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# Hydroponic Systems for Arabidopsis Extended to Crop Plants

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## Abstract

When using Arabidopsis grown hydroponically for gene and drug discovery, a method for translating this approach to crop (and weed) species needs articulation and investigation. In this review, we describe existing inexpensive, frequently aseptic, hydroponic systems for Arabidopsis and compare them to other hydroponic methods for gene and drug discovery in crop plants. Besides gene and drug discovery, an important use of hydroponic analysis is for understanding growth in controlled, enclosed systems, such as during spaceflight and in simulated extra-terrestrial environments. When done initially with Arabidopsis, will these results apply to the growth of other species? We highlight the strengths and weaknesses of existing translational hydroponic approaches whereby results with Arabidopsis extend to other plant species. We find that the existing or slightly modified hydroponic approaches used in Arabidopsis research extend well to crop plants that grow upright about 40 cm in height, e.g., monocots, such as rice, and dicots, such as soybean. However, other, taller species such as maize, or vining species such as tomato, require extensive modification to provide larger enclosures and root stabilization.

**Keywords:** translational research, drug discovery, herbicide discovery, gene discovery, bioregenerative systems, speed breeding, fast generation cycling systems

## 1. Introduction

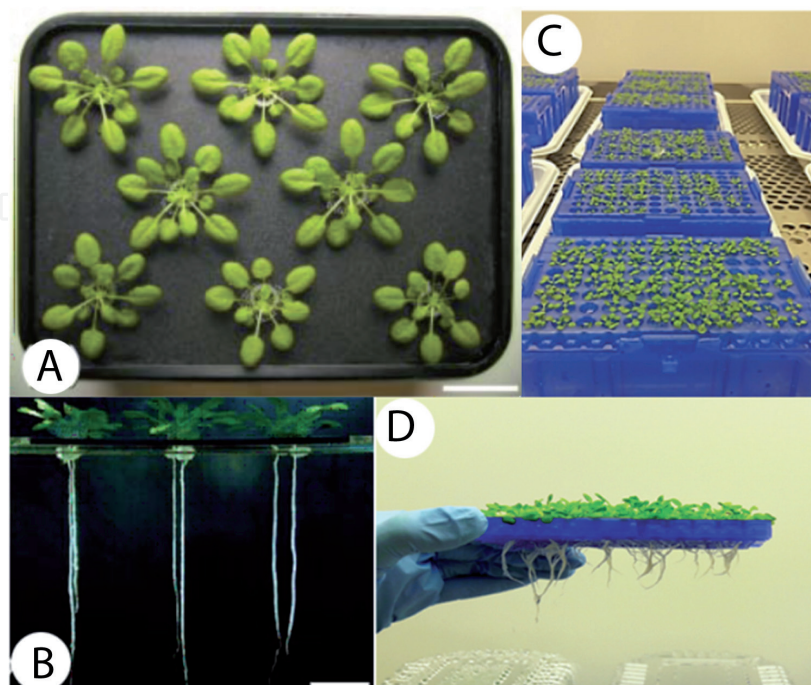
*Arabidopsis thaliana* (hereafter referred to as Arabidopsis) is a model plant system and, unlike most other plants, has a very large number of sequenced chemically induced mutations and libraries of insertional mutations in genes of known and unknown function [1]. This genetic power of Arabidopsis makes it a continuing resource for studying the functions of genes under a variety of conditions. Often, these conditions are best-controlled using hydroponic systems to control nutrients or other abiotic (e.g., drugs, light, solute stress) or biotic (microbes) interactions in the rhizosphere. Furthermore, with tight control of rhizosphere conditions using hydroponics, other experiments on the shoots, leaves or flowers can proceed.

## 2. Hydroponic methods for Arabidopsis

Tocquin et al. [2] briefly review earlier approaches to Arabidopsis hydroponics. More recent studies have developed low cost, efficient systems, which are based on

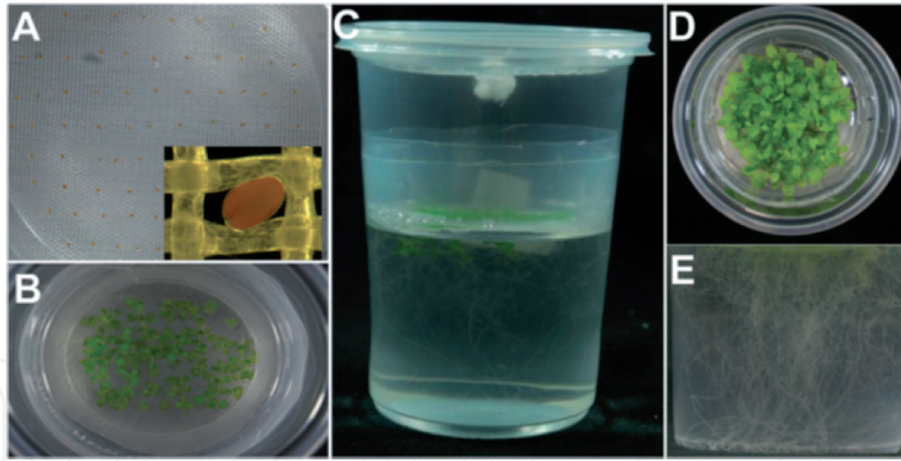
the use of reusable and sometimes sterilizable plastic materials [3–7]. These systems differ in whether they offer (a) aseptic conditions (b) synchronous, rapid growth, (c) growth to maturity and (d) low cost for set-up and maintenance. All of them grow the plants in simple, defined liquid media (usually a ½-strength Murashige and Skoog medium or nitrogen-supplemented ¼-strength Hoaglands solution). They also provide access to the rhizosphere for root phenotyping and drug delivery. As shown in **Figures 1, 2**, the root system is readily available for imaging or for biochemical analysis. Efficient harvest of intact roots is difficult in soil-grown plants, whilst hydroponics provides clean, and potentially aseptic, harvest of roots. However, hydroponics depresses the formation of root hairs and produces developmental changes in other root tissues (reviewed in [8]).

Tocquin et al. [2] and Monte-Bello et al. [7] describe systems where the plants grow in agar-filled end-clipped mini-tubes placed either in the holes of an autoclavable pipette tip holder [7], or in holes drilled into an opaque plastic bin [2], **Figure 1**. The setup by Tocquin et al. [2] is not aseptic and uses polyethylene plastic, which is not autoclavable, but does provide synchronous, rapid growth using optimized nutrients based on the modifications of Hoagland's medium [9]. Although not aseptic, the dark plastic sheet discourages the growth of mold and algae at the surface. The media is not circulating or artificially aerated, known in the popular literature as Kratky-type hydroponics [10]. Groups of a dozen or so plants grow in a single tray and the equipment is scalable to larger plantings (trays) or to larger plants (see below). By lifting the plastic sheet, the roots are easily harvested, **Figure 1**. The cost is low, but the cut and drilled plastic sheets are not available commercially. Unlike the similar system [7], it provides media and space required to grow the plants to maturity. *Arabidopsis* has a shorter generation time in hydroponics than in soil [2], where single-pulse long day lighting induces flowering in 6–7 weeks with hydroponics and 8 weeks in soil [11]. On the other hand, the Monte-Bello setup provides only enough room to grow plants hydroponically to a 4-leaf stage (3–4 weeks), but under aseptic



**Figure 1.**

*Tray-based hydroponics for Arabidopsis. (A) Top view of plants grown by technique of Tocquin et al. [2]. (B) Side-view of root systems grown [2]. (C) Top view of plants grown in pipette holders by the technique of Monte-Bello et al. [7]. (D) Side view of plants grown by Monte-Bello et al. [7]. A&B from Tocquin et al. [2], scale bar = 3 cm. C&D from Monte-Bello et al. [7].*



**Figure 2.** Cup-based hydroponics for Arabidopsis. (A) Seeds sown on mesh, inset = magnified view of Arabidopsis seed on mesh. (B) Top view of 12-day-old seedlings. (C) Side view of 12-day-old seedlings. (D) Top view of 21-day-old seedlings. (E) Side view of 21-day-old seedlings [5].

conditions, **Figure 1**. It is unclear whether, at this stage, it is feasible to transfer plants to other, larger tube systems [4].

Other Arabidopsis hydroponic systems use an insert into a plastic box or cup [3, 5, 6, 12]. All produce synchronous growth to maturity. The plastic cup system [5] is autoclavable when using cups of polypropylene. Covering the plants with an autoclavable lid or, in later stages, a tall cup, maintains sterile culture in early stages of growth. The polypropylene cups are very cheap, because they are available commercially as single-use plastic containers. In this cup system, plants germinate on a screen (plus agar with medium) wedged between a smaller upper cup and larger lower cup, **Figure 2**. Lifting out the screen makes the root system available for analysis and harvest, as is also described in the non-sterile hydroponic culture of Arabidopsis on a supported nylon screen in a beaker [13].

Arteca and Arteca [12] and Nguyen et al. [6] use classic Magenta™ GA-7 boxes as the media chamber and float foam squares containing the plants on the surface of the media. These are modifications of one of the earliest hydroponic culture systems, consisting of a water or nutrient reservoir, an air pump, tube, and a floating platform [14, 15]. Nguyen et al. [6] aseptically pre-germinated the plants on agar and then gently wedged them into foam holders. Robison et al. [3] report another version of this using rock wool and inexpensive food container boxes. More handling of the delicate plants occurs when there is transplantation of plants initially grown in agar. Germination directly on rock wool plugs (with a 0.15% w/v agar) is also possible [16]. Once in the foam or rock wool holders and open to the environment, plant growth is no longer aseptic. One procedure [6] also includes aeration of the media with a bubble stone. Others [12] show that there is no effect of bubble stone aeration on growth.

### 3. Closed and semi-closed systems for Arabidopsis

Some hydroponic systems are open systems that add new media and do not reuse or recycle old media [17]. Providing a continuous supply of defined nutrient or drug-containing solution makes open systems costly and does not take advantage of the ease with which hydroponic nutrients can be recycled or continuously re-used. In several of the above Kratky-type methods for Arabidopsis hydroponics, there is regular, but infrequent (weekly) modification of the nutrient solution and they provide a semi-closed, constant, uncycled medium. Closed and semi-closed systems

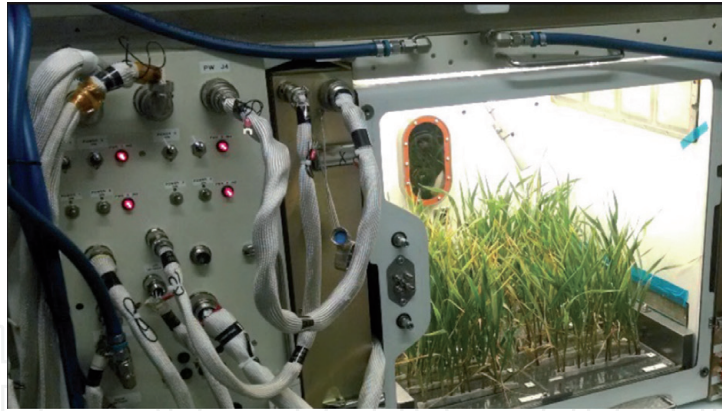
that use flow, such as the nutrient film technique (NFT), deep flow technique (DFT) and aeroponics (misted nutrient solution sprayed on the roots), cycle a larger volume of nutrient medium. However, both cycled and uncycled hydroponics share the problem that the ionic balance or salinity within the nutrient medium can change over time [18, 19]. Computational approaches based on continuous read-out from ion-selective electrodes provide real-time optimization of hydroponic media [20–22]. Such optimization in closed systems require, as part the enclosure, additives or scrubbers (such as ion exchange resins) to add or remove certain ions or nutrients.

Semi-closed and closed, aseptic systems for *Arabidopsis* growth are useful for (1) drug discovery, (2) gene discovery, (3) plant-microbe interactions, and (4) growth in non-terrestrial environments. The advantage of closed or semi-closed hydroponic systems for discovery of drugs effective against plants is that, unlike the situation in animals where target animals are not available for ethical or logistical reasons, it provides a method to evaluate the living target organism from early to late stages of growth [23]. If done on sufficient scale, it can be a form of high throughput *in vivo* screening. Whole organism screening covers important drug or herbicide properties such as uptake, efficacy, and breakdown. However, the frequent use of *Arabidopsis*, and other model organisms, e.g., duckweed (*Lemna* spp.) and the model grass, *Brachypodium distachyon*, as test species for drug discovery, particularly for herbicides, is problematic [24]. Model species may not have the same mechanisms for uptake, delivery, and metabolism of the drugs as agricultural weeds and crops. They have different life cycles and environmental preferences and constraints. Therefore, it is important to extend the work on closed and semi-closed systems using *Arabidopsis* to crop species and weed species, as described below.

This is also true for gene discovery. Identifying and preliminary mapping of multi-genic quantitative trait loci (QTLs) that produce desirable phenotypes is possible with recombinant inbred lines (RILs) and near isogenic lines (NILs) of *Arabidopsis*, if there is minimal environmental contribution to the character. The standard conditions of closed or semi-closed hydroponics seem ideally suited for these studies. When soil is used, it could be a source of irreproducibility. Growth conditions using soils produce irreproducible *Arabidopsis* leaf phenotypes in different labs, even controlling for many environmental variables and nutrient conditions [25]. Similar multi-lab reproducibility experiments with hydroponically grown *Arabidopsis* plants are not available, but provide an exciting opportunity for new study. Exploiting closed or semi-closed hydroponics tested with *Arabidopsis* could be an important step in speed breeding and the analysis of RILs or NILs of fast-cycling crop species [26], as described below.

Sequencing-based analysis of bacterial communities on plants reveals the diversity and complexity of the interaction of plants with the microflora of the rhizosphere [27]. Hydroponic approaches to analysis of *Arabidopsis*-microbe interactions provide a way to monitor how multiple bacterial species colonize the root or interact with each other to form these complex interactions [28]. These approaches require aseptic culture of *Arabidopsis* and controlled introduction of monocultures of bacteria into the media. Harris et al. [28] adapted the simple, inexpensive, closed hydroponic system described above [5] to analyze the colonization of *Arabidopsis* roots with *Pseudomonas*, *Arthrobacter*, *Curtobacterium*, and *Microbacterium* species.

Completely enclosed, but not necessarily hydroponic, systems were common for early studies on the growth of plants in extraterrestrial environments (see review, [29]). The cultivation system of choice is recirculating, enclosed hydroponics, however, for future space flights that would include plants in a life support system [30]. The new recirculating enclosed system by NASA and ORBITEC, however, has Arcelite as a root support matrix with porous tubes to pump the nutrient solution [31].



**Figure 3.** *Advanced plant habitat of NASA showing growing wheat plants in an enclosed chamber with an Arcelite substrate (<https://www.nasa.gov/sites/default/files/atoms/files/advanced-plant-habitat.pdf>).*

The initial tests of the Plant Habitat-01 in the International Space Station will be studies on *Arabidopsis*. Future experiments will include durum wheat, as shown in **Figure 3**. Studies on soil-less bioregenerative life support systems funded by the European Space Agency use the nutrient film technique (NFT) of hydroponics [32], with the caveat that the implementation of such a technique in microgravity is yet to come. All of these life support systems are gas-tight enclosures that will monitor gases emitted by the plants, because the recycling of carbon dioxide and oxygen by plants or other photosynthetic organisms will be a necessity in long-term flights such as those to Mars.

#### **4. Extending closed and semi-closed systems of *Arabidopsis* to crop species**

A semi-closed hydroponic system that is very close to those described above for *Arabidopsis* is the single-tube hydroponics of Kuroda and Ikenaga [33]. As in the procedures for *Arabidopsis* used by Nguyen et al. [6] and Robison et al. [3], the plants initially germinate on an agar (gelrite) medium containing  $\frac{1}{2}$  strength Murashige and Skoog medium. They grow a variety of crop plants, i.e., rice, soybean, Azuki beans, and corn, instead of *Arabidopsis*. At 2 weeks, Kuroda and Ikenaga [33] transplant the intact germinated seedlings into 12 ml polypropylene tubes with two holes cut into the sides to allow the entry and exit of hydroponic medium (1/10 strength Murashige and Skoog medium). A covered outer tray contains the medium and a rack for the tubes in which the plants grow. The tube supports the plant during culture and contains the root ball of each plant, thereby facilitating removal for analysis without damaging the roots. The size of the culture tube is larger than that used by Tocquin et al. [2] for *Arabidopsis* because the seeds and new roots of crop plants are much larger, but the tray system for growth in hydroponics is very similar. For soybean, an additional prop supports the plant during growth. Rice and soybean plants grown in single tube hydroponics produce high viability seed with seed weights equal to or exceeding plants grown in the field. Single tube hydroponics facilitate analyzing and screening the T1 seeds from the transgenic plants with shorter generation times and small amounts of seeds.

The hydroponic system of Conn et al. [4] directly translates an *Arabidopsis* hydroponic culture system to crop plants. They use a system of hole-punched plastic trays to start the plant, followed by transplantation of the seedlings into a plastic tube that can be set in a larger tray with aeration. The 50 ml tube in this case, although it did not confine the root as much those used by Kuroda and Ikenaga [33],

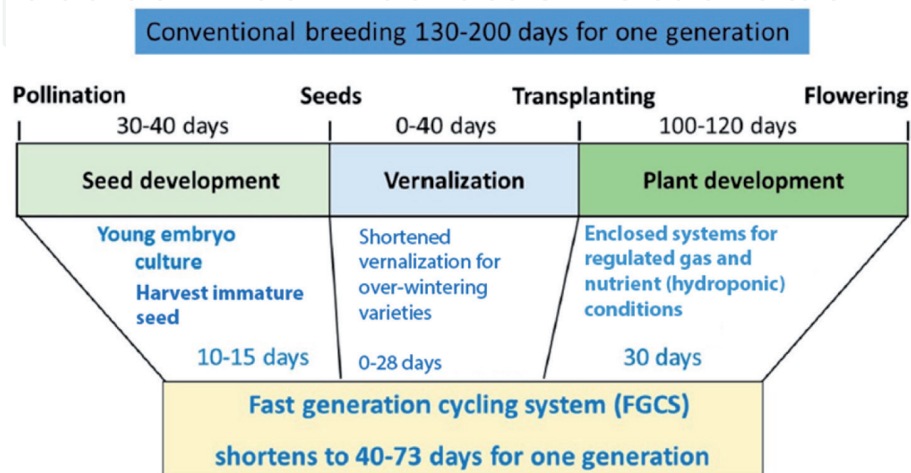
kept the roots of separate plants free from tangling, thereby facilitating measurement and analysis. Although *Arabidopsis* was the main test plant for this system, wheat, cucumber, and tobacco also successfully grew.

Although both techniques [4, 33] require transplantation of newly germinated plants, which has the downside of more manipulation, transplantation may be desirable for crop plants with low germination rates and for studies on post-emergent drug treatments. It has the additional advantage of protecting the plant from water molds and other contaminants because the initial germination is aseptic.

It becomes apparent in studies that translate work on hydroponically grown *Arabidopsis* to crop plants that just the difference in physical size of the seeds and plants dictates some of the modifications. In contrast to *Arabidopsis*, experiments involving larger plants require root stabilization. When larger crops, such as *Zea mays*, grow in hydroponic conditions, lack of support for the root system can result in breakage and damage of the lateral root system [34]. When growing wheat varieties to test the effects of salinity, Munns and James [35] used quartz rock as a stabilizing substrate in a hydroponic flow system. More complex, but definable, substrates may be necessary because they interact with nutrients and help determine their availability, e.g., a defined clay substrate for corn [36, 37].

The generation time for soil-grown *Arabidopsis* decreases by 1 to 2 weeks when grown hydroponically on defined medium [2, 11]. Accelerated breeding programs for crops facilitate the production of RILs and NILs for gene discovery. In fast generation cycling systems, **Figure 4** [26], plants with long generation times, such as crop plants, are sped up using a variety of technologies. Speed breeding can produce generation times that are a third to a half the time [38, 39]. One of the technologies used in speed breeding is *in vitro* growth. With a neutral rhizosphere support medium, such as agar for *Arabidopsis* [40], speed breeding for *Arabidopsis* translates to speed breeding in wheat [41]. Besides achieving fast growth of the seedling, an important feature of many fast generation cycling systems is overcoming seed dormancy with early stage embryo culture *in vitro*, **Figure 4** [26, 42] or harvesting immature seed and drying it [38, 39]. With embryo culture, there will always be aseptic transplantation of agar-grown embryos, but for immature seeds, transplantation is not necessary.

Complete enclosure of the growing crop is one of the features of recent speed breeding technologies [38, 39, 43]. Soybeans grown hydroponically using NFT in the completely enclosed bioregenerative life support systems have a 110–133 day



**Figure 4.** Schedules for speed breeding in fast generation cycling systems showing the timesaving steps to reduce generation times (adapted from [26]).

generation time [32]. Soil grown soybeans have a generation time of 132 days, but growing them in an enclosed system with elevated carbon dioxide decreases the generation time to 70 days [43]. Combining hydroponics of crop species with other technologies of speed breeding might produce even shorter generation times or higher yields and seed viability.

Complete enclosure of the growing crop is also a feature of crops grown for space exploration. Because soybean is one of the species best suited for growth in space [44], its nitrogen fixing symbiosis with bacteria is of interest. As described above, hydroponic submersion systems inhibit root hair development, upon which the initial stages of nodule formation depend. Hence, it is not a surprise to see that hydroponic inoculation of soybean with its nodulation partner, *Bradyrhizobium japonicum*, does not improve nitrogen use efficiency [44]. However, other kinds of plant growth promoting microbes (PGPMs), including some of those tested in hydroponic systems with *Arabidopsis* [28], produce higher photosystem II efficiency in hydroponically-grown soybean plants [45]. This could be beneficial in speed breeding, which improves with improved photosynthesis achieved with elevated carbon dioxide [43]. Those plants grown to maturity in enclosed hydroponics and inoculated with PGPMs show stabilized microbial communities over time [46].

## **5. Conclusions: translational research on hydroponics from Arabidopsis to crops**

As described in Woodward and Bartel [1], research on *Arabidopsis* can sometimes directly translate into discoveries in crops. One example that they use is the expression of MYB12 in tomatoes, which derived from initial discoveries in *Arabidopsis* revealing increased production of flavonoids upon overexpression. The overexpression of MYB12 in tomatoes produces so much flavonoids, the color of the fruit changes from red to orange [47]. However, this small mustard family plant has a growth habit and life cycle so different from most crop plants, can lessons learned from hydroponic studies on *Arabidopsis* be translated to crops? The answer is: mostly. Most of the technical approaches used with *Arabidopsis* translate to crop plants with minor modification, except for those crops that are very large and need extra support for growth. The benefits of using *Arabidopsis* for investigating the different techniques of hydroponics are those that make it valued as a model organism, i.e., its size, well-characterized genome, and short generation time. In fact, given the depth of knowledge on gene function in *Arabidopsis*, current research on *Arabidopsis* hydroponics could apply more widely to studies on fast breeding crop plants for gene discovery, on target plants for herbicide and drug discovery, and on plants used for bioregenerative life support systems in space.

Adoption of some the techniques used in *Arabidopsis* hydroponics could decrease the cost and size (important for space studies) of enclosed test systems without changing the viability and yield of the crop plants grown in those systems. For example, the effects of space travel are varied and complicated. However, most of the work done to date has focused on the microgravity component of space flight without the proper control of having a 1-g set of plants growing in the same space vessel [29]. Because *Arabidopsis* is small and well characterized, the initial tests for the design and implementation of these proper controls may be more feasible (and the data achieved more insightful) for *Arabidopsis* than for the crops identified as “the best” space plants, i.e., durum and bread wheat, soybean, and potato. Once done with *Arabidopsis*, the work would translate to these other species.



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