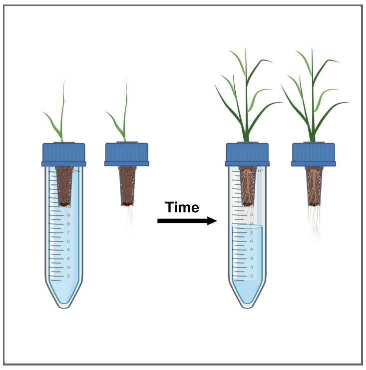

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Closed-chamber hydroponics for whole-plant phenotyping

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Whole-plant phenotyping

Daniel Ginzburg

ABSTRACT

Noninvasive phenotyping can quantify dynamic plant growth processes at higher temporal resolution than destructive phenotyping and can reveal phenomena that would be missed by end-point analysis alone. Additionally, whole-plant phenotyping can identify growth conditions that are optimal for both above- and below-ground tissues. However, noninvasive, whole-plant phenotyping approaches available today are generally expensive, complex, and non-modular. We developed a low-cost and versatile approach to non-invasively measure whole-plant physiology over time by growing plants in isolated hydroponic chambers. We demonstrate the versatility of our approach by measuring whole-plant biomass accumulation, water consumption, and water use efficiency every two days on unstressed and osmotically-stressed sorghum cultivars. Our system can be implemented using cheap, basic components and requires no specific technical expertise. It can, in theory, be used for any non-aquatic vascular plant species.

GUIDELINES

This protocol has been designed for *Sorghum bicolor* (sorghum) seedlings at the third leaf stage. All aspects of the protocol (tube size, nutrient concentration, age of switching from open- to closed-hydroponics, frequency of measuring biomass accumulation and water consumption, etc.) can be modified to suit different species, developmental stages, or biological questions.

Protocol status: Working
We use this protocol and it's working

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PROTOCOL integer ID: 91207

Keywords: noninvasive whole-plant phenotyping, modular phenotyping, water use efficiency, osmotic stress, hydroponics

MATERIALS

For "Construction and operation of ebb & flow system for "open" hydroponic cultivation" and "Closed-system hydroponic growth apparatus construction":

- Electric drill and drill bits
- 50mL plastic tubes with screw-on caps
- 18 gallon plastic reservoir (2x)
- Ebb and flow kit (Botanicare, Vancouver, WA, USA): threaded and barbed bulkhead fitting, threaded height extender, threaded, plastic debris screen
- Submersible pump (8W, 800 liter/hour is recommended for 18 gallon reservoirs)
- PVC plastic tubing
- Programmable timer
- Scissors
- Rapid-Rooter starter plugs (General Hydroponics, Santa Rosa, CA, USA)
- Potting soil
- Waterproof electrical conductivity meter
- All-purpose soluble fertilizer
- Tap water

For "Transitioning samples from open- to closed-hydroponic conditions" and "Quantifying biomass accumulation, water use, and change in media pH":

- All-purpose soluble fertilizer
- Tap water
- pH meter
- Scale

SAFETY WARNINGS

The motor of a submersible pump can overheat if the pump is operating in the absence of (sufficient) water. Carefully watch the water level in the bottom reservoir while the system is irrigating to ensure the pump is always sitting in water. If there is insufficient water, the pump with audibly sound like it intermittently trying to pump water. If this happens, unplug the pump and add more water to the bottom reservoir.

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Construction and operation of ebb & flow system for "open" h...

1 Drill a ¾ inch diameter hole into opposite corners of an **18 gallon** plastic tote (reservoir). 5m

2 Place the reservoir with holes drilled onto a second, **18 gallon** reservoir without holes. 10s

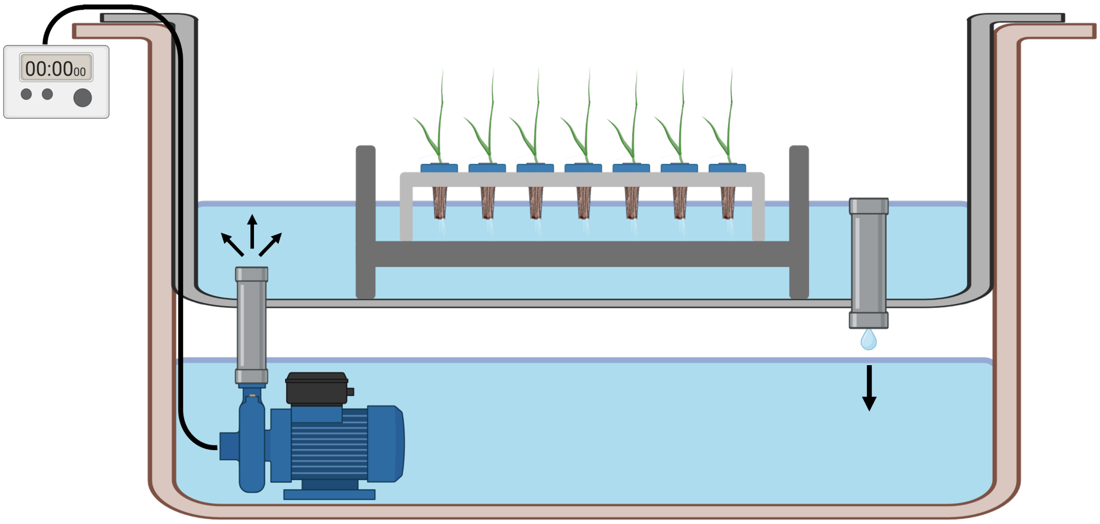
3 Insert a **¾ inch** threaded and barbed bulkhead fitting (Botanicare, Vancouver, WA, USA) from above int 2m

one of the drilled holes and fasten from below with a rubber washer.

4 Water in an ebb and flow system needs to reach, but not submerge, the seedlings in the upper reservo 2m
Thus, to increase the irrigation height the water reaches in the upper reservoir before draining back down, screw a threaded height extender (Botanicare, Vancouver, WA, USA) into this first hole from above.

5 Fit a threaded, plastic debris screen (Botanicare, Vancouver, WA, USA) into the second hole which was 1m
drilled into the upper reservoir to allow water to gently pump up into the top reservoir from below.

6 Place an **8W, 800 liter/hour** submersible pump in the bottom reservoir and connect the pump to the 5m
barbed end of the bulkhead fitting with PVC plastic tubing.



Schematic of an ebb & flow system with two reservoirs, timer-controlled submersible pump recirculating water throughout, and seedlings situated in the top reservoir such that the water level reaches the soil-filled tips but does not submerge the seedlings.

7 In order for water to constantly recirculate during irrigation, sufficient water must be added to the ebb 5m
flow system to allow for filling the top reservoir up to the drainage height and to ensure enough water is in the bottom reservoir to be pumped back up.

Safety information

The motor of a submersible pump can overheat if the pump is operating in the absence of (sufficient) water. Carefully watch the water level in the bottom reservoir while the system is irrigating to ensure the pump is always sitting in water. If there is insufficient water, the pump will audibly sound like it is intermittently trying to pump water. If this happens, unplug the pump and add more water to the bottom reservoir.

8 To control the duration and number of irrigation events each day, connect the pump to a digital programmable timer. 15s

Closed-system hydroponic growth apparatus construction

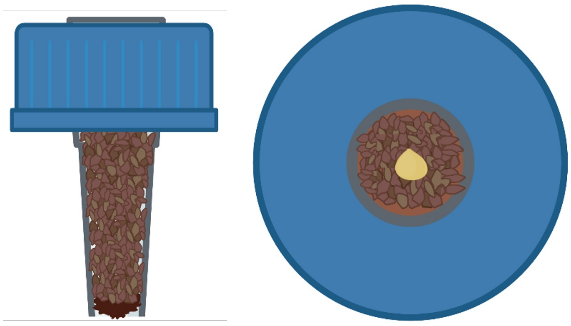
9 Using a **21/64 inch** drill bit, drill holes into the centers of 50mL Falcon tube caps. 30m

10 Using scissors, cut **1250 uL** pipette tips ~3 cm from the tip (narrower) end and insert cut tips into the pre-drilled Falcon tube caps so that the tips fit snugly into the holes in the caps and rest almost flush with the top of the cap. 30m

11 To prevent unnatural water retention and pressure buildup in the filled tips, create pinholes with a nee or small pair of scissors into the tips ~25% from the bottom. 15m

12 Using scissors, cut Rapid-Rooter starter plugs (General Hydroponics, Santa Rosa, CA, USA) into rectangular slices of ~0.5cm length and ~0.9cm width and insert 1 slice into the top of each tip and then gently push slices to the bottom of the pipette tip. 20m

13 Fill caps with tips with potting soil. Ensure the soil is not overly compact as this will inhibit seedling growth. Additionally, ensure that there are no clumps in the soil which would prevent water from reaching the soil from below due to capillarity. 30m



Schematic of screw-on caps with cut pipette tips filled with soil and rapid rooter starter plugs at the bottom of the pipette tips. The yellow circle in the overhead view represents an individual seed.

14 Place soil-filled caps with tips into tube racks, and leave to soak overnight in tap water which reaches 12h bottom of the tips but does not submerse the caps.

15 The next day, plant individual sorghum seeds into soil-filled tips and then put all samples into the ebb 30m flow system.

Pre-phenotyping hydroponic growth conditions

16 Ensure that the drainage height of the ebb & flow system reaches the bottom ~2-3cm of the cut pipet 5m tips during irrigation.

17 Before seedling emergence, program the timer to irrigate samples **three times daily** with tap water 8h 5m 00:05:00 every 08:00:00 .

18 Upon root emergence (root tips growing below the bottom of the pipette tip) of 50% of the samples, 15m adjust the programmable time to irrigate seedlings every hour with a single, 00:15:00 irrigation event. Once roots emerge from below the soil-filled pipette tips, the primary source of water for the roots will no longer be water held within the soil but rather the recirculating water of the ebb & flow system. It

is therefore important to ensure exposed roots are irrigated frequently to prevent desiccation, but not constantly to encourage downward root growth and to increase root oxygenation.

19 At this point, add soluble, all-purpose fertilizer to the lower water reservoir using a plastic scoop up to an electrical conductivity (EC) of **1200 mS/cm** while the water is being circulated. Measure EC with a waterproof electrical conductivity meter. 10m

20 Upon root emergence of all samples, increase the duration of irrigation events to be 00:45:00 every hour followed by 00:15:00 of no irrigation to increase root zone oxygenation. 1h

21 Empty the reservoir every **3-4 days** and refill it with fresh nutrient solution. 3d

Safety information

An ebb & flow system is open to the air and thus will lose water over time due to evaporation. This will occur to a greater extent if growing plants in a hot or dry environment. Because the motor of a submersible pump can overheat if the pump is operating in the absence of (sufficient) water, check the water levels in the system more regularly to ensure there is always sufficient water.

Transitioning samples from open- to closed-hydroponic condi...

22 Once 50% of samples reach a pre-determined stage of development (i.e. the third true-leaf stage), prepare a bulk nutrient solution (EC of **1200 mS/cm**) such that 40 mL of solution can be allocated for each sample in 50mL Falcon tubes. 10m

23 If evaluating the effects of osmotic stress on plant growth, split the bulk nutrient solution into two and add an osmolyte (i.e. mannitol) into one of the solutions up to a pre-determined concentration (i.e. **10mM**). 1m

24 Record the pH of both control and osmolyte-supplemented solutions after fertilizer and osmolytes have been added. 2m

25 Fill 50 mL tubes with 40 mL of either control or osmolyte-supplemented nutrient solution and th 20m
wrap tubes in aluminum foil to prevent algal growth.

26 Without caps on, weigh foil-wrapped tubes with solution. 20m

27 Firmly screw caps containing seedlings onto individual tubes. 15m

28 Weigh tubes with seedlings screwed on to determine initial seedling weight. If the roots of any samp 20m
not reach the nutrient solution when fully closed onto the cap, add additional solution to the tube and reweigh with and without caps. If roots do not reach the water level in the tube, plants will desiccate.

29 Evenly space out samples into 50mL tube racks and place them in their designated growing environr 10m
(i.e. a greenhouse).

Quantifying biomass accumulation, water use, and change in...

30 Every **two days**, prepare fresh nutrient solution and measure pH as described above for both control and osmolyte-supplemented solution.

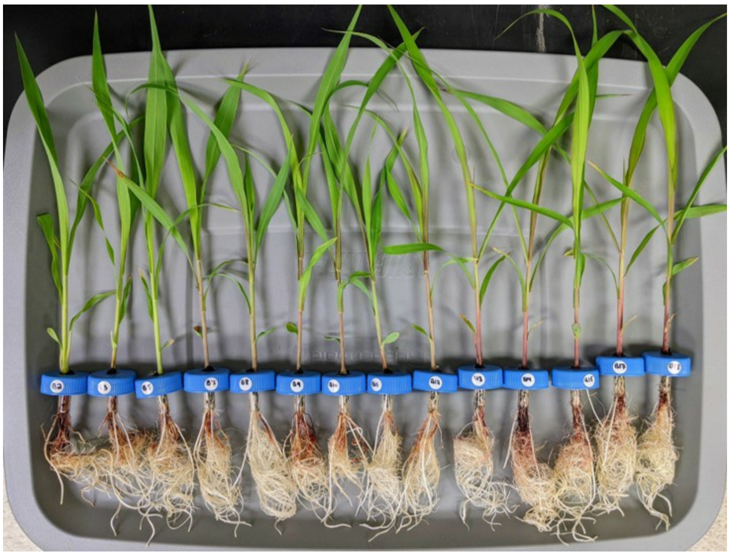
31 Unscrew seedlings and caps from their tubes and place seedlings back into the ebb & flow system 15m
(operating with constant irrigation) to keep the roots hydrated while tubes are weighed. Be very gentle when removing plants from tubes and putting them into racks in the ebb & flow system as roots will be exposed and thus vulnerable to mechanical damage.

32 Screw 50mL tube caps **without holes** onto each tube to prevent evaporation. One sample at a time, 30m
remove the cap and weigh the tube. Before screwing the cap back onto the tube, measure pH of the solution in the tube. Then screw the cap back onto the tube and repeat for all tubes.

33 Empty nutrient solution from each tube and then refill each with either fresh control or osmolyte-supplemented solution up to 40 mL as described above. 20m

34 Without caps, weigh tubes again and then screw caps back on to prevent evaporation while seedlings weighed. 20m

35 Carefully remove seedlings from the ebb & flow system. Weigh each seedling and then screw it back onto its corresponding tube before returning samples to their growing environment. 30m



Sorghum seedlings grown using this method.

36 Biomass accumulation is calculated as the seedling weight at the current time of measurement minus the seedling weight at the previous time of measurement. 1m

37 Water consumption is calculated as the tube weight at the previous time of measurement minus the tube weight at the current time of measurement. 1m

38 Change in media pH is calculated as the pH of fresh media minus the pH of media in tubes after plant 1m

growth.

Determination of evaporative loss in closed-hydroponic syste...

1w 3d 0h 55m

39 Grow plants hydroponically in soil-filled pipette tips as described above. 1w 3d

40 When plants reach the stage at which they are transferred from the ebb & flow system to closed tube 10m cut off the roots and shoots from the bottom of the tips and the base of the cap, respectively to prevent plant water uptake and transpiration.

41 Fill an equal number of tubes as there are cut-off samples with **1200 mS/cm** nutrient solution and we 15m the tubes as described above.

42 Screw caps from which roots and shoots were cut off onto nutrient-filled tubes. 5m

43 Place these samples in the same growing environment as all other samples. 5m

44 Each day (for the duration of the growth experiment), remove caps from tubes weigh tubes to determ 20m average daily and cumulative water loss due to evaporation.