and analytical challenges[11,12].

Most of these HTP platforms involve the assessment of phenotypic traits through electronic sensors or automated image acquisition[13,14]. Advanced field phenomics involve the deployment of proximal sensors and imaging technologies in the field, as well as a high-resolution, precise and large-population scale of measurement[15]. Sensor and image data need to be integrated with other multi-omics data to create a holistic, second-generation phenomic approach[16]. However, methodological advances in data acquisition, handling and processing are becoming increasingly important, as the challenges of translating sensor information into knowledge have

been grossly underestimated during the first years of plant phenomics research[13]. However, the reliability and accuracy of currently available imaging techniques for in depth phenotyping of dynamic genotype–environment interactions and plant stress responses are questionable[17,18]. Moreover, the results from controlled environments are often very different than those observed in the field, especially when it comes to drought-stress phenotyping. This is due to differences in the situation the plants experience in terms of soil volume, soil environment and mechanical impedance due to declining soil moisture during drought stress. Therefore, results from controlled environments are difficult to extrapolate to the field[19]. Finally, the entry price of image-based HTP systems is very high, not only due to the price of sensors, but also due to the robotics, conveyor belts and gantries, which also require higher standards of growth-facility infrastructure and significant maintenance (many moving parts working in a greenhouse environment).

In this paper, we present an HTP-telemetric phenotyping platform designed to solve many of the problems mentioned above. Telemetry technology enables the automatic measurement and transmission of data from remote source(s) to a receiving station for recording and analysis. Here, we demonstrate a nondestructive HTP-telemetric platform that includes multiple weighing lysimeters (a gravimetric system) and environmental sensors. This system can be used for the collection and immediate calculation (image-analysis is not needed) of a wide range of data, such as whole-plant biomass gain, transpiration rates, stomatal conductance, root fluxes and water-use efficiency (WUE). The real-time analysis of the big data that is directly fed to the software from the controller in the system represents an important step in the translation of data into knowledge[14] that has great value for practical decision-making, substantially extending the knowledge that can be acquired from controlled environment phenotyping experiments, in general, and greenhouse studies of drought stress, in particular.

Other advantages of the telemetry platform are its scalability and ease of installation and its minimal growth-facility infrastructure requirements (i.e., it can be easily installed in most growth facilities). Moreover, as this sensor-based system has no moving parts, maintenance costs are relatively low, including both the entry price and long-term maintenance costs. For example, the price of a 20-unit gravimetric system, including the feedback fertigation system for each plant, meteorological station and software, will be similar to the price of one portable gas-exchange system of a leading brand.

Rice (*Oryza sativa* L.) was used as a model crop and drought was the examined treatment. Rice was chosen as it is a major cereal crop with wide genetic diversity and it is the staple food for over half of the world's population[20]. Drought is a major environmental abiotic stress factor that can impair plant growth and development, leading to reduced crop yields[21]. This crop–treatment combination was used to demonstrate the platform's capabilities and the amount and quality of data that it can produce. For more information regarding the theoretical background for this method, please see [22].

PROTOCOL:

In this protocol, we referred to 4 L pots loaded on 20 cm x 20 cm scales, with each pot containing one plant. The same protocol is easily scalable and can be used with much bigger pots (up to 25 L loaded on 40 cm x 40 cm scales, with only a linear adaptation to the protocol measures) and several plants per pot. Thus the protocol can be easily adapted for plants of many types and sizes. Please refer to **Figure 1** and **Figure 2** for the system components.

1. Prepare the pots for the experiment

1.1. Insert the soil filter. Spread the nylon mesh (net) on top of the whole pot and place the net-holder on top of the net. With a hand, slowly push the net holder half way down the inside of the whole pot. Make sure that the net remains uniformly spread as it is pushed down between the two pots.

1.2. Insert the fiberglass stick (pole) 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 as well and does not push the net.

1.3. 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 of the pot once the net holder has been fully inserted (**Figure 2CI**).

1.4. Slide the gasket ring from the bottom of the pot set-up described above, a third of the way up the side of the pot. Make sure that the slits of the ring open toward the bottom of the pot (**Figure 2CII**).

1.5. Repeat steps 1.1-1.4 for all of the experimental pots before continuing on to the next step. Randomize the location of the plants (**Figure 2D**; in either a randomized block design or a fully random design) using the Array Randomizer application.

NOTE: To download the free program and for more information, please see the link: https://drive.google.com/open?id=1y4QbTpxRK5Lx430xzu1RFdrlcL8pz_1q).

1.6. Label the pots according to their locations in the array inside the greenhouse. For example, the label “B10D” corresponds to a pot located on Table B in Column 10 and Row D. Prepare three additional pots for each table for soil-water-content measurements (please see Section 7.1).

2. Grow the plants

2.1. Choose the growing (potting) medium that best suits the experiment. Choosing the right medium for the experiment is crucial and the correct choice depends on several factors (see Discussion). For first-time users, we strongly recommend using a porous, ceramic, small-sized

medium. Please refer to **Table 1** and **Table 2** for more information to help choose the right medium for the experiment**.**

2.2. Germinate the seeds in cavity trays with the desired potting medium. 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 inside that greenhouse.

2.3. If the seedlings were not germinated in trays, transplant them into cavity trays containing the potting medium. Plant one seedling in each cavity and let it grow until its roots are dense enough to take the shape of the cavity (root-soil plug).

2.4. Leave 5–7 cavities without seedlings for soil weight measurements (only potting medium; **Figure 3**). For more information, please see Section 5.9.

3. Improve the signal-to-noise level

NOTE: The following steps improve the quality of the measurements and reduce the noise levels.

3.1. Calibrate the lysimeter.

3.1.1. Use a spirit level to check that all of the lysimeters are level and then start the weight calibration process. Use two standard weights (1–10 kg). Perform the calibration while the green container, including all plugs, is on the load cell.

3.1.2. Put the first (lighter) calibration weight on each load cell.

3.1.3. In the operating software, go to the **Calibration** tab and choose the weight for the first point. Then, select the load-cell position where the weight was placed and click **Get point1** (**Supplementary Figure 1A**). This step can be applied on several load-cells simultaneously.

3.1.4. Repeat for the second weight and click **Get point2**.

3.1.5. Click **Apply calibration**.

3.2. Ensuring a sufficient quantity of plants with an appropriate size for the experiment

NOTE: The smaller the plant, the weaker its signal will be (e.g., weight of water transpired in a day versus the pot weight). The following steps will help to improve the signal-to-noise ratio.

3.2.1. Start the experiment when the plant transpires approximately 10% of the maximum pot water capacity.

NOTE: For example, if working with a sandy medium that contains approx. 1 L of water at pot capacity (see **Table 2**), start the experiment when the plants transpire approx. 100 mL per day. If working with a peat-based medium that holds about 2 L of water at pot capacity (see **Table 2**), start the measurements when the plants transpire about 200 mL per day.

3.2.2. Estimate the initial plant daily transpiration before loading it onto the system by measuring (manually) morning vs. evening weight differences in a few seedlings.

3.2.3. When working with small plants, put several plants in each pot (e.g., six Arabidopsis plants in one 3.9 L pot[23], to reach the recommended minimal level of transpiration)*.

4. Setting up the experiment

NOTE: The process of setting up the experiment is designed to take into account the weight of all the parts of the system, namely, the weight of the potting medium (including the soil-water weight at pot capacity) and the initial weight of the seedlings. Follow the steps below:

4.1. If possible, work with similar static components that have similar weights. Static weight components include pot sets, soil probes and other plastic parts.

4.2. To start a new experiment, open the operating software. Open the **Experiments** tab in the menu on the left side of the screen. Click on **Create New** or duplicate the experiment properties from a previous experiment by right-clicking on the desired experiment and choosing **Duplicate**. Rename the experiment (**Supplementary Figure 1B**).

4.3. Make sure that no unit is being used in a different experiment currently running in the system. Check that all of the plants in the Plants table match the experimental design. If not, change the table according to the design (please see Sections 5.18, 6 and **Supplementary Figure 1C**).

4.4. Start the experiment by clicking on the experiment name and then clicking **Start**.

4.5. Take manual measurements of the pre-prepared empty pots (double pot, net, stick and black gasket ring). If using parts that are similar to one another, the average weight of 10 of them will be sufficient.

4.6. Mix the potting medium thoroughly with plenty of water, for at least 1 h, so that it breaks down into homogeneous particles and is saturated, to ensure uniformity and homogeneity. For first-time users, we strongly recommend using a porous, ceramic, small-sized medium (see **Table 1** and **Table 2**). As a second option, use coarse sand.

4.6.1. Use a mechanical mixer (e.g., a concrete mixer).

4.6.2. When a highly homogenous medium (i.e., industrial sand) is being used, skip step 4.6.1.

4.7. Fill all of the pots uniformly for the experiment with the appropriate potting medium (e.g., sand, soil or peat).

4.8. Insert a cast of a cavity mold (**Figure 3B**) that is similar in shape and size to the root-soil plug of the seedlings (from cavity tray) into the middle of the potting medium. Push it in completely. Tap the bottom of the pot against the floor a few times to make sure that the potting medium is well distributed in the pot. Repeat for all pots.

4.9. Water the pots well and rinse off the outside of the pots. Allow the pots to drain for 30 min before continuing on to the next step. Make sure that the pots drain freely. If the potting medium drains too slowly (e.g., dense peat), premix it with an airy substrate (e.g., perlite; please see also **Table 1** and **Table 2**) to ensure faster drainage.

4.10. After the drainage has completely stopped, place all of the filled pots on the lysimeter array (in the green containers that are already there) according to the experimental design (**Figure 2A**).

4.11. Check that the green containers are properly fitted into the load cell cover and not touching one another.

4.12. In the operating software, open the **Experiment** tab and select the **Measure Components** tab. Click on **Measure object**. Name the measurement "1st measurement" (**Supplementary Figure 1D**).

4.13. Place the irrigation drippers, probes and pot covers on each pot. Make sure that the lines for the multi-outlet drippers and the probe cables are supported by their respective stands (attached to the units for each lysimeter scale; **Figure 1E**) before placing them in the pots. Make sure that all of the drippers, probes and covers are securely positioned.

4.14. Wait up to 3 min for a new measurement to be taken (data are collected automatically every 3 min) and then open the **Experiment** tab. Select the the **Experiment** tab and click the **Experiment**. Meta-tag this measurement to the "1st measurement" taken and name it "Static components" (**Supplementary Figure 1E**). Meta-tags are used when wanting to record a weight value that is determined by subtracting one measured value from another.

4.15. After making any necessary adjustments to the system, wait for a new data point to be recorded (every 3 min) before taking the next measurement.

4.16. Check the **Static Components** column to confirm that the values recorded in the Plants table do not include outliers. If any of the weights recorded are too low or too high, check for any interference with the load cell (e.g., make sure that nothing is touching it) and then take a new measurement (after the system was still for 3 min).

4.17. Click the **Plants** tab. Export the Plants table as a spreadsheet, add the average pot weight (from Step 4.5) to the measurement of the static components - "Tare weight". Save and upload the file (import tab).

4.18. Make sure that all of the drippers are securely inserted into the potting medium and to the pipe coming from the controller. Back in the operating software, in the **Experiment** tab, select **Treatments Scenarios**. Click **Create New** to make a new "Plan".

4.19. In the plan, choose the first step (create a new step if needed) and open it. Choose "Test" for Treatment and "Never" for Termination. In the step option, choose any treatment that is listed in the Irrigation Treatments tab above **Experiments** (**Supplementary Figure 1F;** please see also Step 4.21). Press the **Apply** tab.

4.20. Extract the Plants table as a spreadsheet, add "Plan" to the Treatment column and add "1" to the Step column. Save and upload the file.

4.21. Under the **Irrigation Treatments** tab, choose the "Test" treatment and set it to an irrigation time of 4–5 min [with the exact amount of time depending on the volumetric water content (VWC) of soil used] to enable drainage. Set the time 2 min ahead and go to the pots in the greenhouse. Other treatments can also be created. (See the detailed explanation in Step 7.4.)

4.22. Check visually that all of the drippers are working and that water is dripping out of the perforated drain plug of the green container.

4.23. In the experiment, change the irrigation treatment on Plan "X", Step 1 (please see step 4.19-4.20) to the desired irrigation treatment. Make sure that each night irrigation (with fertigation; see **Table 3** for the fertigation components used) is divided into several short pulses (events) with substantial pauses between them (at least three events every night), to ensure that the soil reaches its field capacity before dawn.

4.24. Let the irrigation program run for 1 or 2 days to let the soil reach its field capacity and continue on to the next phase.

5. Starting the experiment

NOTE: The data collected at this stage will be used as reference values for the rest of the experiment. Therefore, it is important to follow the next steps carefully.

5.1. Repeat Steps 4.18 through 4.20. Alternatively, start the process in the early morning, not long after the latest irrigation step.

5.2. 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 bath.

5.3. Remove the green, unperforated plug (from the lowest orifice) of the green container and let the water drain out completely. Then, put the plug back in its place (**Figure 1D**). If working on "drainage 0" (i.e., with the bottom hole open/the hollow drainage plug connected on the lowest hole), skip this step.

5.4. In the operating software, open the tab for the experiment and go to **Measure Components**. Click **Measure Object** and name the measurement as "Cast-pre". Gently remove all of the casts from the pots and then wait 3 min for a new measurement to be recorded (**Supplementary Figure 1D**).

5.5. Click **Measure Object**, name it "Cast-post" and meta-tag the measurement to "Cast-pre". The option will automatically calculate the difference between the two measured values and give the cast weight to verify the weight sensitivity.

5.6. Check the weight values in the Plants table. The difference between the "Cast-post" measurements should be no more than 20 or 30 g.

5.7. To measure the weight of the wet soil, in the operating software, go to the **Measure Components** tab in the experiment and select the **Measure Soil Wet Weight** option. Take the measurement by clicking **OK** when asked. Check the Soil Wet Weight measurements in the Plants table of yotheur experiment. The weight will appear in the "Soil Wet Weight" column (**Supplementary Figure 1D,G**).

5.8. If some of the measurements seem to fluctuate inappropriately, please do the following:

5.8.1. Confirm that each pot is positioned correctly and is not touching any neighboring pot(s).

5.8.2. Disconnect the first controller on the table from the electricity (the rest of the controllers are serially connected to one another and thus will shut down as well) for 2 min and then reconnect it.

5.9. Measure manually the average weight of a few (5–10) cavities with potting medium (from Step 2.3) without seedlings (soil plug). In the **Measure Components** tab, press **Set Seedling Bulk-Soil Weight** and fill in the average weight (**Supplementary Figure 1D**).

5.10. Click Measure Plant Net Weight. This first measurement is a reference point before planting (**Supplementary Figure 1D**).

5.11. Make sure that the seedlings in the cavity trays are well irrigated (i.e., to field capacity after drainage). Gently pull the seedlings with their root-soil plug from the cavities, making sure not to injure them, and place them carefully into the cavities made by casts in the pots,

according to the experiment design. It is preferable to transfer the plants at dawn or dusk, in order to minimize the stress to the plants (i.e., to minimize wilting).

5.12. Click **Measure Plant Net Weight** again. This second measurement is the plant net weight. Meta-tag the measurement to the first one (the reference point). The software will calculate the difference between the two measurements and subtract the Seedling Bulk-Soil Weight. The result is the plant net weight.

5.13. Check the measured values in the Plants table of the experiment to make sure that they fall within a reasonable and logical range (**Supplementary Figure 1C**).

5.14. Saturate the soil by repeating Steps 4.18 through 4.20.

5.15. Make sure that all of the pots are draining properly. If not, repeat the saturation process. Wait 30 min for the drainage to cease. (See also **Table 1** regarding the correct choice of potting medium.)

5.16. Under the **Measure Components** tab, click **Measure Reserved Water Weight** (**Supplementary Figure 1D**).

5.17. Extract the Plants table as a spreadsheet, subtract the measured Plant Net Weight and Seedling Bulk-Soil Weight from the reserved water weight measurementat (the "Reserve Water Inventory" column). Upload the file (**Supplementary Figure 1C)**.

5.18. Confirm that the time period during which daily transpiration will be recorded is appropriate for the goals of the experiment. Fill in the values in the experiment general tab as appropriate for the project (**Supplementary Figure 1H**).

5.18.1. Fill in zero hour: The time at which the software will check whether it needs to move to the next step in the treatment scenario.

5.18.2. Fill in daily transpiration values: Daily transpiration is calculated as the difference between two weight windows during the day, for all days. The daily transpiration start time is the time at which the software will begin to measure the average weight.

5.19. Monitor the plants for 1–2 days before starting a new experiment (duplicate and rename the experiment).

6. Change the Plants Table

6.1. Extract the Plants table as a spreadsheet and change the table according to needs. **Do not change the Plant IDs, Names or Positions.** Save and upload the file.

6.2. Labeling (grouping) columns: To present or analyze (please see Step 8) grouped plants based on common labels (e.g., treatment, line), add a new column and label starting with # (e.g., #Treatment). In this column, make a notation for each plant (e.g., for "#Treatment" label, mark the plants as drought, control, etc.; **Supplementary Figure 2**).

NOTE: The protocol presented above is the most advanced and comprehensive protocol for this system. However, first-time users may want to start with the simplified protocol (see Supplementary MS). The simplified protocol yields information about fewer traits and may lead to higher noise levels. But, at the same time, it provides a way to more easily become acquainted and familiar with the most important experimental procedures, hardware and software.

7. Run the experiment

7.1. Calculate the soil gravimetrical water content/soil water content (SWC value).

NOTE: Gravimetric soil water content is different from volumetric soil water content (VWC).

7.1.1. The SWC value is the ratio between the dry weight of the soil and the wet weight of the soil. To calculate SWC, use the three extra soil-filled pots (Step 1.3) without plants that were previously prepared and placed on a side table inside the greenhouse for a few days and irrigated regularly. Weigh the wet soil in an aluminum tray in the early morning, as soon as possible after the last irrigation event.

7.1.2. Dry the aluminum tray with the soil in an oven (at 105 °C) for 4–5 days. Verify that the soil is completely dry by taking two consecutive weight measurements at least 60 min apart. If the weights are identical, the soil is indeed dry and the last measurement can be recorded as the dry soil weight.

7.1.3. In the operating software, go to **Measure Components** and click on the **Calculate Soil Dry Weight** tab. Fill in the soil wet and dry weights for each sample, click **Apply** and **Finish** (**Supplementary Figure 3**).

7.2. Manually calculate SWC using equation shown below.

$$\mathrm{SWC} = \frac{Wet\ weight - Dry\ weight}{Dry\ weight}$$

7.3. Average the two SWC measurements taken manualy from at least three pots. Select the **Measure Components** tab and click on **Calculate Soil Dry Weight** the θg [g/g] value, click **Apply** and **Finish** . The soil dry weights of all of the experiment pots will be calculated automatically by the software (assuming that all of the pots in the experiment contain the same medium; **Supplementary Figure 1D** and **Supplementary Figure 3**).

7.4. Apply the irrigation treatments. Irrigation scenarios can be applied by composing a step-by-step treatment plan.

7.4.1. To compose a new irrigation treatment plan, go to **Irrigation Treatment**, click on **Create New**, and name the new treatment. Open the specific treatment in the list of irrigation treatments and click the on the default "00:00".

NOTE: In the main window (**Supplementary Figure 4A**), "Time" indicates the time the valve will open (i.e., the beginning of the irrigation treatment). "Valve" is the valve to be opened (A or B, depending on the valve that is connected to the desired solution). "Command Type" indicates the type of data that will be used to determine when the valve will be closed:

- By Time – How many seconds the valve will be open.
- By Weight – The weight gain/water (in grams) to be added to the pot via irrigation.
- By Transpiration – Irrigation can be applied differentially to each pot based on the transpiration of each individual plant over the previous day. The user can decide what percentage of the previous day transpiration will be applied during irrigation. (Under the well-irrigated condition, it is suggested to give the plant more than 100%, in order to wash the soil and compensate for plant growth.) Drought-treated plants should be given less water, with exact volumes based on the desired drought stress rate.
- By Sensors – Irrigation can be applied according to a sensor reading, such as apparent dielectric permittivity (which can be used to determine the VWC). Select the sensor type, the desired parameter and the desired parameter value.

7.4.2. All possibilities include a Time Out option that will close the tap even if the set conditions were not reached. Set the Time Out for a period longer than the set conditions.

7.4.3. After defining the irrigation treatments for the experiment, open the desired experiment in the list of experiments, open **Treatment Scenario**, open default **Plan** and select the first step (**Supplementary Figure 4B**).

7.4.4. In **Treatment**, choose an irrigation treatment from the list. Then, in **Termination**, choose the appropriate condition to stop the current step and move on to the next one.

7.4.5. After selecting an irrigation scenario, open the experiment's Plants table (**Supplementary Figure 2**) and input the "Treatment" and "Step" for each plant. "Treatment" is the name of the treatment scenario and "Step" is the event number within the treatment scenario.

7.5. Plan a drought treatment.

7.5.1. Each individual plant has a unique transpiration rate based on its size and location in the greenhouse. To enable a standard drought treatment (i.e., similar drying rate for all pots during

the treatment), plan a drought scenario and control it via the system's feedback-irrigation tool (**Supplementary Figure 5**).

8. Analyze the data using data analysis software

8.1. Open the Data Analysis software (e.g., SPAC Analytics). Click on the top right corner to select **Control** system and the name of the experiment (**Supplementary Figure 6A**). In the column of the left side of the screen, select **Experiments** (**Supplementary Figure 6B**) and type the name of the experiment in the Name bar under the Search section. The name of the experiment will appear below the Search section, in the Experiments section (**Supplementary Figure 6C)**. Click on the experiment to open the Info and Plants sections (**Supplementary Figure 6D**).

8.2. In the Info section, edit the WUE start and WUE end dates for a period of at least 3 (preferably more) days before the start of the drought treatment and then click **Update**. The WUE and the R^2 value for every pot will appear in the Plants section. Choose to exclude any scale with a negative WUE value or an R^2 value of less than 0.5 by clicking on the "eye" symbol under the Active column, which will then turn red. This will exclude the selected scale (plant) from all further calculations. The data can be exported by clicking on the **Export Data** button in the Plants section (**Supplementary Figure 6D**).

8.3. In the column on the left side of the scree, click on **Analysis**. Different subsections will then appear: Graph viewer, Histogram, T-test, ANOVA and Piecewise linear curve.

8.4. Click on **Graph viewer**. In the **Filters** section, set the dates for the experiment.

8.4.1. Click on **Labels** (please see Step 6) to select the combination of experimental groups (genotype) and treatment(s). Automatically, all of the pots in the selected group will appear in the Plant subsection. In that subsection, deselect any pots (plants) by clicking on them. Up to two different parameters of choice can be selected at one time as the "Y1 parameter" and "Y2 parameter". Finally, click on **Show Graph** (**Supplementary Figure 5**).

8.4.2. A line graph of the values of the selected parameter will appear in the Graph Viewer window for each plant. Remove data from individual plants or add to the graph by clicking on their legend symbols on the right of the graph. In the top right corner, there are also options for exporting the data as a spreadsheet and for enlarging the Graph Viewer window to fill the full screen (this raw data download function is relevant to all other windows). More options to modify the graph will appear if the cursor is moved to the top right corner of the screen (**Supplementary Figure 5**).

8.5. The histogram module presents the distribution of a single trait in and between populations for a given time period. To use this module, click on **Histogram**.

8.5.1. In the Filters section, set the date and time, parameter, labels and plants as explained in step 8.4.1. Select multiple labels (groups) by clicking on the **+** symbol. Finally, click on **Show Graph** (**Supplementary Figure 7**).

8.5.2. The histogram will appear in the Histogram section, in which there is the option to change the "Bins" and "Date" at the top of the screen. In the top right corner, there are various options as described in step 8.4.2. In the Location Diagram section, the actual location of the plants on the experimental table and their respective trait values can be seen (**Supplementary Figure 7**).

8.6. Click on **T-test**. To statistically compare the means of any measured trait of two groups, enter the dates, labels, plants and parameters in the "T-test Parameters" section, as explained in step 8.4.1.

8.6.1. Set the range of hours to calculate the average values of the data points within the time period of interest (the default is a continuous 24-h presentation). Finally, click on **Show Graph** (**Supplementary Figure 8**).

8.6.2. Two windows will appear on the right side of the screen. The top one is the Graph Viewer section for all of the plants selected from both groups. Below that window is the T-test section, in which will appear the comparison of the two groups as the *t*-test of the physiological parameter selected. Levels of significance can be adjusted by changing the α-value in the top left corner of the screen. A red dot will appear under values that are significantly different. In the top right corner, view various options, as described in step 8.4.2 (**Supplementary Figure 8**).

8.7. Click on **ANOVA**. To statistically compare the means of any measured trait across more than two groups, enter the dates, labels, plants and parameters in the "Filters" section, as explained in step 8.5.1.

8.7.1. Select multiple labels (groups) by clicking on the **+** symbol (as in step 8.5). Set the range of hours. Finally, click on "SHOW GRAPH" (**Supplementary Figure 9**).

8.7.2. In the ANOVA section, use an ANOVA test (Tukey's HSD) to compare the physiological parameters of the different groups. Bars represent the standard errors (±SE). In the top right corner of the screen, there are various options as described in step 8.4.2. Click on the line graph to view a bar-graph comparison for a particular day. Different letters indicate groups that are significantly different from one another (**Supplementary Figure 9A**).

8.8. Presenting the relationship between whole-plant transpiration kinetics or stomatal conductance and VWC is a more accurate way to compare the physiological responses of different plants to drought, as compared to a time-based approach. Present this relationship using the "Piece-wise Linear Curve" function.

8.8.1. Click **Piecewise linear curve**. Enter the dates, labels, plants and parameters (both the x-axis and the y-axis) and then set the range of hours in the "Filters" section, as explained above.

NOTE: The "from" date should be as close as possible to the treatment start date.

8.8.2. Set the x-axis parameter to be VWC and the y-axis parameter as the physiological parameter of choice (e.g., transpiration rate, stomatal conductance, etc.). Finally, click on **Show Graph**. In the "Filter" section, click on **Select all recommendations** and then click on **Show Graph** (**Supplementary Figure 10**).

NOTE: Other physiological parameters (e.g., normalized transpiration, transpiration rate, plant net weight, stomatal conductance, root flux, etc.) and environmental parameters (e.g., temperature, relative humidity, etc.) are easily obtained via the SPAC software (e.g. **Supplementary Figure 9C)**. For more information regarding the theoretical background of their calculations, please see Halperin et al. (2017).

REPRESENTATIVE RESULTS:

The duration of the experiment was 29 days. The experiment was conducted in August, when the local weather is warm and stable and the days are long. Two different irrigation scenarios were used to demonstrate the capability of the phenotyping platform for comparing the physiological behavior of three different varieties of rice (i.e., Indica, Karla, and Risotto) in the presence of drought stress. There were two drought-stress treatments: (i) optimal irrigation [until each pot reached its pot capacity at night after irrigation (control)] and (ii) a drought that started 5 days after the experiment started, lasted for 14 days, and was followed by a 10-day recovery period (optimal irrigation, Days 19–29). For the sake of simplicity, not all of the varieties and groups are shown in the figures presented here. The results showed that the HTP-telemetric system can efficiently measure changes in atmospheric conditions, the soil and the physiology of the plants.

Environmental conditions

Environmental conditions [photosynthetically active radiation (PAR) and vapor pressure deficit (VPD)] were monitored throughout the experiment by an atmospheric probe. The collected data indicate that PAR and VPD remained similar over the different days and over the course of the day (**Figure 4**).

The VWC of the drought-treated pots was measured by soil probes throughout the experimental period. The VWC data collected from one drought-treated cv. Indica plant is plotted in **Figure 5**.

Physiological parameters

The daily transpiration gradually increased in all four treatments (Karla-control, Karla-drought, Risotto-control and Risotto-drought) during the first stage of the experiment, during which all of the plants were well-irrigated. Later, there was a reduction in transpiration that was associated with the drought period (Day 5 to Day 18) in the two water-deprived treatments.

Subsequently, during the recovery period (from Day 18 onward), the daily transpiration increased again in the two water-deprived groups, but to a much lower level than that observed before the drought treatment (**Supplementary Figure 9B**).

The mean calculated plant weight (i.e., rate of plant weight gain) 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 (Days 1–5). When the drought treatment was applied to the cv. Karla plants (Days 5–18), 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. In contrast, the weights of the Karla-control plants increased continuously throughout the experimental period (**Figure 6**).

FIGURE AND TABLE LEGENDS:

Figure 1: Components and setup of the gravimetric phenotyping system. (**A**) Weighing lysimeter. The lysimeter includes the load cell, which converts the mechanical load of an object into an electrical charge, and a metal platform that covers the upper and lower parts of the load cell, so that the object's weight can be properly measured. (**B**) The lysimeter is covered with a polystyrene block and a plastic cover for heat insulation. (**C**) Scale parts. A water reservoir (green container) is placed on the lysimeter cover to collect the liquid that drains from the pot. The green container is coupled to a green cover, which has a large round opening through which the pot is inserted. A black rubber gasket ring is attached to one side of the green cover and the pot is attached to the other side, to minimize water loss via evaporation from the container. The green cover has two sampling holes (small and big) above the drainage extension, which are sealed with rubber plugs. (**D**) Plugs. The container has a drainage extension with four holes (with plugs) at different heights, which can be used to adjust the water level in the container after the drainage through a particular hole stops (the reserve water volume). The desired water volume will depend on the plant species, the type of potting medium being used and the water requirements of the plants (i.e., estimated daily transpiration volume). (**E**) The control unit consists of a green rectangular box that contains the electronic controller and solenoid valves. There are holes through which fertigation solution can enter and exit the pots, as well as sockets for connecting the load cell and different sensors. Different treatments, such as different levels of salinity or different mineral compositions, can be applied via the fertigation solution. A metal stand is connected to the controller, to hold the pipes and cables and prevent them from touching the pots and adding weight**.** The other components required are (**F**) soil probes (e.g., moisture, temperature and EC sensors - 5TE), optional (**G**) multi-outlet drippers (for fertigation and/or treatment applications) and (**H**) atmospheric probes [for measuring vapor pressure deficit (VPD) and radiation]. (**I**) Fully equipped single array. (**J**) Fully equipped array in the greenhouse, yellow arrows pointing the atmospheric probes which enables the stomatal conductance normalization based on the local atmospheric conditions.

Figure 2: Parts required for a single pot set-up. (**A–C**) The following components are needed: one 4 L pot, one 4 L pot with no bottom to serve as a net holder, one circular piece of nylon

mesh (pore size = 60 mesh) with a diameter double that of the bottom of the pot, one cover with designated holes for plant and irrigation drippers, one 60 cm, white fiberglass stick (pole) and one black gasket ring. (**D**) Example of a table plan in which the pots have been randomized. In the greenhouse, each table had 1–18 columns and four rows, here we used 24 positions. However, the array structure can be easily adjusted to any shape based on the size of the own greenhouse.

Figure 3: Pot set-up**.** (**A**) Plants growing in cavity trays. (The tomato seedlings shown here are only an example; many other plant species could be grown in the same way). (**B**) Casts of molds for (**C**) creating cavities in the potting medium that will (D) closely fit the root-soil plugs of the seedlings, to ensure the successful transplanting of (E) the seedlings into the pots.

Figure 4: Atmospheric conditions over the course of the experiment. The y-axis on the right shows the daily vapor pressure deficit (VPD) and the y-axis on the left shows the photosynthetically active radiation (PAR) over the 29 consecutive days of the experiment. This graph was produced by the Data Analysis software.

Figure 5: Volumetric water content (VWC) measured by a soil probe over the course of the experiment. The data represent the VWC values for one cv. Indica plant that was subjected to the drought treatment for the entire experiment period, including recovery. This graph was produced by the Data Analysis software.

Figure 6: Whole-plant weights (means ± SE) over the entire experimental period for cv. Karla under well-irrigated (control) and drought conditions. Groups were compared using ANOVA (Tukey's HSD; $p < 0.05$). Each mean ± SE represents at least four plants. The graph and the statistical analysis were produced by the Data Analysis software.

Supplementary Figure 1: **Operating software windows for setting up an experiment**.

Supplementary Figure 2: 'Plants' table as a spreadsheet; Operating software.

Supplementary Figure 3: Software window for calculating the soil dry weight; Operating software.

Supplementary Figure 4: Software window for setting up an irrigation treatment; Operating software.

Supplementary Figure 5: Data Analysis Graph Viewer window. In our experiment, we used three cultivars of rice (i.e., Indica, Karla, and Risotto) and two different irrigation scenarios, well-irrigated (control) and drought. The raw data revealed variation in the weight of the plants over the course of the experiment. Each line represents one plant/pot. During the day, the plants transpired, so the system lost weight, as can be seen in the slopes of the daily curves. The pots were irrigated every night to full capacity, as represented as the peaks in the curves. The irrigation event was followed by drainage of any excess water after the potting medium

had been saturated. Initially, all plants were well irrigated (control). From 7 August 2018, half of the plants were subjected to a drought treatment. At the same time, the rest of the plants continued to receive optimal irrigation. Differential recovery was achieved by restoring the irrigation to the drought-treated plants, beginning on 20 August 2018 (allowing each plant to experience a similar degree of stress) and continuing through the experiment end.

The system's feedback-irrigation tool enables the user to design irrigation programs for each individual pot based on time, pot weight, data from a soil sensor (e.g., VWC) or plant transpiration over the previous day. Each plant can be irrigated individually in a customized manner based on its own performance. This differential irrigation minimizes the differences between the plants' soil water contents, so that all of the plants are exposed to a controlled drought treatment regardless of their individual water demands.

Supplementary Figure 6: Data Analysis window for the data analysis.

Supplementary Figure 7: Data Analysis histogram window. This figure shows a graphical representation of the distribution of daily-transpiration values in the three different rice cultivars (i.e., Indica, Karla, and Risotto) under well-irrigated (control) conditions. The bottom diagram represents a heat-map visualization of the plants daily transpiration based on the physical location of the pots on the table.

Supplementary Figure 8: Data Analysis T-test window. Lines represent the differences in daily transpiration (a fundamental and important physiological trait) between two rice cultivars (i.e., Karla and Risotto) under well-irrigated (control) conditions. The window shows the daily transpiration of the individual plants (top right) and a comparison of the means ± SE of each group conducted using Student's *t*-test (bottom right). The statistical analysis was performed automatically by the software. The red dots represent significant differences between treatments according to the Student's *t*-tests; $p < 0.05$.

Supplementary Figure 9: Data Analysis ANOVA window. (A) Graphical representation of the differences in daily transpiration between two rice varieties (i.e., Karla and Risotto) under well-irrigated (control) and drought conditions over the entire experimental period. The drought treatment was started 5 days after the experiment started. Clicking on any day will present the **(B)** Groups comparison using ANOVA (Tukey's HSD; $p < 0.05$), here on AUG the 12th. Each mean ± SE represents at least four plants. The same groups could be also presented as a **(C)** Continuous whole-plant transpiration-rate (Means ± SE) over the entire experimental period. The graphs and the statistical analysis were produced by the Data Analysis software.

Supplementary Figure 10: Data Analysis piece-wise linear curve window. This window shows the piece-wise linear curves of three rice cultivars (i.e., Indica, Karla and Risotto) under drought conditions. The software can perform a piece-wise linear fit analysis of the relationship between any physiological parameter (here, daily transpiration) and the calculated volumetric water content (VWC) of the plants subjected to the drought treatment.

Table 1: Potting media

Table 2: General characteristics of 9 different potting media and their compatibility with the gravimetric platform. The measurements were taken using 4-L pots filled with 3.2 L of medium at field capacity (pot capacity). Data are shown as means ± SE. Different letters in the columns indicate significant differences between the media, according to Tukey's HSD test ($P < 0.05$; $3 \leq n \leq 5$).

Table 3: Fertigation components

DISCUSSION:

The genotype–phenotype knowledge gap reflects the complexity of genotype x environment interactions (reviewed by[18,24]). It might be possible to bridge this gap through the use of high-resolution, HTP-telemetric diagnostic and phenotypic screening platforms that can be used to study whole-plant physiological performance and water-relation kinetics[8,9]. The complexity of genotype x environment interactions makes phenotyping a challenge, particularly in light of how rapidly plants respond to their changing environments. Although various phenotyping systems are currently available, most of those systems are based on remote sensing and advanced imaging techniques. Although those systems provide simultaneous measurements, to a certain extent, their measurements are limited to morphological and indirect physiological traits[25]. Physiological traits are very important in the context of responsiveness or sensitivity to environmental conditions[26]. Therefore, direct measurements taken continuously and simultaneously at a very high resolution (e.g., 3 min intervals) can provide a very accurate description of a plant's physiological behavior. Despite those substantial advantages of the gravimetric system, the fact that this system has some potential disadvantages must also be taken into account. The main disadvantages result from the need to work with pots and in greenhouse conditions, which can present major challenges for treatment-regulation (particularly the regulation of drought treatments) and experimental-repeatability.

In order to address these issues, one should standardize the applied stresses, create a truly randomized experimental structure, minimize pot effects and compare multiple dynamic behaviors of plants under changing environmental conditions within a short period of time. The HTP-telemetric functional phenotyping approach described in this paper addresses those issues as noted below.

In order to correlate the plant's dynamic response with its dynamic environment and capture a complete, big picture of complex plant–environment interactions, both environmental conditions (**Figure 4**) and plant responses (**Supplementary Figure 9B**) must be measured continuously. This method enables the measurement of physical changes in the potting medium and atmosphere continuously and simultaneously, alongside plant traits (soil–plant–atmosphere continuum, SPAC).

To best predict how plants will behave in the field, it is important to perform the phenotyping process under conditions that are as similar as possible to those found in the field[18]. We

conduct the experiments in a greenhouse under semi-controlled conditions to mimic field conditions as much as possible. One of the most important conditions is the growing or potting medium. Selecting the most suitable potting medium for the gravimetric-system experiment is crucial. It is advisable to choose a soil medium that drains quickly, allows for the rapid achievement of pot capacity and has a highly stable pot capacity, as those features allow for more accurate measurements by the gravimetric system. In addition, the different treatments to be applied in the experiment must also be considered. For example, treatments involving salts, fertilizers or chemicals call for the use of an inert potting medium, preferably one with a low cation-exchange capacity. Drought treatments applied to low-transpiring plant species would work best with potting media with relatively low VWC levels. In contrast, slow drought treatments applied to high-transpiring plants would work best with potting media with relatively high VWC levels. If the roots are required for post-experiment analysis (e.g., root morphology, dry weight, etc.), the use of a medium with relatively low organic matter content (i.e., sand, porous ceramic or perlite) will make it easier to wash the roots without damaging them. For experiments that will continue for longer periods, it is advisable to avoid media that are rich in organic matter, as that organic matter may decompose with time. Please see **Table 1** and **Table 2** for more detailed information on this topic.

Field phenotyping and greenhouse phenotyping (pre-field) have their own objectives and require different experimental set-ups. Pre-field phenotyping assists the selection of promising candidate genotypes that have a high probability of doing well in the field, to help make field trials more focused and cost-effective. However, pre-field phenotyping involves a number of limitations (e.g., pot effects) that can cause plants to perform differently than they would under field conditions[18,27]. Small pot size, water loss by evaporation and heating of the lysimeter scales are examples of factors in greenhouse experiments that may lead to pot effects[18]. The method described here is designed to minimize those potential effects in the following manner:

(a) The pot size is chosen based on the genotype to be examined. The system is capable of supporting various pot sizes (up to 25 L) and irrigation treatments, which enables the examination of any type of crop plant.
(b) The pots and the lysimeter scales are insulated to prevent heat from being transferred and any warming of the pots.
(c) This system involves a carefully designed irrigation and drainage system.
(d) There is a separate controller for each pot, to enable true randomization with self-irrigating and self-monitored treatments.
(e) The software takes into account the plants' local VPD in calculating the canopy stomatal conductance. Please see the multiple VPD stations localization in **Figure 1J**.

This system involves direct physiological measurements at field-like plant densities, which eliminates the need for either large spaces between the plants or moving the plants for image-based phenotyping. This system includes real-time data analysis, as well as the ability to accurately detect the physiological stress point (θ) of each plant. This enables the researcher to monitor the plants and make decisions regarding how the experiment is to be conducted and how any samples are to be collected over the course of the experiment. The system's easy and

simple weight calibration facilitates efficient calibration. High-throughput systems generate massive amounts of data, which present additional data-handling and analytical challenges[11,12]. The real-time analysis of the big data that is directly fed to the software from the controller is an important step in the translation of data into knowledge[14] that has great value for practical decision-making.

This HTP-telemetric physiological phenotyping method might be helpful for conducting greenhouse experiments under close-to-field conditions. The system is able to measure and directly calculate water-related physiological responses of plants to their dynamic environment, while efficiently overcoming most of the problems associated with the pot effect. This system's abilities are extremely important in the pre-field phenotyping stage, as they offer the possibility to predict yield penalties during early stages of plant growth.

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DISCLOSURES:
The authors have nothing to disclose.

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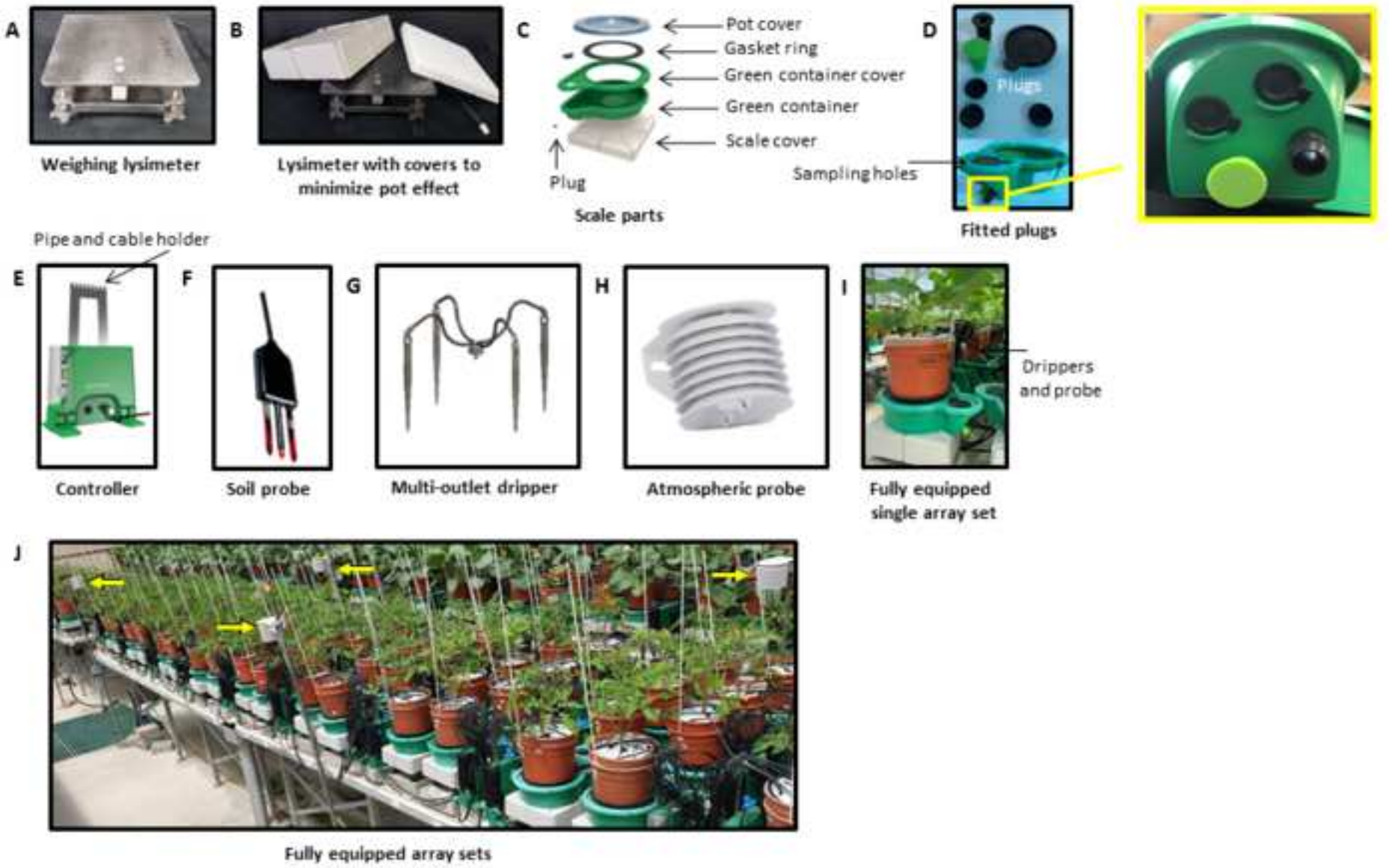
Figure 1



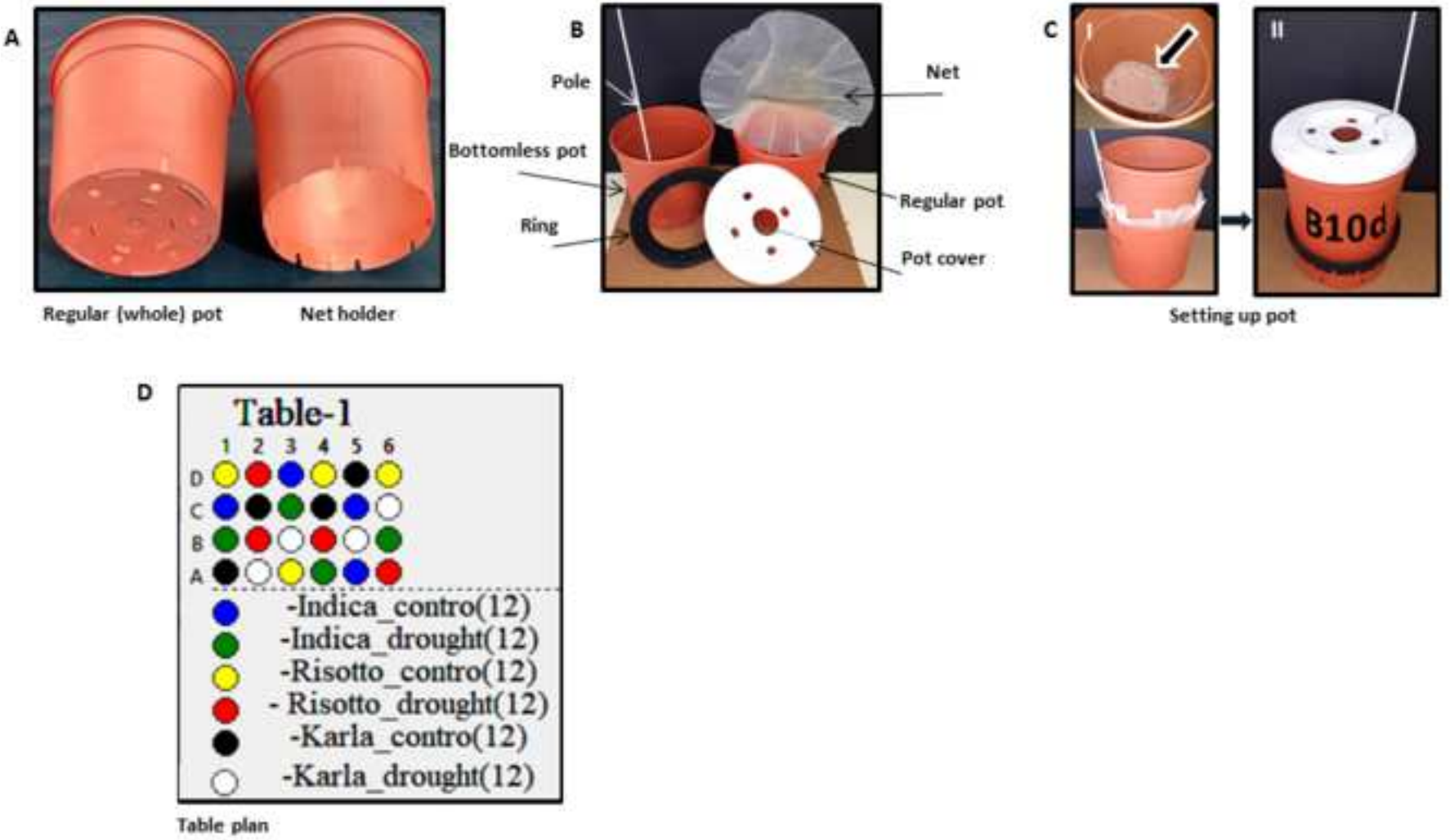


Figure 2

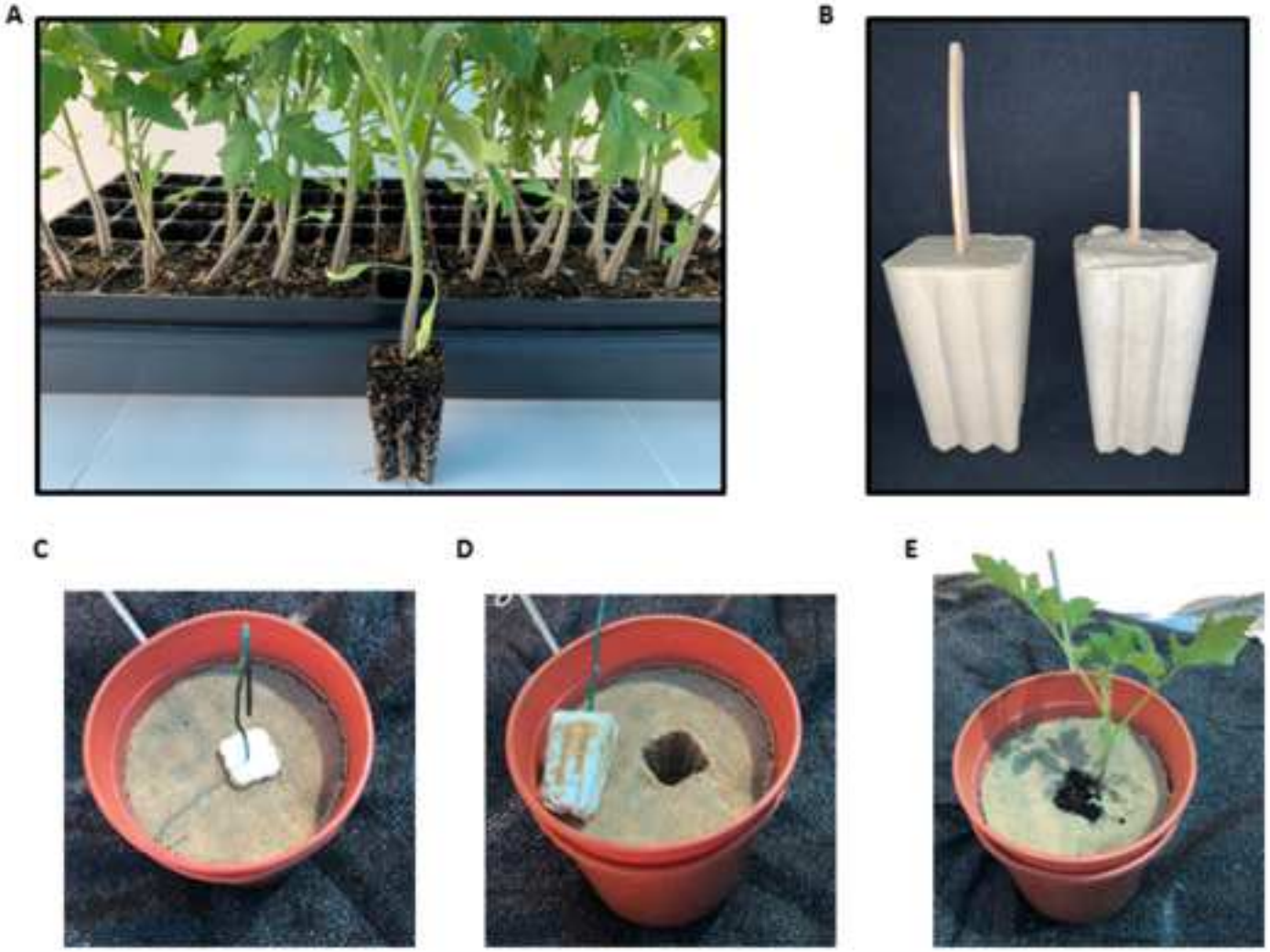


Figure 3

Figure

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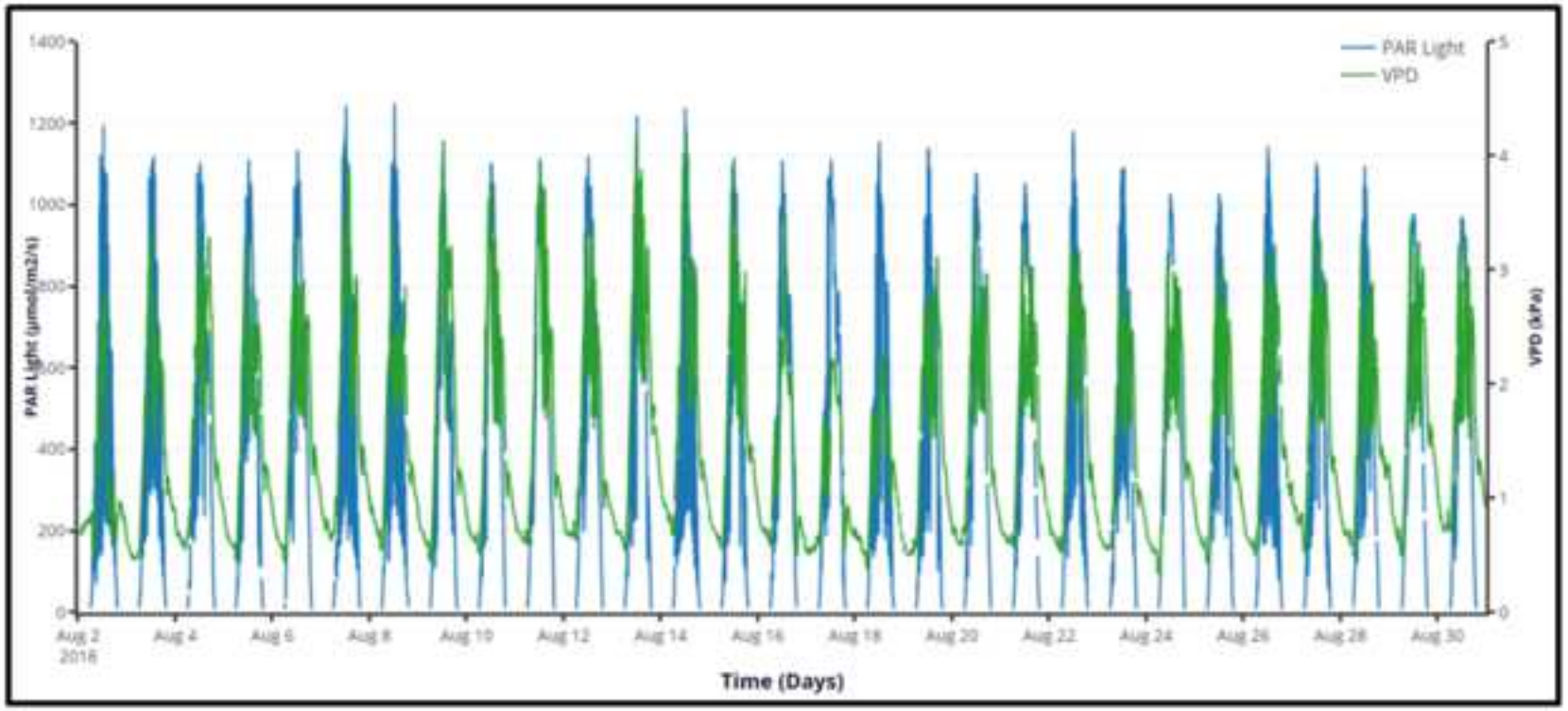

Figure 4

Figure

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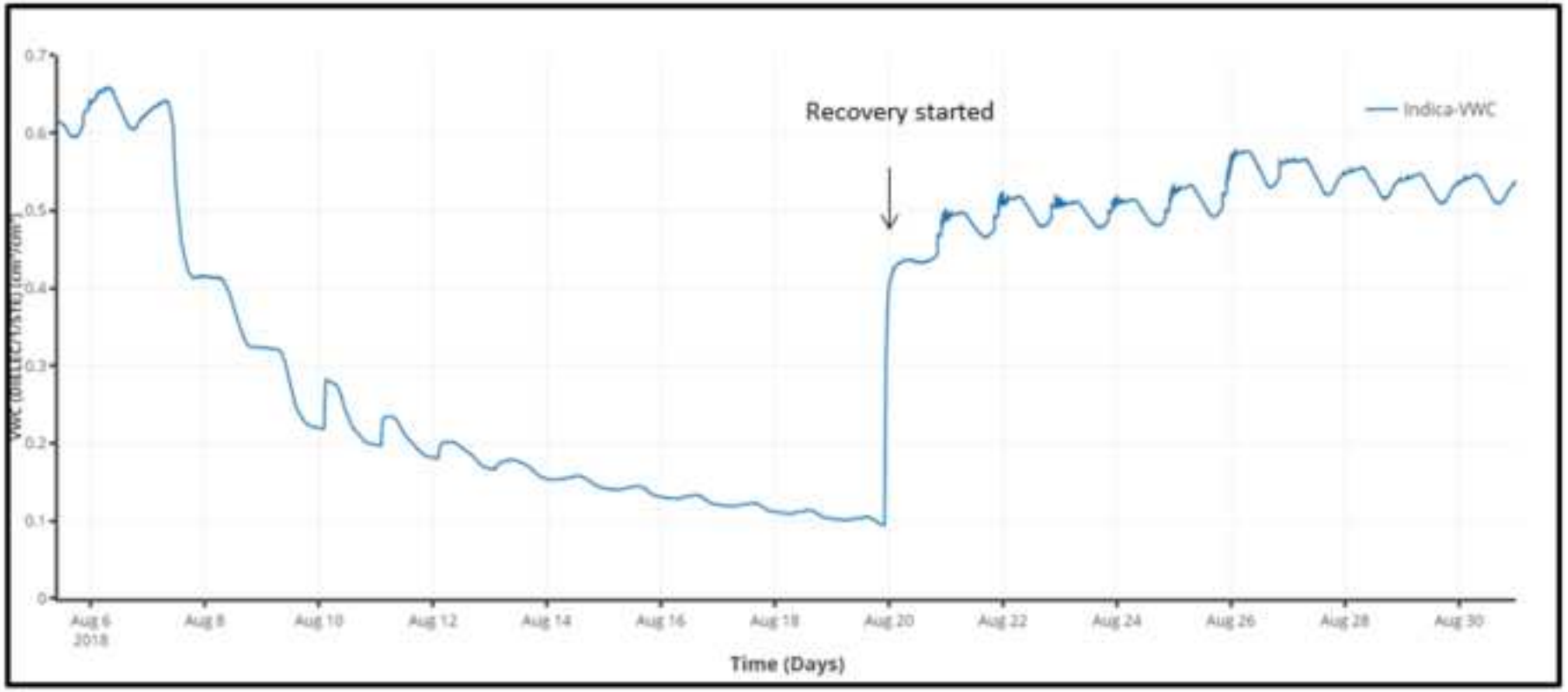

Figure 5

Figure

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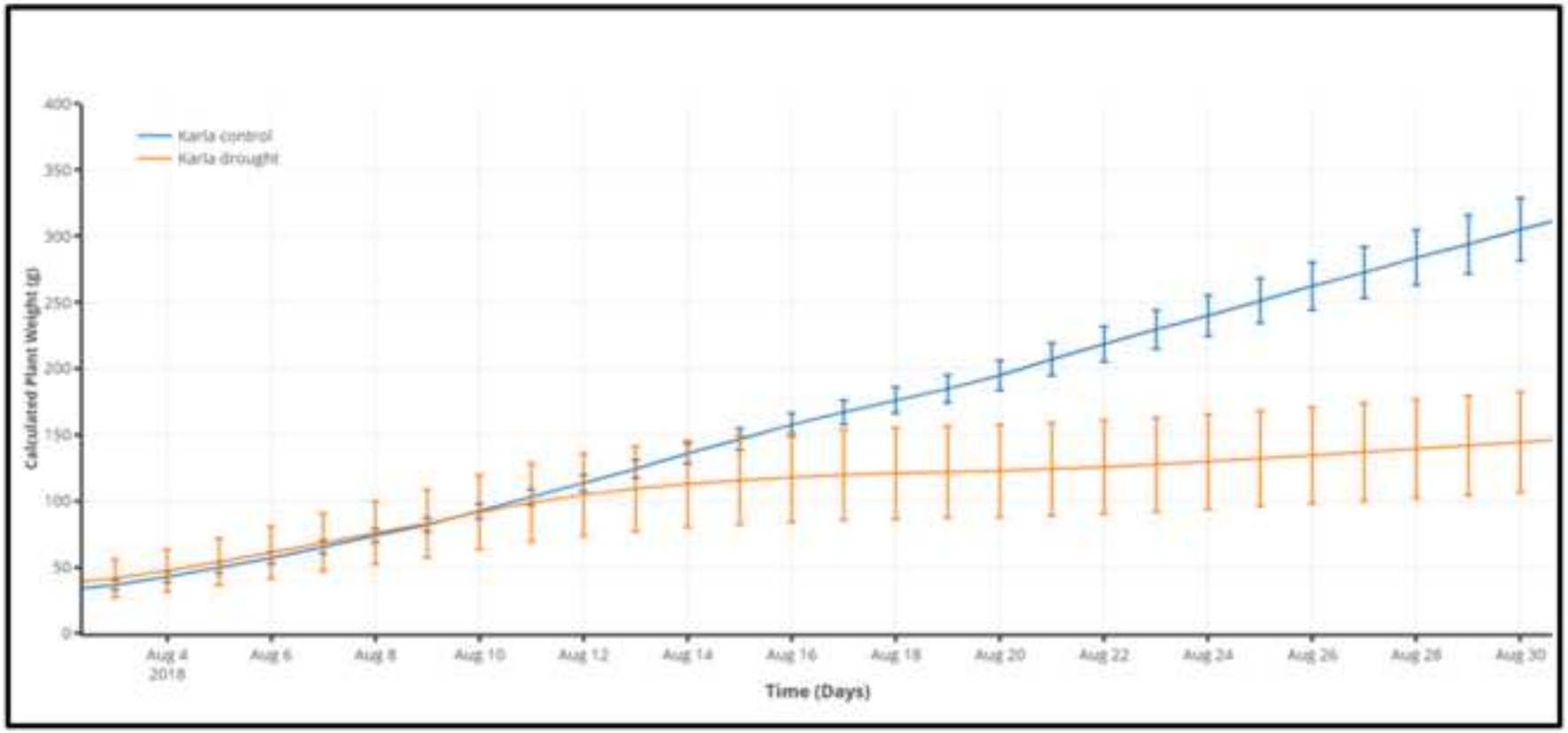


Figure 6

Table 1. Potting media

Medium
Coarse sand
Fine sand
Peat-based soil
Loamy soil (natural soil)
Vermiculite
Perlite
Compost
Porous, ceramic, small-sized medium
Porous, ceramic, mixed-sized medium

Description
Silica sand 20-30 (upper and lower mesh screens through which the sand was passed: 0.841 and 0.595 mm,
Silica sand 75-90 (upper and lower mesh screens through which the sand was passed: 0.291 and 0.163 mm,
Klasmann 686
Sandy loam soil taken from the top layer of a plot at the experimental farm of the Faculty of Agriculture, Foc
Vermiculite 3G
Perlite 212 (Size range: 0.5-2.5 mm)
Bental 11 Potting soil
Profile Porous Ceramic 20-50 (upper and lower mesh screens through which the ground ceramic was passec
Profile Porous Ceramic 50% 20-50 mesh and 50% 20-6 mesh, 0.841– 3.36 mm

respectively)
respectively)
od and Environment, Rehovot, Israel
d: 0.841 and 0.297 mm, respectively)

Table 2. General characteristics of 9 different potting media and their compat

The measurements were taken using 4-L pots filled with 3.2 L of medium at field capacity (p

Soil media type / Parameters	**Coarse sand**	**Fine sand**
Total available water (TAW, ml)	860 ± 7.2 (F)	883.1 ± 24 (F)
Volumetric water content (VWC, ml^3/ml^3)	0.26 (F)	0.27 (F)
Bulk density (BD, g/cm^3)	1.7 (A)	1.6 (B)
Soil weight stability (SWS, g/d)	±2.3 ± 0.3 (B)	±4.3 ± 0.3 (B)
Soil weight stability with reserved water in the bath (g/day; please see Section 6.14)	3 ± 0.4 (B)	3.3 ± 0.4 (B)
Pot capacity gravimetric moisture content (SWC; please see Section 8.2)	0.18 (G)	0.23 (G)
Relative drainage capability	Excellent	Medium
Relative time to reach pot capacity	Fast	Fast
Relative cation exchange capacity (CEC)	Low	Low
Compatibility with:		
Root washing (at the end of the experiment)	++	++
Nutrient/biostimulant treatment	++	++
Salinity treatments	++	++
Accurate measurement of growth rates	++	++
Physical soil structure recovery after drought	+++	+++

* Total available water (TAW) = soil wet weight (at pot capacity) – soil dry weight. Volumetric water c
Bulk density (BD) = soil dry weight/soil volume. Soil weight stability (SWS) = Average change in soil we
Pot capacity gravimetric moisture content (SWC); for the calculation, please see Section 7.2.

tibility with the gravimetric platform

ot capacity). Data are shown as means ± SE. Different letters in the columns indicate significant

Loamy soil	Perlite	Vermiculite	Porous ceramic mixed-sized	Porous ceramic small-sized	Peat-based soil	Compost
1076.3 ± 35.9 (E)	1119.9 ± 8.5 (E)	1286 ± 22.4 (D)	1503.6 ± 15.4 (C)	1713 ± 25.9 (B)	1744.3 ± 8.2 (B)	2089.6 ± 61.6 (A)
0.33 (E)	0.35 (E)	0.4 (D)	0.46 (C)	0.53 (B)	0.54 (B)	0.65 (A)
1.5(C)	0.1 (H)	0.2 (F)	0.8 (D)	0.7 (E)	0.2 (G)	0.1 (G)
±2.9 ± 0.9 (B)	±14.9 ± 0.7 (A)	±7.6 ± 2.8 (B)	±1.3 ± 0.1 (B)	±1.9 ± 0.4 (B)	±6.7 ± 0.8 (B)	±4.3 ± 1.2 (B)
3.2 ± 1.2 (B)	6.3 ± 0.5 (A)	2.7 ± 0.8 (B)	1.6 ± 0.3 (B)	1.9 ± 0.3 (B)	10.6 ± 3 (A)	1.5 ± 0.3 (B)
0.23 (G)	3.79 (C)	3.0 (D)	0.74 (F)	0.99 (E)	4.25 (B)	6.13 (A)
Medium-low	Excellent	Excellent	Excellent	Excellent	Low	Medium
Fast	Slow	Slow	Fast	Fast	Slow	Slow
Low	Low	High	High	High	High	High
+	++	+	++	++	-	-
-	++	+	+	+	-	-
+	++	+	++	++	+	-
+	-,+	+	++	+++	+	+
++	+	-	+++	+++	-,+	-

ontent (VWC) = TAW/soil volume.

et weight over 4 consecutive days (medium at pot capacity with no plant).

differences between the media, according to Tukey's HSD test ($P < 0.05$; $3 \leq n \leq 5$).

Table 3. Fertigation components

Fertigation components	Final concentration (ppm)	Final concentration (mM)
$NaNO_3$	195.8	2.3
H_3PO_4	209	0.000969
KNO_3	271.4	2.685
$MgSO_4$	75	0.623
$ZnSO_4$	0.748	0.0025
$CuSO_4$	0.496	0.00198
MoO_3	0.131	0.00081
$MnSO_4$	3.441	0.0154
Borax	0.3	0.00078
C10H12N2NaFeO8 (Fe)	8.66	0.0204
The pH of the final irrigation solution from the dripper (after dilution with tap water) varied between 6.5 and 7.		

Table of Materials

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Atmospheric Probes	SpectrumTech/Meter group	3686WD 40027	Watchdog 2475 VP4
Array Randomizer		None	The software "Array Randomizer" car Free download and more informatior
Cavity trays	Danish size with curved rim for nursery	30162	4X4X7 Cell, 84 cell per tray
Coarse sand	Negev Industrial Minerals Ltd., Israel		
Compost	Tuff Marom Golan, Israel		
Data Analysis software	Plant-Ditech Ltd., Israel		SPAC Analytics
Drippers	Netafim	21500-001520	PCJ 8L/h
Fine sand	Negev Industrial Minerals Ltd., Israel		
Loamy soil (natural soil)			
Nylon mesh	Not relevant (generic products)		
Operating software	Plant-Ditech Ltd., Israel		Plantarray Feedback Control (PFC)
Peat-based soil	Klasmann-Deilmann GmbH, Germany		

Perlite	Agrekal , Israel		
Plantarray 3.0 system	Plant-Ditech Ltd., Israel	SCA400s	Weighing lysimeters
		PLA300S	Planter unit container
		CON100	Control unit
		part of the planter set	Fiberglass stick
		part of the planter set	Gasket ring
			Operating software
			SPAC Analytics software
Porous, ceramic, mixed-sized medium	Greens Grade, PROFILE Products LLC., USA		
Porous, ceramic, small-sized medium	Greens Grade, PROFILE Products LLC., USA		
Pots	Not relevant (generic products)		
Soil	Bental 11 by Tuff Marom Golan		
Soil Probes	Meter group	40567	5TE
		40636	5TM
		40478	GS3
Vermiculite	Agrekal , Israel		

ı be used for creating an experimental design of a randomized block design, or fully random design. It was developed to have better cc
ı, please click on the following link: https://drive.google.com/open?id=1y4QbTpxRK5Lx430xzu1RFdrlcL8pz_1q

https://desch.nl/en/products/seed_propagation_trays/danish-size-with-curved-rim-for-nursery~p92

ɔntrol over the random distribution of the experimental samples (plants) in order to normalize the atmospheric microvariation inside the

greenhouse.

Editorial comments:
Changes to be made by the Author(s):
1. **Comment/s:** Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

Reply: Thank you for your comment. We have reviewed the manuscript to ensure that there are no spelling or grammar issues.

2. **Comment/s:** Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file. Please sort the Materials Table alphabetically by the name of the material.

Reply: Done.

3. **Comment/s:** Please upload each Figure individually to your Editorial Manager account as a .png or a .tiff file. Please combine all panels of one figure into a single image file.

Reply: Done.

4. **Comment/s:** Please remove the mineral table from the Table of Materials.

Reply: That table was made into a separate table (Table 3: Fertigation Components).

5. **Comment/s:** All tables should be uploaded separately to your Editorial Manager account in the form of an .xls or .xlsx file. Each table must be accompanied by a title and a description after the Representative Results of the manuscript text.

Reply: Done.

6. **Comment/s:** Table 1: Please remove the Company column.

Reply: Done.

7. **Comment/s:** Please reduce the number of figures. Consider whether all figures are necessary due to the video. Screenshots can be considered supplemental files to be used only for scripting.

Reply: We reduced the number of figures by removing the screenshots that were originally presented in the protocol section. They are now provided as supplemental files.

8. **Comment/s:** Please revise the title for conciseness.

Reply: The title was revised.

9. **Comment/s:** For in-text formatting, corresponding reference numbers should appear as numbered superscripts after the appropriate statement(s).

Reply: Done.

10. **Comment/s:** Please revise the text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

Reply: We have minimized their use as much as possible.

11. **Comment/s:** Please revise the manuscript text to remove all colloquial language: "Get to know the system components, etc.

Reply: Done.

12. **Comment/s:** Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note." However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.

Reply: Done.

13. **Comment/s:** The Protocol should contain only action items that direct the reader to do something. Please move the discussion about the protocol to the Discussion.

Reply: The discussions about the protocol were all moved**,** either to the Discussion section or the figure legends.

14. **Comment/s:** The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please simplify the Protocol so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step.

Reply: We have simplified the protocol as much as possible. However, some steps required one or two sentences more. For the sake of clarity, some information could not be moved to the Discussion. We think that this information is essential for readers to easily understand and follow the protocol.

15. **Comment/s:** Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.

Reply: Done.

16. **Comment/s:** 3.1: What is the growing medium used? Please specify and present a specific example instead of a generalized protocol.

Reply: In our experiment, compost was used. However, the particular growing medium (the term *potting medium* is now used throughout the MS) to be used depends on the plants to be used and the goals of the particular experiment. We elaborate on this issue in Section 2 of the Discussion and provide recommendations of specific media to be used under specific conditions. Those media are described in Tables 1 and 2, as well as in Sections 2.1 and 3.2.1–3.2.3 in the protocol.

17. **Comment/s:** What seeds are used?

Reply: We used three different commercial varieties of rice seeds: Indica, Karla and Risotto.

18. **Comment/s:** Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

Reply: Done.

19. **Comment/s:** Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in the imperative tense.

Reply: Done.

20. **Comment/s:** Please do not abbreviate journal titles.

Reply: This has been corrected.

Reviewers' comments:
Reviewer #1:
Manuscript Summary:
The paper deals with the actual topic of plant/crop phenotyping and describes the methodology adopted for a novel phenotyping platform based on continuous gravimetric measurements of plants in parallel with data analysis/processing in real time. The paper is focused on phenotyping for drought stress responses and aims at describing a phenotyping platform that permits robust data gathering and analysis/processing in real time.

Optimizing methodologies and protocols to make easier and faster plant stress monitoring and phenotyping for selection of different genoypes is of outmost importance and will help to retrieve robust data and promote a more efficient selection procedure, in a user-friendly way at preferably lower costs. However, nothing is mentioned about the costs of the described platform/methodology (installation and maintenance), which would be also relevant for readers and potential users of such a platform. The methodological approach here described represents and alternative solution to the use of imaging in phenotyping and selection under

controlled conditions.

Major Concerns:
Comment/s: Authors must explain better why the use of imaging is questionable in plant/crop phenotyping (see Line 83). Indeed, this is a major point raised by authors and needs a more detailed explanation, than only the two supportive references (one belongs to the same group of authors/lab). Therefore, I suggest to use other papers to provide a broader review of the pros/cons of imaging in plant/crop phenotyping (see e.g. Roitsh et al. 2019 Plant Science; Tardieu et al., 2017 Current Biology; Singh et al., 2019 Front. Plant Sci., 03 April 2019; Merlot et al., 2002). On the other hand, the problems associated with phenotyping under controlled environments and/or using potted plants are not optimal when dealing with water stres (see e.g. Araus and Cairns, 2014). Moreover its is also well accepted that field phenotyping is largely dependent on imaging based methods (see e.g. Gebremedhin et al. 2019 Agronomy; Jones 2004; see info at the site of IPPN - https://www.plant-phenotyping.org/ipps2018).

Reply: We have elaborated on this, as suggested.

Comment/s: The authors should also describe in the introduction related costs of the technology used. Besides data robustness/analysis costs are another major issue when talking about modern phenotyping. Indeed, affordable phenotyping is a major item analysed by the industry and research community working in phenotyping.

Reply: We now discuss this issue in the introduction.

Comment/s: The paper also misses a definition of what is "High throughput phenotyping";

Reply: The topic is now introduced and discussed in the Introduction.

Comment/s: The method seems efficient and robust but the protocol must be improved. It is long and sometimes too detailed which makes difficult to follow some of the procedures. Some points need to be better described, by using less detail or by improving English (see points 3.1. and 3.2, and point 5 for example); Also the number of plates and figures seems excessive and not always clear. The paper misses a clear picture of the overall set up of the platform and some plates could be skipped (ex. potential overlap between fig. 12 and 16). In addition the quality of the figures should be improved, especially when using grey scale.

Reply: Thank you for that comment. The protocol has been modified to make it more concise and to the point.

The detailed parts of the protocol have been moved to the Discussion and/or the figure legends.

The descriptions of Points 3.1, 3.2 and 5 (and other points) have been improved, as suggested.

Figure 16 (currently Supplementary Figure 9C) was modified to explain continuous results and merged with Figure 12 into one (Supplementary Figure 9).

A clear picture of the overall set-up of the platform is presented in Figure 1J.

We improved the quality of the figures wherever required.

Comment/s: Moreover, the possibility of have real time analysis of data, is innovative but the software may have have limitations in showing that graphically in case differences between genotypes are small and/or if the number of genotypes is large and or climate conditions are highly variable in the greenhouse. In fact, I miss a more detailed description of the greenhouse where the platform is installed. This because greenhouse climate control conditions will largely influence evapotranspiration and plant's stomatal behavior, and consequently weight measures. For example, Figure 17, suggests a big variation if we consider the values of SE. Why so large variation? Climate control issues?

Reply: The system can be installed in almost any controlled or non-controlled greenhouse. In our case, we chose to work in a non-controlled greenhouse to enable close-to-field conditions, which are very important for pre-field screenings. The software takes into account the plants' local VPD in calculating the canopy stomatal conductance ((Please see the multiple VPD stations localization in Figure 1J). Moreover, as variation in environmental conditions can be expected in many growth facilities, we strongly recommend using a randomized-block design as a general solution for that issue.

Comment/s: Authors should make more clear what would be the best substrate to be used to optimize procedures/results. This is not clear and the impact of the substrate/substrate mix on the procedures and measurements is mentioned several times (e.g. in point 5.7 authors report the potential impact of substrate on the drainage speed);

Reply: We added a recommendation for first-time users in Section 4.6. We also added a simplified protocol for first-time users as a supplementary file (Supplementary MS). Please see also our reply to Editorial Comment 16.

Comment/s: Regarding figures, Why using "tomato plants" in figure 3, when the protocol is focused on rice? Maybe Fig. 3a could be skipped. Fig. 9 has limited quality. In figure 12, the legend should indicate when the drought treatment was imposed; There is some overlap between Figures 12 and 16; Regarding figure 15, does the figure only shows the volumetric water content after drying out or also after recover by restarting irrigation? In legend of fig. 17, indicate the number of samples used to estimate the SE; Figure 16 and 17 could be combined as Fig. 16a and 16b

Reply: The protocol is versatile and can be used to study any kind of plant. Figure 3A shows a sample cavity tray and a tomato root-soil plug, but other plant species could just as easily be used. We added some clarification to Figure 3 and its legend. For the representative results, we used three different varieties of rice.

The original Figure 9 (currently Supplementary Figure 6) is a screenshot of the SPAC Analytics window for the data analysis. It is meant to provide an overall picture of how the interface looks, rather than a detailed view. The image quality has been improved.

In the legend for what was originally Figure 12 (currently Supplementary Figure 9A), we now include when the drought treatment was imposed.

You are correct regarding the overlap between Figures 12 and 16. Figure 16 (currently Supplementary Figure 9C) was modifaied and noe it demonstrate a continues results. Now merged with Figure 12 into one (Supplementary Figure 9).

The original Figure 15 (currently Figure 5) shows the volumetric water content throughout the experiment period, including recovery. This point is now noted in its legend.

In the legend of the original Figure 17 (currently Figure 6), the number of samples used to calculate the SE is indicated (n = 4).

The original Figure 16 was merged with the original Figure 12 to form the current Supplementary Figure 9. We have kept the original Figure 17 (currently Figure 6) in its original form.

Comment/s: I miss in the discussion section, the discussion of the pros and cons of such method namely for selection/breeding programs and what would be the feasibility of using the platform for horticultural and woody crops? Can this system be applied to these crops? And what would change in that case in the protocol or measurements?

Reply: We appreciate this comment, which highlights the need to discuss the system's signal-to-noise level. The system is capable of supporting various pot sizes (up to 25 L) and irrigation treatments, which enables the examination of any type of crop plant. We added a comment regarding this scalability to the introduction to the protocol, as well as a new section (Section 3) about reducing the noise that may result from non-suitable plant–pot pairings (e.g., a smaller plant in a heavy pot).

Minor Concerns:
Line 101. Rewrite as "System components"

Reply: The section was moved to the figure legends and re-written.

Line 117. "coupled to…";

Reply: Changed, as suggested.

Line 119. …" to minimize water loss via evaporation from the container";

Reply: Changed, as suggested.

Line 160. Indicate the link to the website in the text;

Reply: Done.

Line 162. Why "physical". Write only position;

Reply: We have changed ”physical position” to “location,” which sounds more appropriate. Thank you.

Line 171 and 172. Remove "Choose your growing medium smartly"; Be more direct; Why you state "in principle"? State more clearly what will be the best substrate to be used to allow the best measurements in the gravimetric system;

Reply: We have replaced the word “smartly” with “to best suit your experimental needs” and deleted “in principle”. Regarding the use of the best substrate, please see our reply to Editorial Comment 16.

Line 190. Not so clear . Rewrite as follows "In case seedlings were not germinated in trays, transplant them into cavity trays containing substrate/soil; Plant one seedling per cavity…";

Reply: Modified, as suggested -- thank you.

Lines 177-182. No so clear. Define VWC; What do you mean by "vice-versa";

Reply: Modified, as suggested.

Line 182. Instead of "degraded" write "decomposed";

Reply: Changed, as suggested.

Line 199, correct "are leveled"?;

Reply: Changed, as suggested.

Line 207. "Which operating software" . Indicate the name of it;

Reply: Done.

Line 218. Rewrite sentence. Use the nomenclature consistently. Instead of "growing medium", "potting medium" or write along the paper "substrate";

Reply: Modified, as suggested. We now use the term “potting medium” throughout the manuscript.

Line 225. What is the operating software?;

Reply: Please see our reply to the comment on Line 207 above.

Line 253. How can you be sure that pots drain quickly? This depends on the substrate

or mix used;

Reply: The word “quickly” was replaced with “freely”.

Line 254 "airy substance?" Better to write, Perlite?;

Reply: Perlite is mentioned as an example. We also recommend referring to Tables 1 and 2 for more details.

Line 271. Instead of "few" indicate the number of minutes "3 or 4 or 5";.

Reply: Modified as suggested.

Line 299. Why 250 seconds?;

Reply: This is to enable drainage**.** This was changed to 4–5 min and is now explained in the text.

Line 336. Instead of weight measurements write " Weight values";

Reply: Changed, as suggested.

Line 360. Please explain;

Reply: Explained as suggested.

Line 473. Write "…using SPAC Analytics software";

Reply: Added, as suggested.

Line 475. Indicate the "company that created the software and the web page";

Reply: Done.

Line 559. What does it mean "optimal irrigation" - 100% ETC?;

Reply: This is explained in the text.

Line 561. The authors state that for the sake of simplicity not all data are presented. However, for the sake of a better idea of the capabilities/limitations of the platform all data should be presented. Limiting the comparison to only two varieties is limiting;

Reply: In this paper, we focus on the basic applicative approach of establishing the experiment and getting the basic data. We refer to Halperin et al. (2019), in which the theoretical basis for this approach is thoroughly explained.
This system can be used for any number of samples and comparisons. We think that given the limited space available, our approach provides a sufficiently detailed explanation of how to set up and run an experiment in this system.

Line 570. Please quantify "moderate";

Reply: The sentence was change to: “fluctuations in PAR and VPD were similar over the different days of the experiment and over the course of each day.”

Line 724. This point raised here should move to the introduction in order to clarify the problem and emphasize the need of a more efficient method of data collection and analysis;

Reply: We now present this point in the Introduction, as suggested. However, we feel that is was important to retain it in the Discussion as well.

Line 732. What do you mean by "near-to-field" conditions?;

Reply: This term refers to conditions that are similar to those found in the field. We now use the term “close-to-field conditions” throughout the text.

Line 734. Which are the most relevant problems related to pots experiments? Please indicate them here and eventually at the introduction

Reply: Corrected to “pot effect,” which is thoroughly explained in Point 3 of the Discussion section.

Line 747. Correct references according to journal's rules;

Reply: Done.

Table 2 . Indicate the minimum and maximum of samples (3-???); simplify the table by removing decimals for TAW parameter;

Reply: The sample number (3–5) is mentioned in the table legend. We prefer to keep the decimals for the TAW parameter, as this is an important parameter that requires accuracy.

The legends of figures should indicate the name of the "operating software";

Reply: That information was added, as suggested.

Figure 9. Better quality image is required, at least for grey scale presentation;

Reply: The original Figure 9 (currently Supplementary Figure 6) is a screenshot of the SPAC Analytics window for the data analysis and is meant to present a overall picture of how it looks rather than a detailed view. The image quality was improved.

Finally, there are several minor mistakes along the text (repetitions, less clear wording) which will require careful reviewing of the text;

Reply: The text was carefully reviewed and proofread, as suggested.

Reviewer #2:

Manuscript Summary:
The described protocol refers to the procedures, handling and software settings and measurements needed to conduct and analyse experiments using a high-throughput platform for real-time physiologocal plant screening based on lysimeter approach. Here, each pot is connected with its own lysimeter and do not need to be moved to any measuring station, which allows for real-time phenotyping of plant biomass gain, transpiration rates, water use. Such a system is highly needed to better resolve the responses of each plant or plant stand to e.g. drought and to make comparisons of genotypes based on the same points in drought stress.
The described protocol is very detailed and cleary written, so it is easy to follow the different steps and to avoid pitfalls. Everyone who reads this protocol is enabled to successfully conduct an experiment on this HTP platform and to anaylse the recorded parameters. A use case of a drought experiment with rice is presented after the protocol has been described.

Major Concerns:
none

Minor Concerns:

Comment/s: In the introduction they claim that root fluxes and stomatal conductance (line 89) can be measured but do not explain how throughout the protocol.

Reply: Thank you very much for your comments. In this paper, we focus on the basic applicative approach of establishing the experiment and getting the basic data. Indeed, there are a few trait calculation that we did not show (e.g., stomatal conductance and root flux). However, we refer to Halperin et al.'s (2019) work, which includes clear and detailed theoretical explanations. We think that given the limited space available, our approach provides a sufficiently detailed explanation of how to set up and run an experiment in this system.
.
Comment/s: For point 5.13: is there an automated check for such outliers by the software that produces a warning message and the recommendation that the measurement has to repeated?

Reply: The outliers mentioned in 5.13 (now 4.16) refer to materials that do not change in weight (static components) like irrigation drippers, probes, pot covers, etc. and which can be weighed manually in a smaller experiment. By checking outliers, we meant any unusually high or low values which could only be due to problems with the scales or controller. However, we recommend calibrating the scales at the beginning of the experiment to prevent any such errors.

Comment/s: Point 5.14: I do not fully understand this point: I thought that an advantage of the system is to have the exact extra weight of all components, including the soil. Why does the user now need to export the table with weight of static components and add an average pot weight to have the tare weight and upload this file? Why is that not done by the software that has recorded all the weights anyway? And do we talk of pot weight as weight of empty pot or weight of pot with soil? As you refer to 5.5. which is about filling the pots with soil? Please clarify.

Reply: There are several way of taking the measurement for static components. They can be taken directly from the system or they can be taken separately and added them into the system. Here, we show the longer and more detailed protocol, which could be practical if one is not using the average values, but rather individual values (i.e., non-homogenous pots that were weighed separately or some extra static weight).

We use the term "pot weight" to refer to the weight of an empty pot.

Thank you for the correction. It should read 5.4 (now 4.5) instead of 5.5.

Comment/s: Point 5.20 Define field capacity for the user. How can the user know that this point was really reached for each pot?

Reply: Field capacity or pot capacity is the amount (percentage) of water that the soil in the pot can hold when the soil is fully saturated. This can be known when the weight of the soil-filled pot becomes stable (does not increase any more in weight) after subsequent irrigation and drainage. The user can see this in the SPAC analytics software.

Comment/s: Point 6.7 It is probably misleading to write that the user needs to measure the weight of a few empty cavities. I assume they should measure the weight of a cavity that has no seedling but only soil, correct?

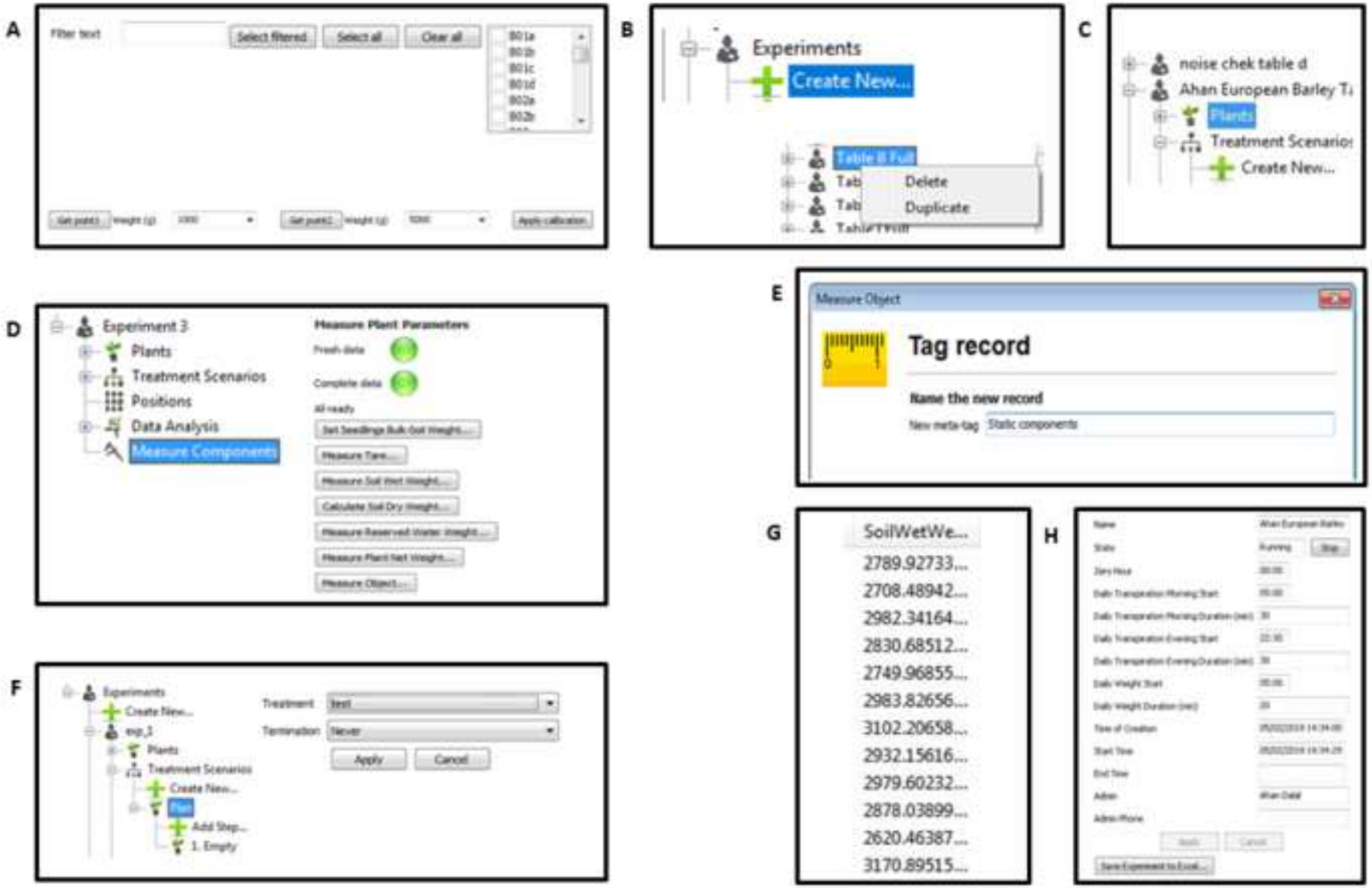
Reply: Yes, thank you for this comment. We have modified the text to clarify this point.

Comment/s: In the analysis section 9 I am missing the information on how the data of a finished experiment will be exported: what is the format (as depending on the duration this file may be very large), how many seperate files are generated (as you have also information from environmental sensors) and how the data are organized in the export file. Also what are the export formats for the graphs that can be generated with the software?

Reply: Thank you for your comments. In the Analysis part of the SPAC Analytics software, different subsections like Graph viewer, Histogram, T-test, ANOVA, and Piecewise linear curve have options for exporting the data as an Excel file in the top right corner. This is explained in Step 8.4.2. The files can be downloaded separately for each analyzed parameter or as raw data in a compressed format.

Figures

Click here to access/download;Supplemental File (Figures, Permissions, etc.);Supplementary Figure1.TIF



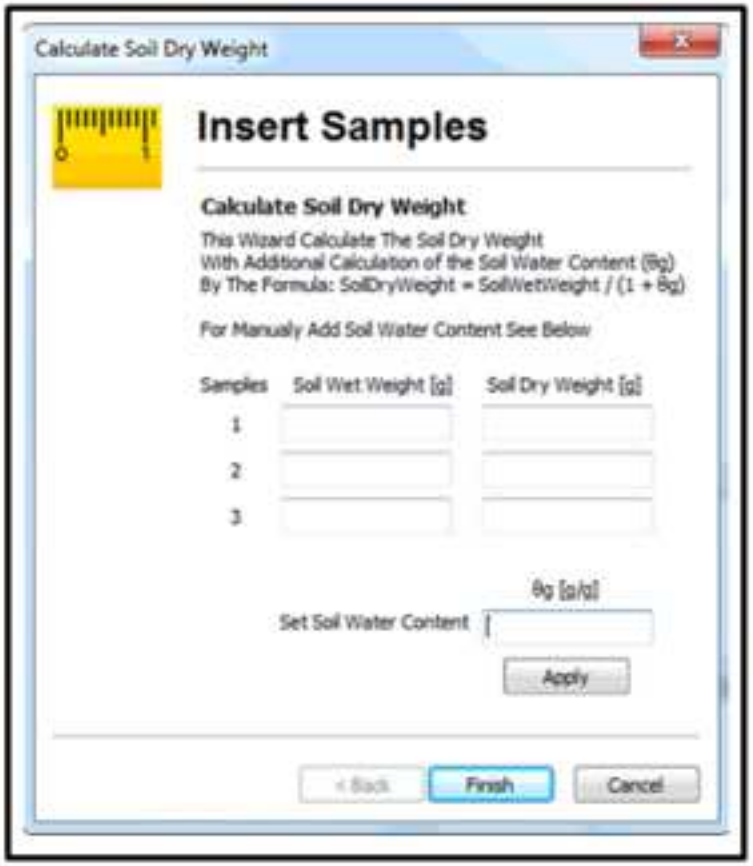
Supplementary Figure 1

Figures

Click here to access/download;Supplemental File (Figures, Permissions, etc.);Supplementary Figure2.TIF

Name	Treatment Plan	Step	Position	Reference Weather Station	Tare Weight	Seedling Bulk Soil Weight	Soil Dry Weight	Soil Wet Weight	Reserve Water Inventory	Soil Type	Soil Volume	Plant Net Weight	#Treatment	# line
Indica-1	65% of prev. day transpiration	2	H10a	H10d	361.6	35.0	402.7	3007.9	352.4	2	3200	20.7	Drought	Indica
Indica-2	65% of prev. day transpiration	2	H11c	H10d	364.2	35.0	338.2	2493.8	374.9	2	3200	14.3	Drought	Indica
Indica-3	Control 3 pulses	1	H11d	H10d	393.3	35.0	392.5	2900.7	308.6	2	3200	19.1	Control	Indica
Indica-4	Control 3 pulses	1	H12b	H13a	356.0	35.0	381.5	2848.7	354.1	2	3200	10.1	Control	Indica
Indica-5	65% of prev. day transpiration	2	H14d	H13a	360.1	35.0	359.7	2673.9	395.2	2	3200	21.5	Drought	Indica
Indica-6	Control 3 pulses	1	H15a	H16d	359.5	35.0	399.0	2982.9	359.1	2	3200	9.6	Control	Indica
Indica-7	65% of prev. day transpiration	2	H16a	H16d	398.3	35.0	393.6	2905.5	362.5	2	3200	28.6	Drought	Indica
Indica-8	Control 3 pulses	1	H17d	H16d	366.8	35.0	366.5	2736.5	391.7	2	3200	17.9	Control	Indica
Karla-1	65% of prev. day transpiration	2	H11a	H10d	365.5	35.0	378.7	2827.5	404.5	2	3200	21.2	Drought	Karla
Karla-2	Control 3 pulses	1	H12d	H13a	361.8	35.0	333.0	2478.1	369.8	2	3200	10.5	Control	Karla
Karla-3	Control 3 pulses	1	H13a	H13a	362.9	35.0	354.0	2641.9	408.9	2	3200	6.3	Control	Karla
Karla-4	65% of prev. day transpiration	2	H13c	H13a	360.4	35.0	379.5	2833.6	337.4	2	3200	14.4	Drought	Karla
Karla-5	65% of prev. day transpiration	2	H14b	H13a	368.1	35.0	332.1	2399.4	448.2	2	3200	11.1	Drought	Karla
Karla-6	Control 3 pulses	1	H15b	H16d	357.2	35.0	389.5	2913.2	398.7	2	3200	16.1	Control	Karla
Karla-7	65% of prev. day transpiration	2	H16b	H16d	360.0	35.0	344.6	2565.7	385.6	2	3200	5.9	Drought	Karla
Karla-8	Control 3 pulses	1	H16d	H16d	367.2	35.0	350.0	2601.0	349.3	2	3200	18.9	Control	Karla
Risoto-1	Control 3 pulses	1	H10d	H10d	362.9	35.0	381.6	2831.4	391.2	2	3200	3.0	Control	Risoto
Risoto-2	65% of prev. day transpiration	2	H11b	H10d	364.8	35.0	382.0	2812.8	382.9	2	3200	2.0	Drought	Risoto
Risoto-3	Control 3 pulses	1	H13b	H13a	358.3	35.0	349.2	2605.6	400.8	2	3200	34.4	Control	Risoto
Risoto-4	65% of prev. day transpiration	2	H13d	H13a	363.8	35.0	347.0	2589.3	364.5	2	3200	32.6	Drought	Risoto
Risoto-5	65% of prev. day transpiration	2	H15c	H16d	334.4	35.0	356.0	2657.6	385.7	2	3200	30.1	Drought	Risoto
Risoto-6	Control 3 pulses	1	H16c	H16d	361.3	35.0	382.8	2859.9	306.2	2	3200	12.4	Control	Risoto
Risoto-7	65% of prev. day transpiration	2	H17a	H16d	364.6	35.0	395.0	2944.9	346.3	2	3200	14.5	Drought	Risoto
Risoto-8	Control 3 pulses	1	H18a	H16d	360.7	35.0	366.4	2742.7	317.7	2	3200	14.0	Control	Risoto

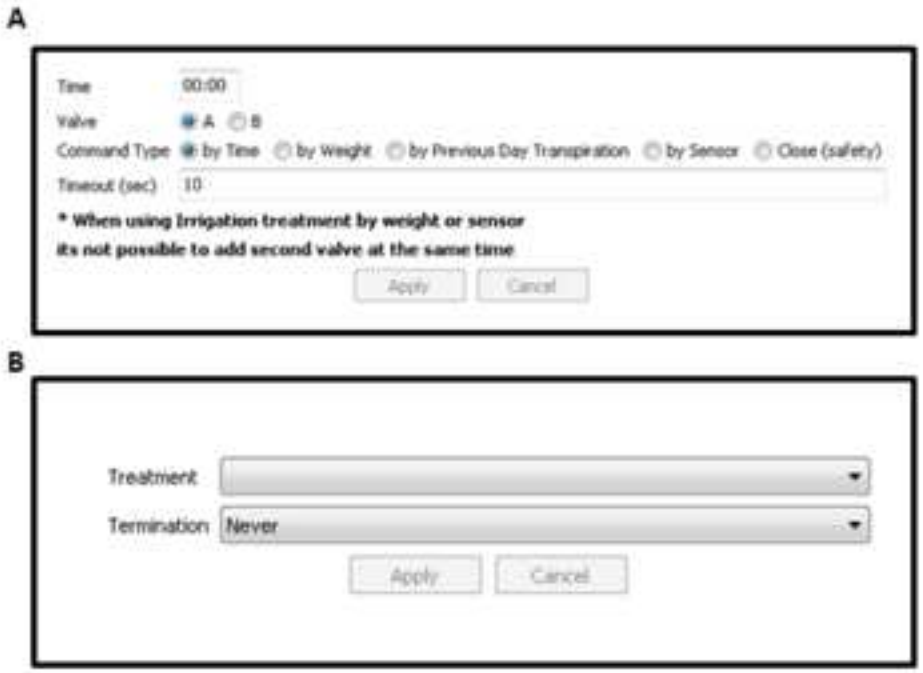
Supplementary Figure 2



Supplementary Figure 3

Figures

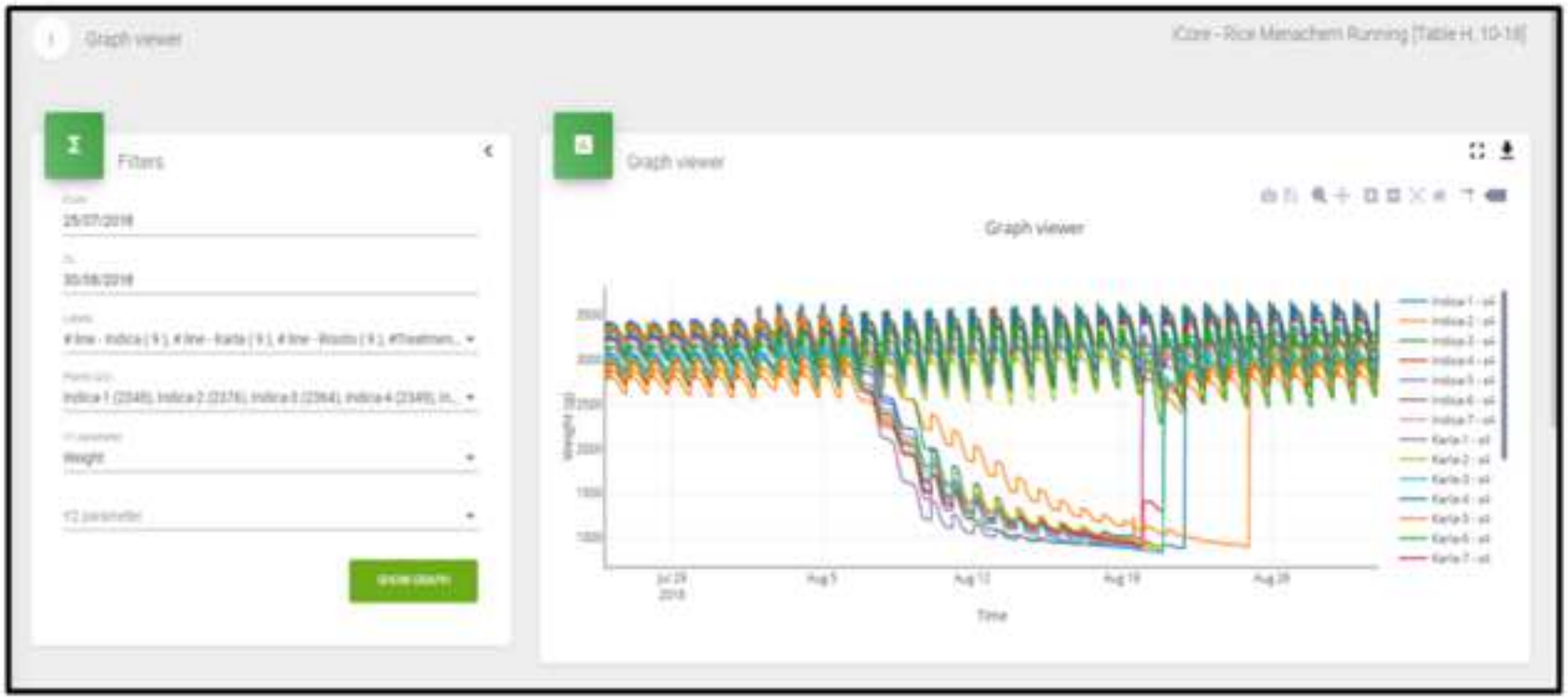
Click here to access/download;Supplemental File (Figures, Permissions, etc.);Supplementary Figure4.TIF



Supplementary Figure 4

Figures

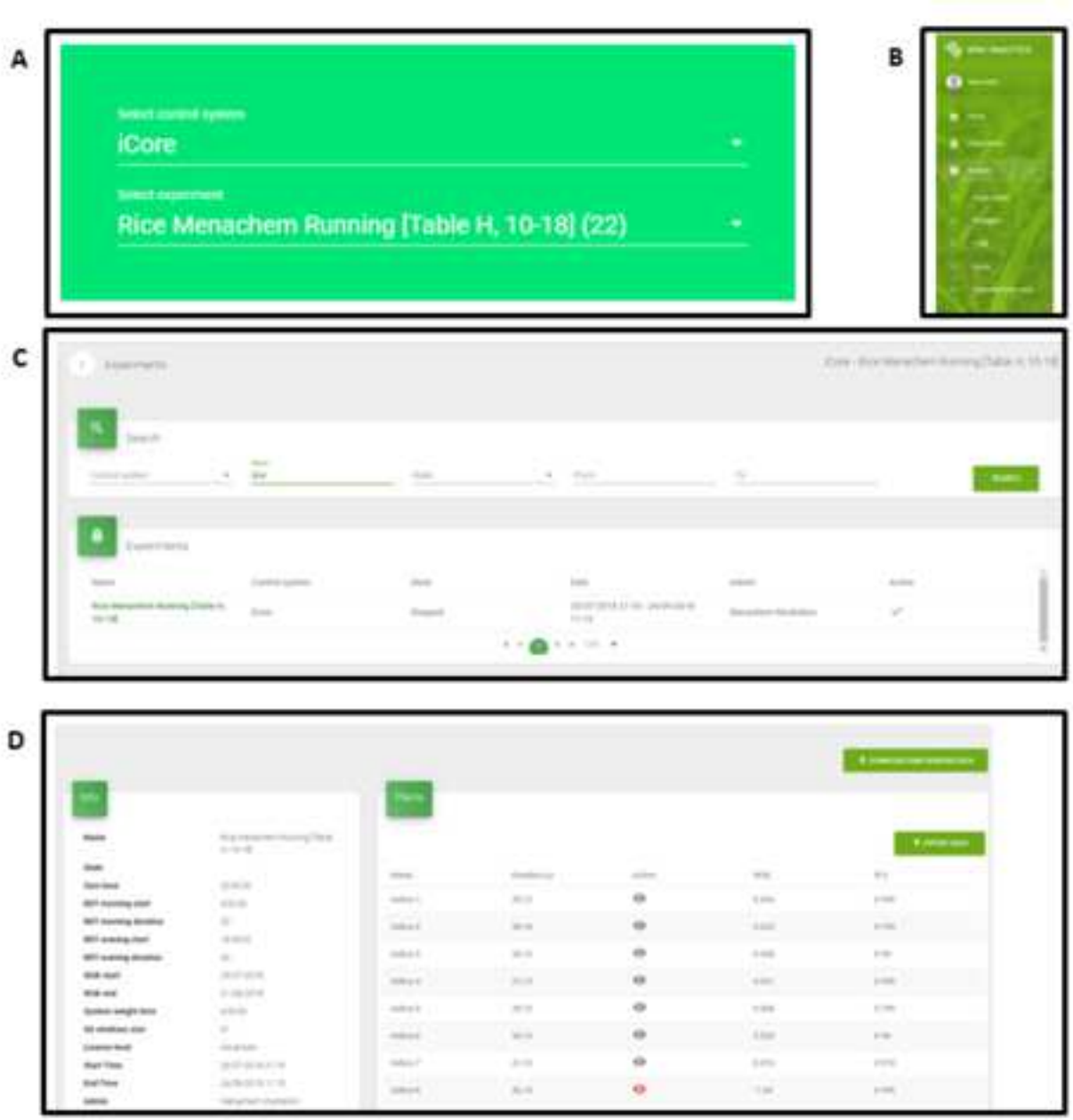
Click here to access/download;Supplemental File (Figures, Permissions, etc.);Supplementary Figure5.TIF



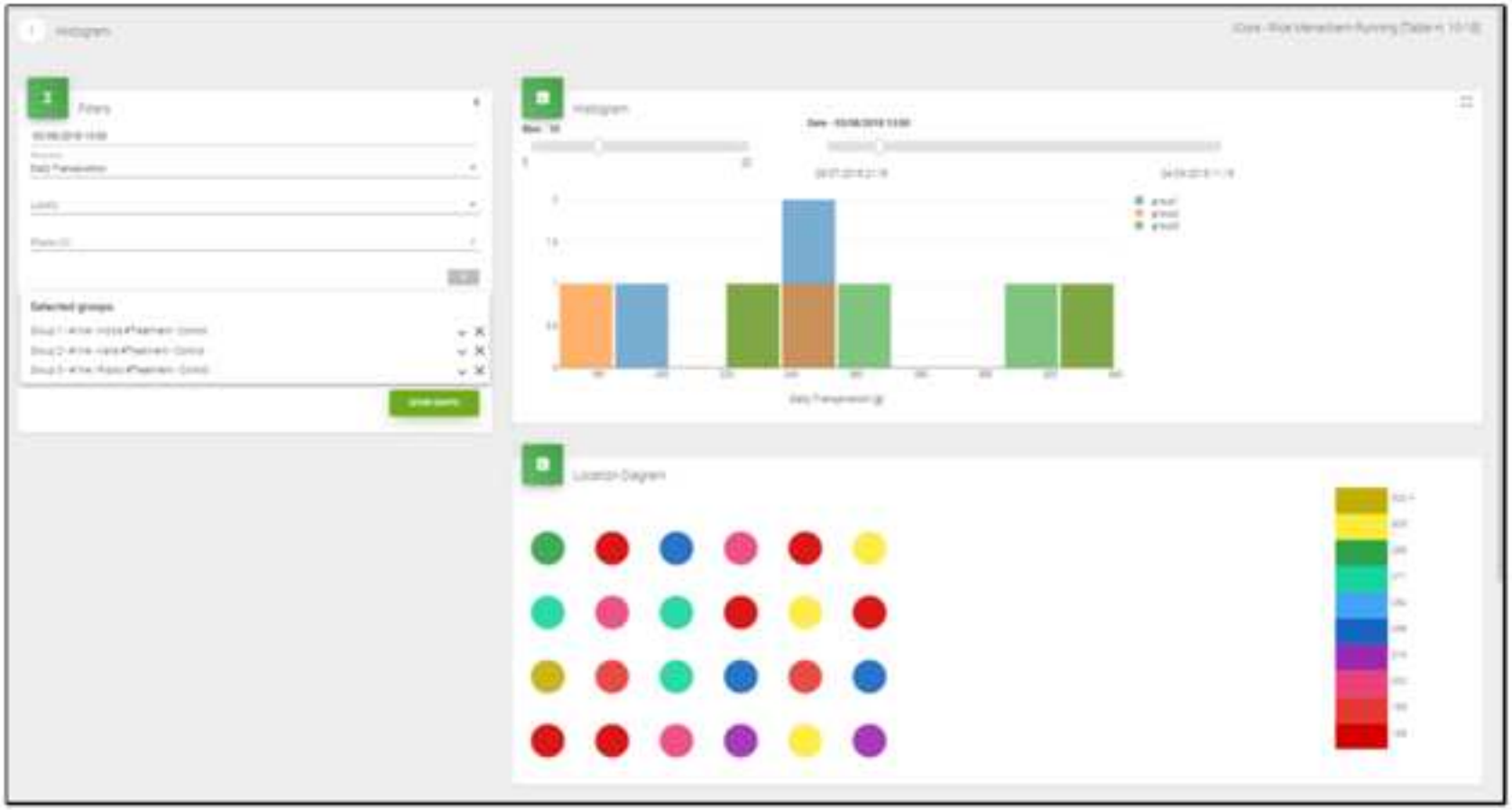
Supplementary Figure 5

Figures

Click here to access/download;Supplemental File (Figures, Permissions, etc.);Supplementary Figure6.TIF



Supplementary Figure 6



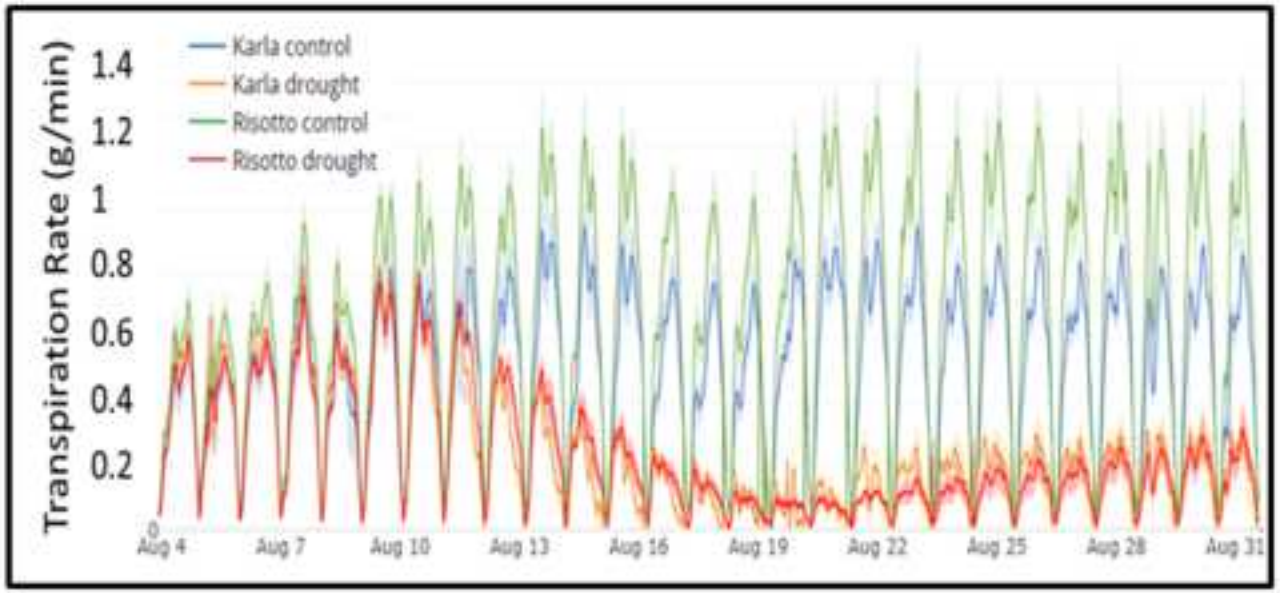
Supplementary Figure 7

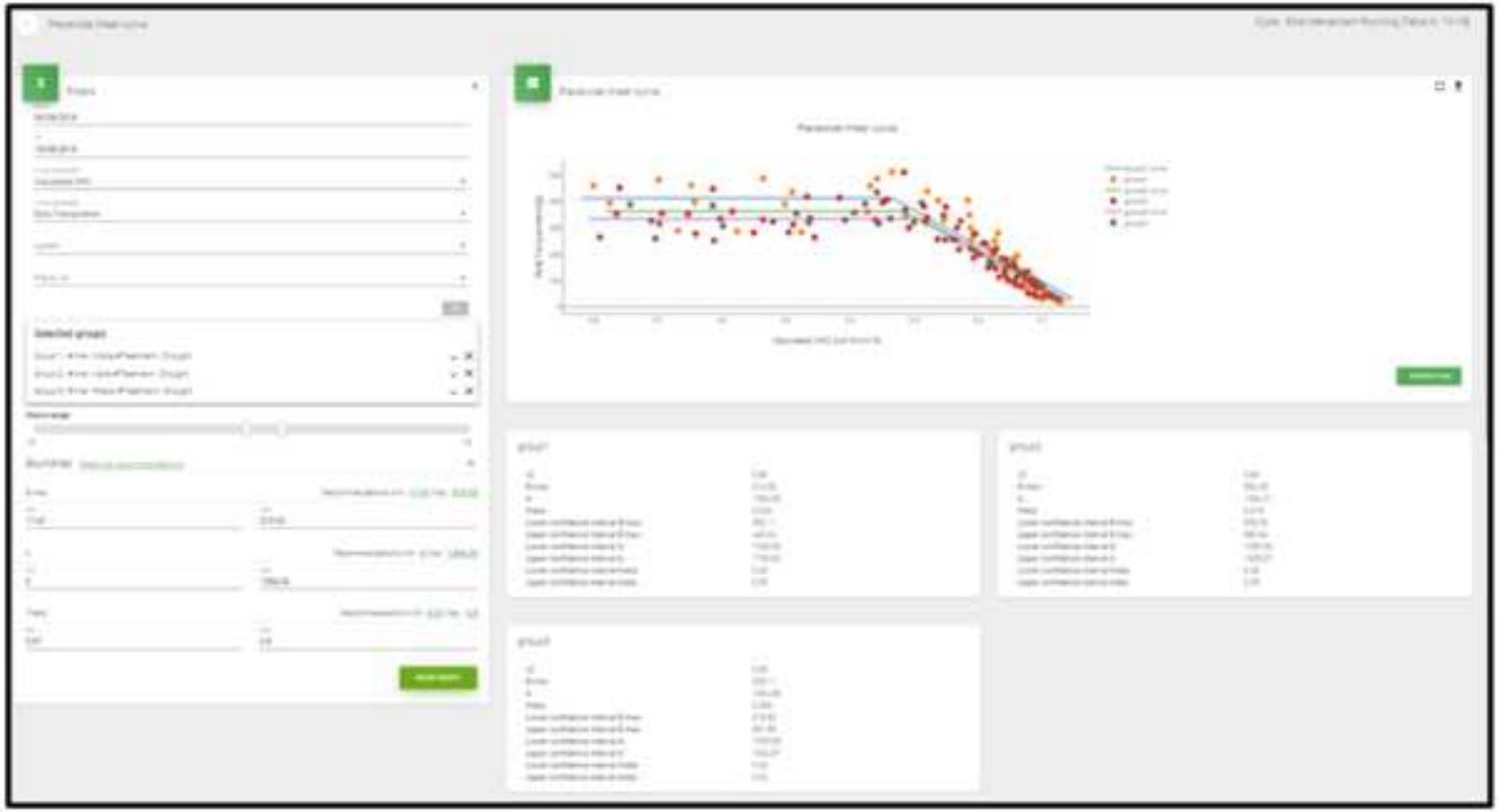
Figures

Click here to access/download;Supplemental File (Figures, Permissions, etc.);Supplementary Figure8.TIF

Supplementary Figure 8

Supplementary Figure 9





Supplementary Figure 10

Simplified Protocol

First-time users may want to start with this simplified protocol.

The main difference between this protocol and the full protocol is that, in this protocol, you will load planted pots onto the system. This will simplify many of the initial preparations and measurements. Using this simplified protocol, you will not be able to calculate the initial seedling weight and soil wet weight for each pot separately. However, you will still be able to assess the following traits: plant growth per day [g/day], daily biomass gain [g/day], daily transpiration [g/day], WUE, transpiration rate [g/min] and root-influx rate [g/min; requires the use of soil sensors].

Before starting this protocol, please read through the full protocol.

1. Prepare dedicated pots (see Section 1 of the full protocol). For each genotype, prepare at least six extra pots (three to be used for the estimation of plant initial weight based on manual measurements, see Section 12 below, and three to enable better selection of similarly sized plants for use in the experiment; Section 4 below).
2. Fill the pots with **homogenized** soil (see Section 2.1 of the full protocol).
3. Plant the seedlings in the pots (use seedlings that are all of a similar size).
4. Grow the plants until they are ready to be loaded onto the system (it is important that the plants be as uniform in size as possible). Try to use seedlings that are about 15 to 20 cm tall (it is important to follow also Section 3.2 of the full protocol).
5. Manually measure the average weights (5–10 repetitions) of the following system components: empty green bath, dedicated empty pot, cover plate, probes, dripper and sticks.
6. Calibrate the lysimeters (see Section 3.1 of the full protocol).
7. Open a new experiment in the control program and run it (see Sections 4.1–4.4 of the full protocol).
8. Put the pots in the green baths on the scales.
9. Add probes, cover-plates, sensors and drippers.
10. Check that each pot is in a stable position and is not touching any of the other pots.
11. Download the Excel file from the system and **manually** add the weight (sum of all the static components; empty pot, cover plate, probes and drippers, taken in Section 5 above) to the “Tare weight” column (see also Section 4.17 of the full protocol).
12. Take the extra pots (make sure the plants are similar in size to the plants on the system), wash the seedlings carefully, gently absorb the remaining drops of water with a tissue paper, and weigh the seedlings manually. **Manually** add their weights to the Excel table in the “initial plant weight” column.
13. Design an irrigation plan for the experiment (see also Sections 4.19–4.24 and 7.4 of the full protocol).
14. Design your treatment (see Section 7.5 of the full protocol).