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# A Simplified Hydroponic Culture of *Arabidopsis*

Houqing Zeng[1, 2], Chao Xia[3], Cankui Zhang[4] and Li-Qing Chen[1, *]

[1]Department of Plant Biology, School of Integrative Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, USA; [2]College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou 310036, China; [3]Maize Research Institute, Sichuan Agricultural University, Chengdu 611130, Sichuan, China; [4]Department of Agronomy and Purdue Center for Plant Biology, Purdue University, West Lafayette, IN 49707, USA

*For correspondence: lqchen77@illinois.edu

**[Abstract]** Hydroponic culture systems are widely used in research due to their intrinsic properties, such as easily altering nutrient composition, applying chemical treatments like metal, salt and hormones in the growth media, and collecting the root sample. Here, we describe a relatively easy and economic hydroponic culture system of the model plant *Arabidopsis thaliana*. It is worthy to note that this simple system can be adjusted and is suitable for other plant organisms.

**Keywords:** Hydroponic culture, *Arabidopsis*, Hoagland nutrient solution, Mineral nutrient, Root growth, Nutritional and physiological analysis, Aeration

**[Background]** *Arabidopsis* has been adopted as a model organism for a long time due to the significant features including its short life cycle, its well-annotated genome, easy transformation, and the availability of different types of mutants. Hydroponic culture has been considered as a very useful system in studying plant responses to nutrient or hormone changes since manipulation of the concentration or composition of mineral nutrients is feasible and phenotype observation of the morphology and architecture or sample collection are also convenient for diverse analyses (Hoagland and Arnon, 1941; Tocquin *et al.*, 2003; Conn *et al.*, 2013). However, the small size, the rosette growth habit, and the sensitivity of shoot apical meristem to flooding make the hydroponic growth of *Arabidopsis* difficult. Several hydroponic culture systems have been developed for the growth of *Arabidopsis* (Gibeaut *et al.*, 1997; Arteca and Arteca, 2000; Schlesier *et al.*, 2003; Tocquin *et al.*, 2003; Norén *et al.*, 2004; Smeets *et al.*, 2008; Conn *et al.*, 2013). Some of the systems have often been designed for a specific purpose and could not be suitable for various experimental purposes (Arteca and Arteca, 2000; Schlesier *et al.*, 2003; Norén *et al.*, 2004); some of the systems have to use rockwool or sponge, which could prevent the collection of root tissues near the root-shoot junction and risk the shoot apical meristem in a flooding stress (Gibeaut *et al.*, 1997; Smeets *et al.*, 2008); some of the systems require specialized materials like seed holder, raft float and container (Arteca and Arteca, 2000; Tocquin *et al.*, 2003; Conn *et al.*, 2013). In addition, algae growing is a common problem when rockwool, sponge or agar-based plugs are used.

In 2013, Conn *et al.* developed an *Arabidopsis* hydroponic system by germinating seeds on the pierced cap of 1.5 ml microcentrifuge tube in the germination solution, and then transferring the seedlings to the modified 50 ml Falcon tubes which are placed in an aerated large container filled with

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the basal growth solution. This system has some advantages of preventing algae growth and ensuring the harvest of the whole root system. However, it is relatively complex to prepare some materials, such as the pierced lids of 50 ml Falcon tubes and two different nutrient solutions (the germination solution and the basal growth solution). Here, we simplified Conn's method by using the materials and equipment that can be easily found in the laboratory or cheaply bought online, like the 0.5 ml microcentrifuge tube, recycled 1 ml pipette tip box, plastic bucket, and the air pump. Particularly, we only use ½ Hoagland nutrient solution during the whole life cycle of the plant to simplify the making of nutrient solutions. The construction of the hydroponic culture system we described here should be much simpler and cheaper compared with the previous systems (Tocquin *et al.*, 2003; Conn *et al.*, 2013). Our system also has the advantages of preventing algae growth, ensuring the harvest of the whole root system and maintaining high uniformity of the plants. In our hydroponic system, the plant can healthily complete the whole life cycle and can be treated and sampled at different growth stages according to research goals. The root system can be harvested totally without any damage. The hydroponic system can be used for various treatments like nutrient deficiencies, hormonal and chemical treatments, for shoot and root growth observation and measurement, and for shoot and root sample collection that can be used for various analyses at physiological, biochemical, metabolic, cellular and molecular levels. The size of a container used for plant growth can be adjusted based on the experimental goal and budget. For example, big bucket can be switched to 1 ml pipette tip box in the hydroponic culture system to save the cost of nutrient solution and chemical treatments if a large amount of plant materials is not necessarily required.

## Materials and Reagents

1. 0.5 ml flat-top microcentrifuge tube
2. Single edge blade (Personna, 0.23 mm)
3. 1 ml pipette tip boxes with racks and covers
4. Scotch tape
5. Air tubing (3/16" in diameter) (Elemental Solutions, catalog number: ECAT2010) (Figure 1B)
6. *Arabidopsis thaliana* seeds
7. 70% ethanol
8. Deionized water
9. Sterilized $dH_2O$
10. Agar (Sigma-Aldrich, catalog number: A1296)
11. Potassium nitrate ($KNO_3$) (Fisher Bioreagents, catalog number: BP368-500)
12. Calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$) (Fisher Chemical, catalog number: C109-500)
13. Ammonium nitrate ($NH_4NO_3$) (Fisher Chemical, catalog number: A676-500)
14. Magnesium sulfate heptahydrate ($MgSO_4{\cdot}7H_2O$) (Fisher Chemical, catalog number: M80-500)
15. Potassium dihydrogen phosphate ($KH_2PO_4$) (Alfa Aesar, catalog number: AAA1214236)
16. NaFe(III) EDTA [conjugated using $FeSO_4{\cdot}7H_2O$ (Honeywell, catalog number: F7002-500G) and EDTA $Na_2{\cdot}2H_2O$ (Fisher chemical, catalog number: O2793-500)]

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17. Boric acid ($H_3BO_3$) (Fisher Chemical, catalog number: A73500)
18. Manganese(II) chloride tetrahydrate ($MnCl_2·4H_2O$) (Sigma, catalog number: M3634-500G)
19. Zinc sulfate heptahydrate ($ZnSO_4·7H_2O$) (Awresco, catalog number: 97061-324)
20. Copper(II) sulfate pentahydrate ($CuSO_4·5H_2O$) (VWR International, catalog number: BDH9312-500G)
21. Sodium molybdate dihydrate ($Na_2MoO_4·2H_2O$) (Alfa Aesar, catalog number: A19222)
22. ½ Hoagland's nutrient solution (pH 5.6) (see Recipes)

**Equipment**

1. Scissors
2. Single-channel pipettes (200 µl, 1 ml)
3. Plastic buckets (one gallon, black) and bucket lids with seven holes drilled (six holes for holding plants, one hole for air tubing)
   Each hole is about 7.5 mm in diameter, and can be drilled using a driller with a bit of 0.25" (https://www.amazon.com/Gallon-Black-Plastic-Pail-Bottom/dp/B06XPQ61X3) (Figure 1A)
4. Air pumps (Elemental, 380 gph) (Elemental Solutions, catalog number: EOP246) (Figure 1B)
5. Air stone (2" in diameter and compatible with 3/16" air tubing) (Elemental Solutions, catalog number: EOCS202) (Figure 1B)

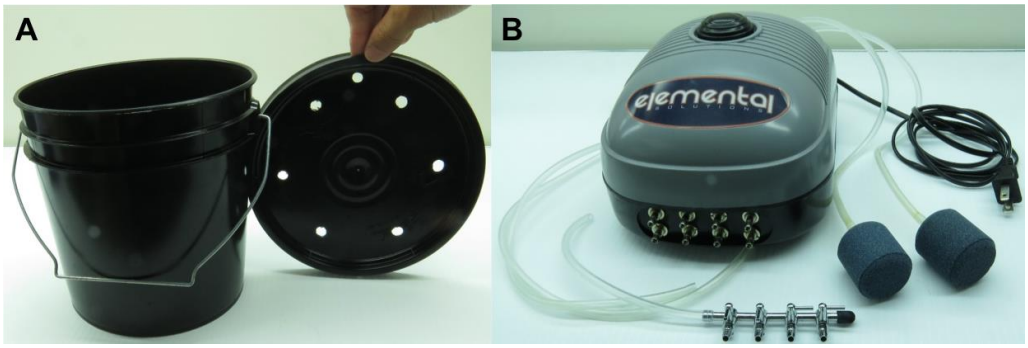


**Figure 1. Parts of the equipment used for hydroponic culture of *Arabidopsis*.** A. Black bucket (one gallon) and lid with seven holes drilled. B. Air pump, air tubing, air stone, and four-way outlet valve.

6. Four-way outlet valve (https://www.amazon.com/dp/B01N2K46XR/ref=sspa_dk_detail_1?psc=1&pd_rd_i=B01N2K46XR&pd_rd_wg=Lf7RJ&pd_rd_r=QDPMR7T6RBNYDGKFAB0G&pd_rd_w=roaMV) (Figure 1B)
7. 24 h plug-in mechanical timer (https://www.amazon.com/Century-Plug-Mechanical-Timer-Grounded/dp/B00MVFF59S/ref=sr_1_9?ie=UTF8&qid=1541631923&sr=8-9&keywords=24-hour+timer+heavy+duty)

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8. Polyethylene tank (25 gallons, 19" Diameter x 26" High) (United States Plastic, Tamco® Industries, catalog number: 3491)
9. Polyethylene tank cover (25 gallons) (United States Plastic, Tamco® Industries, catalog number: 3021)

**Procedure**

1. Sterilize *Arabidopsis* seeds with 70% ethanol for 5 min in a sterilized 1.5 ml microcentrifuge tube, then wash the seeds with sterilized $dH_2O$ for 3 times, and then cover the seeds soaked in water of the tube with aluminum foil and keep at 4 °C for 3 days for stratification.
2. Use scissors or a suitable needle to drill a hole (about 2 mm in diameter) in the center of the cap of the 0.5 ml microcentrifuge tube (Figure 2A). Use a blade to cut off the bottom of the 0.5 ml tube for about 1 cm long (Figure 2B).
   *Note: When drilling holes or cutting the tube, please be careful to avoid injury. Prepare at least 20% more seeds and tubes than what you need for the possible failure of seed germination and seedling growth. After collecting the plant samples, the bottom-removed tubes can be re-used after washing and sterilization.*
3. Invert the prepared tubes with the cap closed on a piece of adhesive tape or clingfilm to seal the hole of the tube cap (Figure 2C). Fill each tube with 150 µl 0.7% agar (made with ½ Hoagland nutrient solution by melting in the microwave oven) (Figure 2D). After the solidification of agar, fully fill each tube with ½ Hoagland nutrient solution (Figure 2E).
   *Note: If you work on nutrient deprivation treatment on the seedlings, you can consider making 0.7% agar with water instead of ½ Hoagland solution.*
4. Fill a 1 ml pipette tip box with ½ Hoagland nutrient solution, and keep the surface of nutrient solution about 0.5 cm away from the rack in the box. Upright and transfer the filled tube to the rack. Each box can hold at least 48 tubes, which are arrayed evenly. The solution in the tube cannot leak out due to the relatively small opening when you handle them gently (Figure 2F).
   *Note: It is highly recommended to sterilize the tube, 1 ml pipette tip box, and the ½ Hoagland nutrient solution in order to avoid algae from growing before the experiment begins. Make sure the tube is fully filled with the nutrient solution, and there are no bubbles in the tube after transferring to the rack.*
5. Use a 1 ml pipette tip to place 2 to 3 *Arabidopsis* seeds to the agar surface in the hole of each tube cap (Figure 2G). Close the pipette box with its own cover and transfer the box to growth room set with a 12:12 h, light:dark cycle, 55%-60% atmospheric humidity, and 120 µmol photons $m^{-2}s^{-1}$ in an irradiance. After 6 days, thin down to a single plant in each tube using tweezers, and leave the box open a little to slowly adapt plants to humidity of the growth room for four days (Figure 2H), and then the cover can be removed or opened completely.
   *Note: The photoperiod can be adjusted according to the purpose of the experiment. For example, if fast growth is needed, long photoperiod (16:8 h) is recommended, and if a large fresh weight*

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*of the plant is needed, short photoperiod (8:16 h or 10:14 h) is recommended. If the body of the pipette box is transparent, wrapping it around with aluminum foil is necessary to prevent the growth of algae (Figure 3A). If the cover of the pipette box is not transparent, plastic clingfilm can be used instead to create a small chamber (Figure 3B). But you need to leave clingfilm at least 20 mm above the plant for growth, and after growing for 6 days in the box, plants can be equilibrated to the humidity of the growth room or chamber by puncturing several holes in the clingfilm, and the clingfilm cover can be removed four days later.*

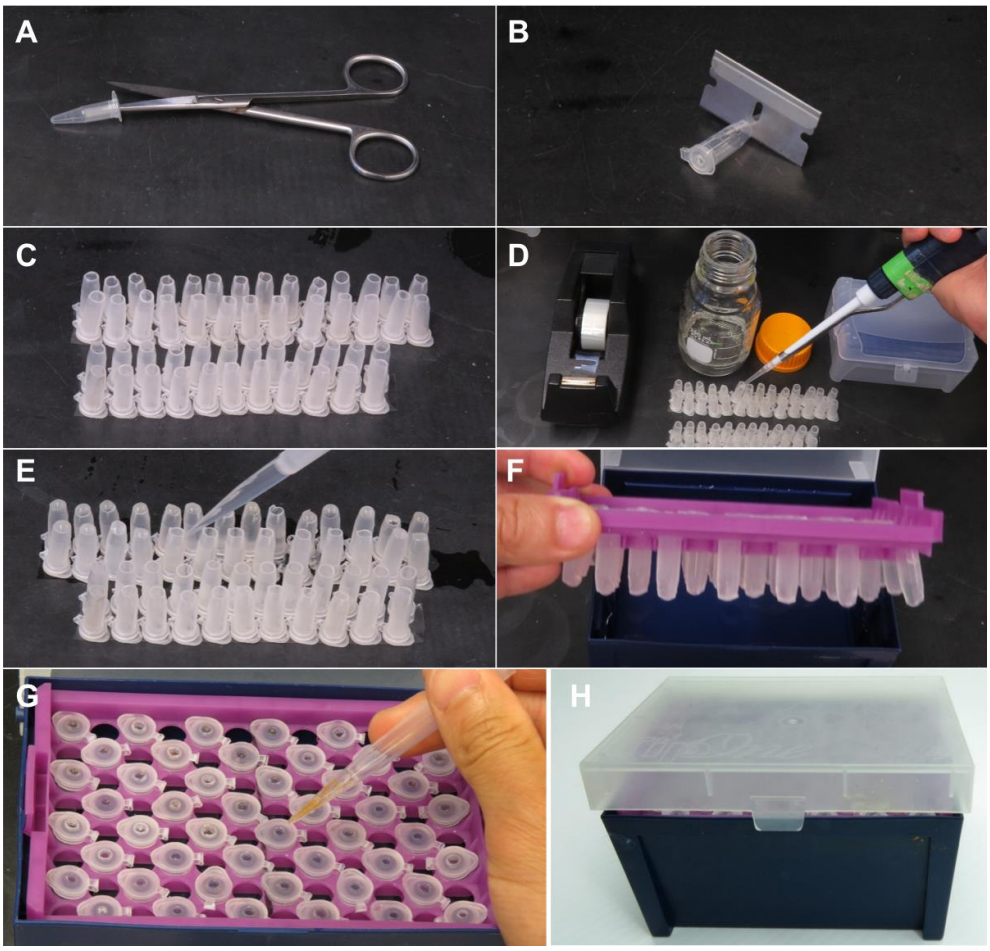


**Figure 2. Preparation of the holding tubes and the seed germination.** A. Drill a hole in the center of the cap using scissors. B. Cut off the bottom of the tube using a blade. C. Invert the prepared tube onto adhesive tape. D. Fill each tube with 150 µl 0.7% agar. E. Fill each tube fully with ½ Hoagland nutrient solution. F. The filled tubes were transferred to the rack in the box. G. Use a 1 ml pipette tip to place *Arabidopsis* seeds to the agar surface of each tube lid. H. The cover of the box was lift a little to equilibrate the plant to chamber humidity.

6. After growing in the pipette tip box for two weeks, the roots of the seedlings emerge out of the bottom of the tube (Figures 3C and 3D). Transfer and fit the tube with a seedling to the hole prepared in the lid of the black bucket that is filled with ½ Hoagland nutrient solution (Figure 3E).

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Make sure the bottom of each tube can touch nutrient solution. Aerate the nutrient solution in the bucket using an air pump that bubbles air through air stones every one hour (Figure 3E). The pump is turned on/off hourly controlled by a 24-h timer during the plant growth period. Change the nutrient solution every two or three weeks with fresh nutrient solution.

*Note: The lid of the bucket typically generates static electricity during the processes of nutrient solution filling, air tubing penetration or manual handling. The static electricity makes the penetration of the slender roots of the seedlings into the hole located in the lid of the bucket difficult. It is better to place the nutrient solution-filled bucket and lid for several hours to reduce static electricity before transferring the seedlings. If a large amount of nutrient solution is needed, the nutrient solution can be made in a big polyethylene tank (e.g., 100 L). After growing for a few weeks, the plants are ready to be treated with chemicals like hormones, or stresses like nutrient deprivation, metal stress and salt stress at the developmental stages according to the purpose of the experiments. To reduce the cost of nutrient solution and chemicals, smaller containers can be chosen to hold plants within your budget range. The 1 ml pipette tip box can hold 4 to 8 plants and allow plants to healthily complete the whole lifecycle (Figures 3F and 3G), which can dramatically reduce the solution volume. Aeration is optional for the hydroponic plants in the box since the plants in the box without aeration can grow very well (Figures 3G). This small and portable system can be served as an ideal option for some expensive treatments if you do not need a huge amount of materials afterward.*

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**Figure 3. Hydroponic plants grown in the pipette tip box and the black bucket.** A. One-week-old seedlings grown in the transparent pipette tip box wrapped around with aluminum foil. B. One-week-old seedlings grown in the pipette tip box covered with plastic clingfilm. C. Two-week-old seedlings grown in the pipette tip box. D. One single two-week-old seedling ready for transferring to the bucket. E. The seedlings located in the cover of the bucket after transferring. F. The pipette tip box was used for further cultivation or treatment (the plants in the box is 24 days old). G. Seven-week-old hydroponic plants grown in the pipette tip box. H. Hydroponic plants grown in the growth room. I. The shoots of plants after transferring and growing in the bucket for 18 days (32 days old, under 12:12 light/dark photoperiod). J. The roots of plants after transferring and growing in the bucket for 18 days. K. Hydroponic plants after transferring and growing in the bucket for 40 days (54 days old, under 12:12 light/dark photoperiod).

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## Recipes

1. ½ Hoagland nutrient solution

| Macronutrients | MW (g/mol) | g to make 1 L stock | Stock concentration (M) | Vol of stock (ml) for 1 L nutrient solution | Final concentration (mM) |
|---|---|---|---|---|---|
| $KNO_3$ | 101.1 | 202.2 | 2.00 | 1.25 | 2.50 |
| $Ca(NO_3)_2{\cdot}4H_2O$ | 236.1 | 236.1 | 1.00 | 1.25 | 1.25 |
| $NH_4NO_3$ | 80.0 | 80.0 | 1.00 | 0.50 | 0.50 |
| $MgSO_4{\cdot}7H_2O$ | 246.5 | 493.0 | 2.00 | 0.50 | 1.00 |
| $KH_2PO_4$ | 136.1 | 136.1 | 1.00 | 0.25 | 0.25 |
| **Micronutrients** | **MW (g/mol)** | **g to make 1 L stock** | **Stock concentration (mM)** | **Vol of stock (ml) for 1 L nutrient solution** | **Final concentration (µM)** |
| NaFe(III)EDTA | 367.1 | 18.4 | 50.00 | 0.50 | 25.00 |
| $H_3BO_3$ | 61.8 | 2.86 | 46.00 | 0.50 | 23.00 |
| $MnCl_2{\cdot}4H_2O$ | 197.9 | 1.81 | 9.10 | 0.50 | 4.55 |
| $ZnSO_4{\cdot}7H_2O$ | 287.5 | 0.22 | 0.77 | 0.50 | 0.39 |
| $CuSO_4{\cdot}5H_2O$ | 249.5 | 0.051 | 0.20 | 0.50 | 0.10 |
| $Na_2MoO_4{\cdot}2H_2O$ | 241.95 | 0.12 | 0.50 | 0.50 | 0.25 |

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## Competing interests

The authors declare no competing interests in regard to this publication.

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