

Plant Growth and Cultivation

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Abstract

There is a variety of methods used for growing plants indoor for laboratory research. In most cases plant research requires germination and growth of plants. Often, people have adapted plant cultivation protocols to the conditions and materials at hand in their own laboratory and growth facilities. Here I will provide a guide for growing some of the most frequently used plant species for research, i.e., *Arabidopsis thaliana*, barley (*Hordeum vulgare*) and rice (*Oryza sativa*). However, the methods presented can be used for other plant species as well, especially if they are related to the above-mentioned species. The presented methods include growing plants in soil, hydroponics, and in vitro on plates. This guide is intended as a starting point for those who are just beginning to work on any of the above-mentioned plant species. Methods presented are to be taken as suggestive and modification can be made according to the conditions existing in the host laboratory.

Key words: Plant growth, *Arabidopsis*, *Thlaspi*, Barley, Rice, Hydroponics, Soil growth, In vitro growth, Seeds sterilization

1. Introduction

Plants require very little for their growth in comparison to animals. As long as they have a substrate with even a small amount of mineral elements, water, and light plants will grow. Growing plants outdoor is subject to environmental conditions which vary greatly in space and time. For example, soil fertility and climatic conditions can rapidly change according the seasons and or geographical position. However, in order to ensure repeatability and uniformity across experiments, specific growth conditions need to be defined and controlled for research purposes. Over time, much interest and resources have therefore been dedicated to optimize the growing of plants indoor, under well-defined and controlled conditions. The optimum type and volume of soil, nutrients, and water dosages, light conditions and photoperiod required by different plant species were studied. Subsequently, hydroponic systems were introduced

with nutrient solutions that offer more uniform mineral composition and are easily adjusted. This has the advantage that the root systems develop in direct contact with the required minerals which are already in their ionic form and uniformly distributed in the substrate. Thus, macro- and micronutrients recipes formulated for the exact needs of each plant species have been developed. The level of control can be further increased by using in vitro plant cultures, especially when grown in dedicated growth cabinets or chambers where light regime, humidity, and CO₂ concentrations can be easily manipulated. In this chapter, I will give detailed protocols for growing some of the most often used plant species for research purposes. All protocols are based on indoor growth but various methods will be discussed such as growth on soil, in hydroponics, and in vitro cultures. The intention is to provide researchers who are starting to work with *Arabidopsis*, barley (*Hordeum vulgare*), or rice (*Oryza sativa*) a beginners guide. Growth conditions presented here can also be customized to suit other plant species and can also be tailored according to the conditions that exist in local growth facilities.

2. Materials

2.1. Growth Facilities

Growing plants indoor requires growth facilities in the form of, e.g., growth chamber, growth cabinet or greenhouse equipped with lighting and temperature control.

2.2. Sowing and Plant Growth on Soil

1. Plant seeds or seedling.
2. Soil/compost. In general, soil/compost is mixed with vermiculite, perlite, or polystyrene pellets in different ratios to prevent the soil from compacting and becoming too heavy after repeated watering and also to reduce the growth of fungi. Some people autoclave the soil before use. However, this practice is not recommended as it can affect the properties of the soil. Pesticides can be used to treat the soil prior to sowing to reduce fungal growth and development of insect larvae (see ref. 8). Soil/compost treated with pesticides is prepared at least 2–4 days in advance of sowing (see ref. 8). If the soil already contains fertilizers no further addition is required, at least, in the early stages. For suggestions regarding the type of soils used for *Arabidopsis*, barley, and rice, see Notes 1–3.
3. Trays/pots. There is a larger variety of trays/pots that are used and their sizes depend on the plant species they are used for. Pots are usually available at any gardening center. Trays/pots should be provided with drainage holes. If trays/pots are to be reused they should be thoroughly washed with detergent, or

even soaked in commercial bleach for about an hour in order to make sure that no fungi, seeds, or soil particles from previous experiments have been left. For suggestions of trays/pots used for growing *Arabidopsis*, barley, and rice, see Notes 1–3.

4. Lighting and temperature control. See Notes 1–3 for recommendations regarding light intensities and temperatures required by *Arabidopsis*, barley, and rice.
5. Water. To ensure repeatability between experiments distilled and deionized water (dIH₂O) is recommended to be used for watering the plants. If mineral composition of plants is also a matter of investigation, then the use of dIH₂O is a must. To ensure a healthy growth, fertilizers can be used.
6. Fertilizer. See Notes 1–3 for recommendations on fertilizers to be used for *Arabidopsis*, barley, and rice.
7. Pesticides. Pesticides are not a must, but if you are working in an institution with large facilities for growing plants, they will be used anyway to prevent plant damages and spread of any pest that is present. For scarid fly and greenfly control, compost can be treated with Intercept 70WG (Levington Horticulture, Ipswich, UK) before use. To control thrips, their mite predator *Amblyseius cucumeris*, can be applied to the leaves as larvae in a sawdust medium. Further thrip control can be provided by spraying with Conserve (Dow AgroSciences, Indianapolis, USA) every 2 months and with Dynamec (Fargro Ltd, Littlehampton, UK). To control mealy bug plants can be sprayed every 2 weeks with Provado (Bayer Garden, Cambridge, UK). Fungal infection can be controlled by using sulfur burners. To provide a broad spectrum pest control plants can be sprayed weekly with Agri 50E (Fargro Ltd, Littlehampton, UK). The active ingredient present in the above-mentioned pesticides can be found in Table 1.

Table 1
List of pesticides used for treating the soil used for plant growth. Active ingredients and providers are also listed

Pesticide	Active ingredient	Manufacturer
Intercept 70WG	Imidacloprid 70 % w/w	Levington Horticulture, Scotts
Agri 50E	Dodecylphenol ethoxylate 27–33 % Tetrahydrofurfuryl alcohol 13.5–16.5 %	Fargro
Conserve	Spinosad 120 g/L (11.6 % w/w)	Fargro
Dynamec	Abamectin (1.8 % w/v) also contains hexan-1-ol	Fargro
Provado	Thiacloprid 0.150 g/L	Bayer

8. Labels and a permanent marker for labeling.
9. Waxed paper bags to collect the seeds.
10. Insulating tape to seal seed bags.
11. Fine mesh sieve to separate seeds from other plant material if dealing with small seeds such as *Arabidopsis*.
12. White paper for handling the seeds.
13. Support for larger plants such as barley and rice to prevent them from tangling. Optional.
14. ARACONs—harvesting devices used for *Arabidopsis* seeds. Their use prevents cross contamination between adjacent plants (e.g., Arasystem, Belgium; Lehle Seeds, Texas, USA).

2.3. Plant Growth in Hydroponics

1. Plant material. Both seeds and seedlings can be used for hydroponics.
2. Containers used for hydroponics are typically made of plastic. In general containers with lids are used in order to prevent evaporation of the solution, but other tops can also be used as long as the volume of the hydroponics is maintained constant by replacing the evaporated solution. In general, opaque containers are recommended as they reduce the growth of algae and confer appropriate conditions for roots growth. Usually, dark containers (brown, black) are used for this purpose. Dark containers are suitable for growth chamber and cabinets. However, when plants are grown in the greenhouse, white or light color nontransparent containers are recommended as they prevent over heating of the solution by reflecting the sunlight. In general, any plastic container can be used for hydroponics and there are numerous methods to adapt them for hydroponics. For example, transparent containers can be made opaque by either painting them with vinyl paint, or covering them with black insulating tape or aluminum foil. When using aluminum foil care should be taken in order to avoid its contact with the solution and thus prevent aluminum contamination. Containers can be washed with detergent every time the solution is changed, but often they are washed during an experiment only if required, i.e., if formation of algae or deposition of salts on the walls of the containers occurs.

With respect to the size of the containers there is no uniformity in the literature. However, there are a few things that need to be considered when choosing a container. If the plants are to be analyzed separately and especially if the roots are to be analyzed (for biometric measurements or for mineral or biochemical analysis) then a deeper container should be used to allow the root system of each plant to develop without getting entangled. In this case, also the spacing between the plants should be considered.

A method for growing *Arabidopsis* in hydroponics using Magenta GA7 boxes and without the need of aeration (see ref. 4) was described by Arteca and Arteca (1). Indeed, the Magenta vessels work well. However, the downside of using these boxes is that they can only sustain growth of a very limited number of plants (about 4 plants). In many experimental contexts, (e.g., growth of wild-type plants is compared with that of mutant plants) a larger number of plants per box would be preferable. Therefore, people tend to use larger containers to be able to grow a larger number of plants within the same container. This ensures that both types of plants that are compared are exposed to exactly the same growth conditions.

3. Holders for plants. For holding the plants polyurethane foam, rockwool, or cotton wool cylinders of about 3 cm height can be used. Precut polyurethane foam stoppers of different diameters can be acquired from Fisher (<http://www.fishersci.com>). Rockwool can be bought from any gardening center as it is a very common material used for hydroponics or aquacultures. It is made of mineral fibers and has a water- and air-holding capacity of up to 80 and 17 %, respectively. To some people, rockwool can be a skin irritant. Rockwool can be difficult to cut when dry, so wetting the material may help. Before placing the seeds or the seedling within the rockwool cylinders they should be well soaked with nutrient solution to allow the pH of the rockwool to adjust to that of the solution.
4. Aeration system for the nutrient solution. Nutrient solutions should be well aerated. Any aquarium air pump or any commercial aeration pump from a gardening center and any rubber tubes for tubing can be used.
5. Reagents for nutrient solution. Reagents used to prepare the nutrient solution are to be of high purity (e.g., Fluka, Sigma, Supelco from Sigma-Aldrich <http://www.sigmaaldrich.com>) and solutions should be prepared in distilled and deionized (dH_2O) water. Nutrient solutions used for plant growth are, in general, modified Hoagland's solution (2) and many variations exist (3). Stock solutions of nutrients (100 or 1,000 \times concentrated) can be prepared in advance and can be stored at 4°C. Below are given some examples of nutrient solutions used for different plants. For all the nutrient solution presented below, the concentration of the stock solution, the volume of the stock solution required for 1 L of hydroponic solution and the concentration of nutrients in the growth solution is given.
 - (a) Nutrient solution for *Arabidopsis*.
Gibeaut's solution (4) (Table 2). This is a modified Hoagland's solution (2) containing one-third of the concentration of macronutrients of Hoagland and full strength concentrations for the micronutrients. Reduced

Table 2

Nutrient solution used for *Arabidopsis* hydroponics (after Gibeaut et al. (4)). The micronutrients Si and Fe are prepared as separate stock solutions whereas the other micronutrients are mixed together in a single stock solution

	Stock solution concentration	Volume of stock solution to be added to 1 L final solution (ml/L)	Concentration in the hydroponics
<i>Macronutrients</i>			
Ca(NO ₃) ₂ × 4H ₂ O	1 M	1.50	1.50 mM
KNO ₃	1 M	1.25	1.25 mM
Mg(SO ₄) × 7H ₂ O	1 M	0.75	0.75 mM
KH ₂ PO ₄	1 M	0.50	0.50 mM
<i>Micronutrients</i>			
Na ₂ O ₃ Si × 9H ₂ O	0.1 M	1.00	0.1 mM
Fe (Sprint 330) ^a	0.072 M	1.00	0.072 mM
	One single stock solution containing		
KCl	50 mM	1.00	50 μM
MnSO ₄ × H ₂ O	10 mM		10 μM
CuSO ₄ × 5H ₂ O	1.5 mM		1.5 μM
ZnSO ₄ × 7H ₂ O	2 mM		2 μM
H ₃ BO ₃	50 mM		50 μM
(NH ₄) ₆ Mo ₇ O ₂₄	0.075 mM		0.075 μM

^aFe (Sprint 330) is a commercial Fe product containing 10 % DTPA-Fe (DTPA = pentetic acid or *diethylene triamine pentaacetic acid*)

strength for the macronutrients prevents osmotic shock, whereas full strength of micronutrients prevents depletion. Gibeaut et al. (4) have added silicon (Si) into the medium because this is an important element for cell walls which in turn play a vital role in defense against pathogens. Another alternative is presented in Table 3 and is a modified solution after Arteca and Arteca (1). The pH of the hydroponic solution for *Arabidopsis* should be adjusted to 5.7–6.5 using 1 M sodium hydroxide (NaOH) or potassium hydroxide (KOH) or with 1 N hydrochloric acid (HCl).

(b) Nutrient solution for *Thlaspi caerulescens*.

An example of nutrient solution is presented in Table 4 and is also a modified Hoagland's solution. The pH of the hydroponic solution for *Thlaspi caerulescens* is similar to the *Arabidopsis* slightly acidic and it should be adjusted to 5.5–6.0 using 1 M NaOH or KOH or with 1 N HCl.

(c) Nutrient solution for barley.

Several hydroponic solutions for growing barley have been published. One example is given in Table 5. An alternative would be the nutrient solution used by Gries et al. (5).

Table 3
Nutrient solution used for *Arabidopsis* hydroponics modified after Arteca & Arteca (1)

	Stock solution concentration	Volume of stock solution to be added to 1 L final solution (ml/L)	Concentration in the hydroponics
<i>Macronutrients</i>			
KNO ₃	1.25 M	1.00	1.25 mM
Ca(NO ₃) ₂ × 4H ₂ O	0.50 M	1.00	0.50 mM
MgSO ₄ × 7H ₂ O	0.50 M	1.00	0.50 mM
KH ₂ PO ₄	0.625 M	1.00	0.625 mM
<i>Micronutrients</i>			
FeNaEDTA	42.5 mM	1.00	42.5 μM
	One single stock solution containing		
CuSO ₄ × 5H ₂ O	160 μM	1.00	0.16 μM
ZnSO ₄ × 7H ₂ O	380 μM		0.38 μM
MnSO ₄ × H ₂ O	1.8 mM		1.8 μM
H ₃ BO ₃	45 mM		45 μM
(NH ₄) ₆ Mo ₇ O ₂₄ × 4H ₂ O	15 μM		0.015 μM
CoCl ₂	10 μM		0.010 μM

Table 4
Nutrient solution used for *Thlaspi caerulescens* hydroponics

	Stock solution concentration	Volume of stock solution to be added to 1 L final solution (ml/L)	Concentration in the hydroponics
<i>Macronutrients</i>			
KNO ₃	0.5 M	1.00	0.5 mM
Ca(NO ₃) ₂ × 4H ₂ O	0.4 M	1.00	0.4 mM
MgSO ₄ × 7H ₂ O	0.2 M	1.00	0.2 mM
KH ₂ PO ₄	0.1 M	1.00	0.1 mM
<i>Micronutrients</i>			
FeNaEDTA	20 mM	1.00	20 μM
	One single stock solution containing		
CuSO ₄ × 5H ₂ O	0.2 mM	1.00	0.2 μM
ZnSO ₄ × 7H ₂ O	12 mM		12 μM
MnSO ₄ × H ₂ O	2 mM		2 μM
H ₃ BO ₃	10 mM		10 μM
Na ₂ MoO ₄ × 2H ₂ O	0.1 mM		0.1 μM

Table 5
Nutrient solution used for spring barley (*Hordeum vulgare* cv. Golden Promise) hydroponics

	Stock solution concentration	Volume of stock solution to be added to 1 L final solution (ml/L)	Concentration in the hydroponics
<i>Macronutrients</i>			
KH_2PO_4	0.4 M	1.00	0.4 mM
K_2SO_4	0.4 M	1.00	0.4 mM
$\text{MgSO}_4 \times 7\text{H}_2\text{O}$	0.6 M	1.00	0.6 mM
NH_4NO_3	1.0 M	1.00	1 mM
$\text{Ca}(\text{NO}_3)_2 \times 4\text{H}_2\text{O}$	2.0 M	1.00	2 mM
<i>Micronutrients</i>			
$\text{FeCl}_3 \times 6\text{H}_2\text{O}$ or FeNaEDTA	75 mM	1.00	75 μM
	One single stock solution containing		
$\text{MnCl}_2 \times 4\text{H}_2\text{O}$	7 mM	1.00	7 μM
ZnCl_2	3 mM		3 μM
$\text{CuSO}_4 \times 5\text{H}_2\text{O}$	800 μM		0.8 μM
H_3BO_3	1.6 mM		1.6 μM
$\text{Na}_2\text{MoO}_4 \times 2\text{H}_2\text{O}$	0.83 mM		0.83 μM

The pH of the hydroponic solution for barley should be adjusted to 5.5–6.0 using 1 M NaOH or KOH or 1 N HCl.

(d) Nutrient solution for rice.

One example of nutrient solutions used for growing rice in hydroponics is given in Table 6. An alternative nutrient solution that works well for rice is the one used for *Arabidopsis* and described in Table 3. The pH of the hydroponic solution for rice should be adjusted to 5.5–6.0 using 1 M NaOH or KOH or 1 N HCl (6, 7).

6. Concentrated 1 N hydrochloric acid (HCl).
7. 1 M solution of sodium hydroxide (NaOH) or potassium hydroxide (KOH). (Dissolve 2 g of NaOH in 50 ml dH_2O or 2,8 g KOH in 50 ml dH_2O).
8. Bottles and dark bottles for preparation and storage of stock solutions.
9. Tweezers for handling larger seeds or seedlings.
10. Knife or sharp scalpel to cut the plant holders.
11. pH meter to check the pH of the hydroponic solution.
12. Magnetic stirrer to mix the nutrient solution when prepared.
13. Fridge or cold room to store the stock solution.
14. Labels and permanent marker for labeling.

Table 6
Nutrient solution used for rice (*Oryza sativa*) hydroponics (after Miyamoto et al. (16))

	Stock solution concentration	Volume of stock solution to be added to 1 L final solution (ml/L)	Concentration in the hydroponics
<i>Macronutrients</i>			
(NH ₄) ₂ SO ₄	0.09 M	1.00	0.09 mM
KH ₂ PO ₄	0.05 M	1.00	0.05 mM
K ₂ SO ₄	0.03 M	1.00	0.03 mM
MgSO ₄ × 7H ₂ O	0.07 M	1.00	0.07 mM
KNO ₃	0.05 M	1.00	0.05 mM
Ca(NO ₃) ₂ × 4H ₂ O	0.06 M	1.00	0.06 mM
<i>Micronutrients</i>			
Fe-EDTA	0.11 M	1.00	0.11 mM
	One single stock solution containing		
MnSO ₄	1.8 mM	1.00	1.8 μM
ZnSO ₄ × 7H ₂ O	0.3 mM		0.3 μM
CuSO ₄ × 5H ₂ O	0.3 mM		0.3 μM
H ₃ BO ₃	4.6 mM		4.6 μM

2.4. Sowing and Growing on Plates

- Plates (sterile Petri dishes: round or square, the latest being used especially if plants are to be grown vertically, either for root measurements or to ease the removal of the seedlings for transfer to a different substrate), e.g., Square 100 × 100 mm (Sterilin Ltd, UK) or 120 × 120 mm (Greiner—Sigma-Aldrich; Sarstedt, Sarstedt AG & Co., Germany).
- Reagents for preparation of media. Media used for in vitro culturing are based on Murashige and Skoog (1962) formula (6), thus named MS. It can nowadays be bought as a powder including all the minerals. Different variations exist in order to assist all needs.
 - ½ MS minimal medium (1 L) (half-strength MS, no sucrose (7)).
 - 1/2 MS basal salt mixture (without micronutrient and vitamins) (weigh half of the manufacturer's recommended amount for 1 L) (e.g., Sigma; Duchefa, The Netherlands, <http://www.duchefa.com>).
 - 0.8 % plant agar for horizontal growth and 1.5 % for vertical growth (e.g., Sigma; Duchefa).
 - 1 × MS medium (1 L).
 - 1 × MS basal salt mixture (without micronutrient and vitamins) (weigh the manufacturer's recommended amount for 1 L) (e.g., Sigma; Duchefa).

- 1× Gamborg's vitamins (weigh the manufacturer's recommended amount for 1 L, to be filter sterilized and added after autoclaving) (e.g., Sigma, Duchefa).
 - 2.56 mM MES-KOH (e.g., Sigma) ((MES—KOH—Monohydrate 2-(N-morpholino)ethanesulfonic acid) adjusted to pH 5.7 with 1 M KOH).
 - 1 % sucrose (to be added after autoclaving from a filter-sterilized 20 % stock solution). (The percentage of added sucrose can be increased).
 - 1.5 % phyto or plant agar (Duchefa).
3. dIH₂O (distilled and deionized water).
 4. Filter-sterilized 20 % sucrose solution (Weigh 20 g sucrose (e.g., Formedium, UK, <http://www.formedium.com>) and add dIH₂O up to 100 ml volume. Add a magnet and place it onto a magnetic stirrer until dissolved. Within a laminar hood filter the solution through a sterile syringe-driven filter with 0.22 μm pore size (e.g., Millipore) into a sterile recipient (e.g., 2 × 50 ml Falcon-type tube or an autoclaved empty Duran bottle)).
 5. Solutions for sterilization of seeds:
 - (a) 70 % ethanol/ methanol or Mikrozid.
 - (b) 96–100 % ethanol.
 - (c) 30–50 % domestic bleach (e.g., Domestos) or 2.625 % sodium hypochlorite in final solution made in H₂O (0.02–0.05 % Triton X-100 or Tween 20 (Sigma) can be added to reduce surface tension and allow better surface contact).
 - (d) Concentrated hydrochloric acid (HCl) (for Subheading “Sterilization of Large Numbers of *Arabidopsis* Seeds with Chlorine Gas”).
 - (e) Sterile dIH₂O (autoclaved water dIH₂O).
 - (f) Sterile top agar (0.1 % agar prepared in dIH₂O and autoclaved) (Duchefa).
 6. Duran bottle to autoclave the media and solutions.
 7. Duran bottle for the liquid waste (make sure you label it appropriately) and a recipient to dispense the pipette tips.
 8. 1.5 ml Eppendorf-like tubes or, if large numbers of seeds are to be handling, 15 ml Falcon-like tubes.
 9. Racks for the tubes.
 10. Labels and a permanent marker for labeling.
 11. White paper for handling the seeds.
 12. Tweezers for handling larger seeds or seedlings, or toothpicks (cocktail sticks) for small seeds.
 13. Scissors.

14. 200 ml glass beaker (for Subheading “Sterilization of Large Numbers of *Arabidopsis* Seeds with Chlorine Gas”).
15. Plastic box or lid large enough to cover the 200 ml beaker and the rack with seeds (for Subheading “Sterilization of Large Numbers of *Arabidopsis* Seeds with Chlorine Gas”).
16. Pipettes (for up to 1,000 μ l).
17. Sterile pipette tips (autoclaved tips; the box of tips should only be opened inside the sterile laminar hood).
18. Micropore tape (3 M) to seal the plates.
19. Timer.
20. Autoclave.
21. Laminar flow hood, possibly also fume hood (for Subheading “Sterilization of Large Numbers of *Arabidopsis* Seeds with Chlorine Gas”).
22. Table-top centrifuge for 1.5 ml Eppendorf-like tubes or centrifuge for 15 ml Falcon-like tubes.

3. Methods

3.1. Sowing and Growing in Soil

Seeds can be either placed directly onto soil and thus germinated in pots or they can be first germinated and then seedlings can be transferred to soil. There is no need to sterilize the seeds as long as they are not to be used for in vitro culturing.

3.1.1. *Arabidopsis thaliana*
(This Method Also Works
for Other Small Seed
Species Such As *Thlaspi*
*caerulescens** and
Nicotiana sp.)

1. If the seeds are to be germinated directly onto soil in single cells/pots, then one seed should be placed in the center. *Arabidopsis* seeds are very small and sometimes it is difficult to place only one seed onto soil. If more than one seed is placed and germinated, just remove the extra seedlings.
2. To ease the sowing the soil should be water-saturated, and water should be seen in the tray as well. Also, water can be sprayed directly onto the surface of the soil.
3. Seeds can be placed on white paper and single seeds can be taken using a wet wooden tooth pick (cocktail stick) and placed onto the surface of the soil. Another method to place seeds onto soil is to first put them into a 0.1 % agar (e.g., plant agar Duchefa) solution and then pipette them. Seeds require light to germinate, therefore there is no need to cover them with a lot of soil or push them deep into the soil.
4. After sowing, trays should be covered to maintain moisture either with tops (if the trays are provided with incubation tops, transparent plastic tops) or wrapped in transparent foil. If using the incubation lids then the vents should be closed.

5. If seeds have not been stratified prior to placing them onto soil, then the trays should be placed at 4°C for 48 h before moving them to growth cabinets or greenhouse.
6. If seeds are to be first germinated onto wet filter paper or in vitro, then 10–20 day old seedlings (usually two to four leaves) are transferred to the soil. A small hole needs to be made in the center of the pot/cell where seedling should be gently placed using tweezers. The roots of the seedling should be placed entirely in soil. As *Arabidopsis* seedlings are prone to dehydration the soil should be saturated with water and the trays/pots should be covered with the incubation lids with vents closed.
7. After germination, plants should be watered as required. In general, the top soil should be slightly dry before watering. Depending on the conditions in the greenhouse or growth cabinet, administration of water is required every 5–7 days. Both water logging and complete dryness should be avoided.
8. If plants are grown for seeds, in order to obtain a larger number, the first bolt can be simply removed by hand. This will stimulate more bolts to form.
9. To collect the seeds, stop watering the plants once the siliques have developed and turn brown.
10. Bag the entire inflorescence/siliques of a plant by introducing it into a waxed paper bag. Do not remove the plant from the soil, but seal the bag to the stem using insulating tape. Wait for the siliques to dry as this will allow the seeds to mature. When the plants are dead, about 7–10 days after bagging, seeds can be collected by cutting off the inflorescence pedicel with scissors. Optionally, for collecting and avoiding cross-contamination of *Arabidopsis* seeds ARACONs can be used (<http://www.arabidopsis.com/main/cat/arasystem/!as02.html>).
11. To clean the seeds from the rest of the plant material, cut off a corner of the bag and pour its content onto a fine mesh placed above a piece of white paper. For a first rough cleaning a tea sieve can be used followed by sieving through a finer meshed sieve.
12. Once cleaned, seeds can be transferred into vials (e.g., 1.5 ml Eppendorf-like tubes or Falcon-like tubes depending on the amount of seed). Vials should be labeled properly, sealed with parafilm and stored in dry conditions at 2–8°C, usually in dark. For more information on storage conditions of *Arabidopsis* see ref. (8).
13. *Winter annuals or biennial plants such as *Thlaspi* sp. also require a period of cold (about 4°C) in order to flower, a process called vernalization. Therefore, 6–8 weeks old plants should be moved to 4°C for 10 weeks, 8/16 h photoperiod. Before moving to the cold, plants should have developed a

large rosette of leaves as they should have acquired enough carbohydrates in order to survive vernalization. During vernalization plants should be watered once per week, just to make sure the soil does not dry out. Also, plants should not be fertilized before vernalization. Fertilizers should be added together with water only once the plants have been vernalized and moved to long days (16/8 h day/night light regime) and temperatures above 20°C. For *Thlaspi* bolting will start 7–10 days later (9).

3.1.2. Barley and Rice

1. If seeds are to be germinated directly in soil, then place one in the center of each pot at about 2.5 cm depth and make sure you cover it with soil.
2. Alternatively, germination of seeds can be achieved on moist filter paper or wet vermiculate. Wet vermiculate would give the advantage of a solid support and therefore seedlings can grow as they would in soil for up to 2 weeks. Use dH₂O as this will have the least effect on seedlings that are to be used in downstream experiment.
3. Germination can be performed in germinators, plastic boxes or Petri dishes at room temperature (<22°C) for barley and at 28°C for rice. At the time of placing the seeds, the filter paper or the vermiculate should be moist/wet for barley so that the seeds do not end up floating. For rice more water can be added. Cover the germination container to maintain moisture and place it in the dark for 3–5 days. Water can be added during this time if the paper or vermiculate gets dry.
4. Once the coleoptile has emerged, uncover the container and place it in the light (e.g., growth chamber, greenhouse or even window frame if seedlings are to be used only for molecular analysis such as RNA or gDNA extraction). Once the first leaf has emerged and seedlings are 5–10 cm in length, they can be transferred to soil or hydroponics.
5. Plants grown in soil should be watered as required. There is no consensus because this is highly dependent on the type of soil, volume of the pot, number of plants per pot, developmental stage, air-humidity, and temperature. Just avoid water-logging the soil and keep in mind that in plastic pots the soil tends to dry at the surface, but can be very moist deeper within.
6. For barley and rice, after 6–7 weeks of growing, plants require feeding, especially if they are to be grown to maturity and seeds are required. In general, fertilizers are added from the 6th to 7th week, once per week together with water. The type of fertilizer used varies a lot. One possibility is to use a general purpose fertilizer (e.g., “Phostrogen” NPK 14:10:27 that includes also trace elements (PBI Home and Garden Ltd.,

Hertfordshire, UK)). Another option would be “Osmocote Plus” (NPK-Mg, 5:5:11–1.2) also added weekly together with the water. Murray et al. (10) used Aquasol liquid fertilizer (NPK 23:4:18) once per week (5 g/L).

7. Support the plants to prevent tangling when needed.
8. Seed heads should be harvested when they turn brown.
9. Store seeds in waxed paper bags or just paper bags at room temperature in dry conditions.

3.2. Sowing and Growing in Hydroponics

3.2.1. Preparation of Nutrient Solution

1. To ease the preparation of hydroponics, stock solutions of nutrients are prepared beforehand and certain volumes of these solutions are then added to prepare the final hydroponic solution in which plants are grown. In general, macronutrients are prepared as individual stock solutions while micronutrients are mixed together in a single stock solution. However, each microelement should be dissolved separately in water and only then mixed together. It is possible that for ZnCl_2 you might need to add a small drop of concentrated hydrochloric acid (HCl) until the solution is clear. Iron (Fe) is also usually prepared separately and, as it is sensitive to light, the stock solution should be kept either in a dark bottle or covered with aluminum foil. It is also recommended that the stock solutions are kept at 4°C to prevent algae formation. If you see any aggregates floating in the solution discard it and prepare a new one. The pH of the hydroponic solution should be adjusted to slightly acidic using 1 M sodium hydroxide (NaOH) or potassium hydroxide (KOH) if a Na-free solution is needed. The recipient in which the hydroponic solution is prepared can be placed on a magnetic stirrer while the pH is adjusted as required for each plant species.
2. Examples of nutrient solutions used for some of the most commonly used plant species are listed under Subheading 2.3.
3. Once the nutrient solution is prepared proceed to sowing (see Subheading 3.2.2).
4. Then place the hydroponics into the growth chamber/cabinet or greenhouse.
5. Aerate the solution continuously via a needle, injecting compressed air into the solution. However, for *Arabidopsis*, continuous aeration should be avoided as it can inhibit root growth due to agitation (4, 11).
6. Some protocols recommend changing the solution every 3–4 days; however others change the nutrient solution once per week. When changing solution for the first time, check the pH and the level of solution and adjust the changing times according to needs and duration of the experiment.

3.2.2. Preparation of Plant Material and Holders

1. Cut the holder cylinders (foam, rockwool or cotton) by making an incision along their length up to the center. This cut will allow you to place the seedling in the center of the holder and then will allow the plant to develop its root down to the nutrient solution.
2. Before sowing the seeds or transferring the seedlings, wet the holders by soaking them into nutrient solution.
3. Seeds can be sown directly onto wet holding material. Place two to three seeds in the center of the holders. After germination seedlings are thinned to one per plug of holding material.
4. If seedlings are used, seeds need to be germinated and seedlings to be grown to a certain developmental stage. In many cases, for small seeds such as *Arabidopsis*, germination is performed in vitro on half-strength Murashige and Skoog (MS) medium and 10–20 days old seedlings are then transferred to hydroponics. For sowing and getting seedlings in vitro for *Arabidopsis* see Subheadings “Sterilization of Limited Numbers of Small Seeds Such As *Arabidopsis*” and 3.3. For larger seeds such as from cereals, seeds can be germinated on wet substrate (see Subheading 3.1.2) and seedlings, developed to one leaf stage, can be transferred to hydroponics.
5. Place one seedling in the center of the holder and place the holder into one of the holes in the lid/top of the hydroponic container, making sure that the root is coming out from the holder and is reaching the nutrient solution.
6. To avoid algae growth onto the holder you can sprinkle some soil on top of them. Otherwise, just wash the holders with detergent or replace them every time or every second time you change the nutrient solution.
7. Place the containers with the hydroponic solution and the plants in the growth chamber/cabinet, greenhouse.

3.3. Sowing and Growing on Plates

3.3.1. Preparation of Media and Plates

1. Weigh the required amount of reagents needed to prepare the medium and place them in a Duran bottle. Sucrose can be added as a feed source for seeds and plants. However if seeds are healthy they should have enough stored resources to germinate and once the first leaves appear the seedlings are autotrophic. The addition of sucrose can also increase the susceptibility of the media and, in consequence, of the plants to fungal infection. However, on the other hand, sucrose can help to ensure uniformity of germination and of early development of the seedlings. Two examples of media (without and with sucrose) that can be used for general purpose growth of *Arabidopsis* and other plant species are given under Subheading 2.3. The agar or phytigel should be added only after the pH has been adjusted.

2. Add 900 ml of dH₂O and agitate until the reagents added before are dissolved. completely.
3. Adjust pH to 5.6–6.0 with 1 M KOH.
4. Add the agar or phytagel. Mix well.
5. Fill with water to 1 L. (If a certain volume of sucrose and/or vitamins is to be added after autoclaving take into consideration those volumes when adding the water so that, after their addition, the final volume will still be 1 L).
6. Autoclave for 20 min at 121°C.
7. Let the medium cool down and then pour it into plates inside the laminar hood. Plates should only be opened inside the laminar hood to keep them sterile.
8. Let plates dry for 30 min (some people recommend 45 min or even 1 h). If the experiment will involve comparing the plants grown in different batches, then time the drying and use the same time every time you pour the plates.
9. Plates can be stored upside down at 4°C for 1–2 weeks.
10. If plates are to be used immediately leave them covered (lids on) in the hood and proceed to surface sterilization of the seeds and then sowing.

3.3.2. Surface Sterilization of Seeds

1. Sterilization of seeds is required for any in vitro experiments in order to get rid of any pathogens or bacteria that can be present at the surface of the seeds.
2. All steps of sterilization should be conducted in a laminar flow hood, ideally supplied with a UV lamp. The surface of the flow hood should be cleaned, wiped with 70 % ethanol/methanol or Mikrozid. Optional: Place all the solutions and materials (except the seeds) in the hood and keep them under the UV light for 5–10 min.
3. All solutions placed on the seeds during the procedure are to be discarded into the autoclavable waste bottle and tips into the waste bin. It is important, especially when you work with transgenic seeds, that all the liquid and solid waste will be autoclaved, in order to avoid any accidental spread.
4. Make sure you swab you hands with ethanol before starting work in the laminar flow hood or wear gloves.

Sterilization of Limited Numbers of Small Seeds Such As *Arabidopsis*

1. Label the tubes for each batch of seeds you are using.
2. Transfer the required amount of seeds from the storage vial (tube) into a clean 1.5 ml Eppendorf-like tube. This can be done on a bench, in the laboratory. Do this on a white paper so that you can see any seeds that might be spilled. To ease the transfer into the Eppendorf-like tube you can use either a white weighing boat or a folded white piece of paper. Make sure that

there are no seeds spilled on the paper or weighing boat when you change to a different batch of seeds.

3. Take the tubes with the seeds into the laminar flow hood and make sure you have all the solutions and materials ready.

The following steps are to be performed in sterile conditions inside the laminar flow hood, using only sterile materials and solutions.

4. Carefully open the first Eppendorf-like tubes with the seeds (avoid seeds jumping out due to static). Add slowly 1 ml of 96–100 % ethanol. Close the tube and mix by inverting it several times, so that all the seeds get in contact with ethanol.

Do the same for each of the remaining tubes. Seeds should be maintained in ethanol for 1 min. If you have a large number of tubes, handle them in batches so that the seeds do not stay in ethanol longer than 2 min.

5. Remove the ethanol using a pipette. Once you get more experienced you can pour it out, but for beginners it is better to use a pipette. Discard the ethanol into the waste bottle and then the tip into the waste bin.
6. Add 1 ml of 30–50 % bleach (sodium hypochlorite) solution. Make sure the tubes are tightly closed and mix the seeds with the bleach solution by inverting the tubes. Keep the seeds in the bleach solution for 10 min. Do not exceed this time as it can have a detrimental effect on seed germination.
7. Take the tubes to a centrifuge and give them a quick spin so that the seeds are collected at the bottom of the tubes. Then take them back into the laminar flow.
8. Remove all the bleach solution from the tubes by using a pipette and discard it. Do it slowly and make sure you do not take out the seeds as well.
9. Add 1 ml of sterile dH_2O into the tube. Close the tube and mix well by inversion. The aim is to remove any traces of the bleach.
10. Take the tubes to the centrifuge and spin them quickly to collect the seeds at the bottom.
11. Repeat the washing step one or two times.
12. At this stage, seeds can be either sown on plates or seeds can be left in the last washing with dH_2O and placed the tubes at 4°C (in the fridge or cold room) for 2–3 days to be stratified. Seeds can be kept like this for up to 1 week.
13. If seeds are to be sown on plates immediately, proceed to (Subheading 3.3.3).
14. A slight modified version of sterilization would be to use: 70 % ethanol for 5 min, 50 % bleach supplemented with 0.02 % Triton X-100 (Sigma) for 5 min and four washes with dH_2O .

Sterilization of Large
Numbers of *Arabidopsis*
Seeds with Chlorine Gas

If many batches of seeds are to be sterilized in one go, then chlorine gas can be used. This method saves the time required for handling each individual tube of seed. As chlorine is a toxic gas, the sterilization procedure should be performed in a fume hood rather than a sterile hood.

1. Place the seeds in 1.5 ml Eppendorf-like tubes. Tubes should be labeled by writing in pencil on sticky labels as pen writing directly on the tubes will be removed by the chlorine gas.
2. Add 100 ml of bleach in a 200 ml beaker.
3. Place the tubes with the seeds in a rack and open them.
4. Put the tube racks with the seeds and the beaker with the bleach in a box or under the lid in a fume hood.
5. Add 3 ml of concentrated HCl to the bleach. A small quantity of brown gas will evolve.
6. Immediately close the box and leave for about 4 h. Avoid accidental spillage of the bleach from the beaker. Longer times might be used, but exceeding 24 h can cause seed mortality.
7. When the time is up, open the box in the fume hood and leave it for 10 min for the gas to disperse.
8. Close the tubes and transfer them to a sterile hood.
9. Seeds can be placed directly onto MS media or can be resuspended in sterile 0.1 % agar for pipette sowing.
10. For sowing (plating) follow protocol in Subheading 3.3.3.

Sterilization of Large Seeds
(e.g., Barley, Rice, Wheat)

1. Remove awns and take only the seeds. Dehusked or non-dehusked seeds can be used depending on the purpose of the experiment. However, if seeds are intended for transformation or if seedlings are to be kept for longer in vitro, then it is recommended to use dehusked seeds. This can be achieved manually.
2. Place the seeds in a Berzelius or Erlenmeyer (beaker). The size of the Berzelius/Erlenmeyer should be at least twice the volume of the seeds that are to be sterilized. At this stage a magnet can be added to help with agitation in the further steps.
3. Add enough 96–100 % ethanol to cover the seeds completely. Mix by agitating the beaker for a minute, or you can cover the Berzelius/Erlenmeyer with aluminum foil and place it onto a magnetic stirrer for 1 min. An alternative is to place the seeds in a 50 ml Falcon-like tube (no more than half full) and rinse with 96–100 % ethanol for 1 min.
4. Instead of 96–100 % ethanol for 1 min, 70 % ethanol can be used for 3 min.
5. Take the Berzelius/Erlenmeyer back in the laminar flow hood and pour off the ethanol into a waste bottle.

6. Add 20 % bleach solution (or final 1 % sodium hypochlorite) containing 0.02–0.05 % Triton-X or Tween-20. Use about 1.5–2 times the volume of the seeds (e.g., if seeds occupy about 100 ml, then add 150–200 ml of bleach solution).
7. Cover the Berzelius/Erlenmeyer and place it onto a magnetic stirrer (if you had previously added the magnet) for 15–20 min. If no magnet was added, keep the Berzelius/Erlenmeyer in the laminar flow hood and agitate it occasionally. Do not exceed 20 min.
8. Take the Berzelius/Erlenmeyer back in the laminar flow hood and pour off the bleach solution into a waste bottle.
9. Add sterile dH₂O. The volume of the water should be 1.5–2 times the volume occupied by the seeds. Agitate the Berzelius/Erlenmeyer well under the laminar flow hood.
10. Discard the water by pouring it into the waste bottle and repeat the washing steps 5–8 times. The purpose of these washes is to get rid completely of the bleach, as this can later impede the germination of the seeds.
11. After the last wash leave a few drops of water onto the seeds to keep them moist until they are used. If they are not used immediately, cover the Berzelius/Erlenmeyer and place it at 4°C (in the fridge or cold room).

3.3.3. Sowing (Plating on Petri Dishes) and Growing

1. Sowing needs to be performed in the laminar hood and sterilized seeds are to be used.
2. Small seeds, like *Arabidopsis* or *Nicotiana* can be resuspended in 200–1,000 µl of sterile 0.1 % agar. The volume of the agar can be adjusted according to the volume of your seeds. To plate, take the seed suspension into a tip (1,000 µl tip) and transfer them individually onto the solid medium in the plates, by gently touching the surface of the medium with the tip.
3. Larger seeds, like from cereals, can be placed directly onto the solid medium using sterile tweezers.
4. Once sowing is done let the plates dry under the hood for about 5 min to evaporate the water placed onto the agar together with the seeds.
5. Seal the plates with micropore tape (3 M) and place them into a growth chamber to allow them to germinate. Micropore tape is a porous material that allows water to evaporate and therefore reduced water condensation is observed in comparison to parafilm.
6. Some seeds require stratification (e.g., *Arabidopsis*). Stratification can be performed by placing the sterile seeds at 4°C for 2–3 days before or after sowing. If stratification is done after sowing, the plates (Petri dishes) are covered with aluminum

foil and placed at 4°C (in the fridge or cold room) and only afterwards moved to the growth room.

7. Plates can be placed either horizontally or vertically. Vertical growth is usually preferred if roots are intended for further investigations. Vertical position prevents roots to enter into the agar. Thus the roots can be easily measured for length (e.g., for root phenotypes) or removed for further investigations. Seedlings intended for hydroponics can also be grown upright as they are easier to be removed from the plate and the root system has place to develop vertically.

4. Notes

Notes presented are with regard to growth conditions used for *Arabidopsis*, barley and rice. With respect to light regimes and temperatures used for plant growth some alternatives are listed here, though they should be considered as consultative. Also, if plants are to be grown only for the extraction/preparation of nucleic acids there is no need to strictly follow or adapt your laboratory/greenhouse protocol to the growth conditions presented in this section.

1. *Light intensity*. Approximate 100–200 $\mu\text{mol}/\text{m}^2 \text{ s}$ from fluorescent bulbs, although lower light intensities have also been used (e.g., 40–60 $\mu\text{mol}/\text{m}^2 \text{ s}$ (12)). Also, higher light intensities of 250 $\mu\text{mol}/\text{m}^2 \text{ s}$ provided by mercury vapor lamps have been reported (13).

Photoperiod. Long days >12 h accelerate the reproductive cycle—plants grown under long day regimes produce a small biomass (few and small leaves) while development of inflorescences occurs early. One example is 16/8 h day/night regime at 23/18°C. Short days <12 h favor vegetative growth—a 10 h/14 h day/night regime provides a good compromise between sufficient shoot biomass production and pre-flowering growth (4).

Temperature. 20–22°C day/17–18°C night. The temperatures required for *Arabidopsis* growth depends on the ecotype. In general, temperatures around 20–22°C during the day and 17–18°C during the night are fine for any of the ecotypes. However, *A. thaliana* Landsberg erecta grows better at day temperature around 23°C, whereas Columbia 0 (Col 0) shows stress symptoms at this temperature (4).

Stratification of seeds. *Arabidopsis* seeds, depending on the ecotype, can germinate without stratification. However, stratification of seeds ensures a more uniform germination and therefore a better comparison of results between different lots of seeds that are used in an experiment. Stratification of seeds is performed at 4°C for 2–4 days depending on the ecotype. Three

days stratification periods would suit any of the *Arabidopsis* ecotypes. Stratification can be performed before sowing by placing the seeds into a vial in water or after sowing by placing the plates (in vitro experiments) or the pots with soil at 4°C. Seeds can be kept at 4°C for up to a week.

Humidity. High humidity is required for *Arabidopsis* seeds to grow, e.g., 65 % (7), 75 % (4), 60/75 % day/night (13).

Soil. In general, any standard peat-based soil/compost would work for *Arabidopsis*. Here are two examples: GS90 (composition: peat, clay, coconut fiber, 2 g/L salt, 160 mg/L N, 190 mg/L P₂O₅, 230 mg/L K₂O, pH 6; supplied by Werner Tantau GmbH & Co. KG, Germany) (14) and Levington F2 seed and modular compost pH 6 (Green-tech, UK). Soil is mixed 1:1 v/v with vermiculate or perlite.

Trays/pots. The size of trays/pots used for *Arabidopsis* growth depends on the number of plants that is needed for the experiment and available space for growing. In general, people use flat cell trays if individual plants are required or large trays if plants are to be pooled. For trays with individual cells or pots a square of around 7 × 7 cm works well. However smaller cells/pots (36 cells trays) also give good results.

Containers for hydroponics. One example would be to use a 5 L container 35 × 35 × 20 cm, holding up to 36 plants, with a diameter of the hole of 1.5–2.0 cm and a distance between the holes of 4–5 cm.

2. *Light intensity.* Barley requires high light intensities and therefore the light quality can significantly influence the performance of the plants. 350–500 mmol/m² s photosynthetically active radiation (5, 15) is adequate.

Photoperiod. 16/8 h light/dark photoperiod is used most of the time for barley.

Temperature regime varies between experiments and may also depend on whether the conditions in the greenhouse or growth chamber can be adapted for barley or whether other plant species are to be grown. However, keeping the temperature in the low range 10–20°C would be beneficial. High temperatures should be avoided. If plants are to be grown for the whole life cycle then ~15°C/10°C day/night temperatures gives good results (15). Other day/night temperature regimes that can be used are: 20/15°C, 20/17°C, 21/18°C, 23/17°C (5) or 24/18°C.

Humidity. Relative air-humidity varies between different published data, but in general is between 60 and 80 %.

Soil type. There are different types of soil that can be used to grow barley, but here are three possibilities that all work well: (1) Standard sphagnum (or sphagnum mixed with polystyrene pellets in a 2:1 ratio), (2) Levington F2 seed and modular compost pH 6 (Green-tech, UK) mixed with perlite in a 3:1 ratio or (3) compost and perlite in a 4:1 ratio (10).

Pots size. A 2 L pot works well. However, smaller pots (15 × 15 × 15 or 13 × 13 × 13 cm square) can also be used, but if there are no space constraints, then larger pots would work better.

Containers for hydroponics. The volume of the containers varies largely between experiments. In general, if the plants are to be grown in hydroponic solution up to maturity and seeds are required 1 L hydroponic solution/ plant is recommended. Alternatively smaller containers can be use such as 5 L containers that can hold up to ten plants. Diameters of the holes should be 2.0 cm and a distance between the holes of at least 5 cm.

3. *Light intensity.* Rice, like barley, requires high light intensities and therefore the light quality can significantly influence the performance of the plants. 300–500 $\mu\text{mol}/\text{m}^2 \text{ s}$ photosynthetically active radiation (16, 17).

Photoperiod. 16/8 h light/dark photoperiod is used most of the time for rice. However 12/12 h day/night regime can also be used.

Temperature regime varies between experiments. However, rice prefers higher temperatures, in general above 20°C. Some examples of day/night temperatures used are: 25/20°C (17) or 27/22°C (16).

Humidity. Relative air-humidity varies between different published data, but in general is between 40 and 80 %.

Soil type. There are different types of soils that can be used to grow rice; in general clay loam mineral soil should be avoided. Levington F2 seed and modular compost pH 6 (Green-tech, UK) mixed with vermiculite or perlite in a 1:1 v/v ratio works well for rice.

Pots size. See barley (Subheading 4.1.2).

Containers for hydroponics. The volume of the containers varies largely between experiments. In general, 0.4–1 L hydroponic solution/ plant is recommended (e.g., 20–24 plants/8–10 L container (16, 17)). Alternatively smaller containers can be use such as 3 L containers that can hold up to 16 plants. Diameter of the hole is 2.0 cm and a distance between the holes of at least 5 cm should be used.

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