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A Simple and Versatile 2-Dimensional Platform to Study Plant Germination and Growth under Controlled Humidity

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Abstract

We describe a simple, inexpensive, but remarkably versatile and controlled growth environment for the observation of plant germination and seedling root growth on a flat, horizontal surface over periods of weeks. The setup provides to each plant a controlled humidity (between 56% and 91% RH), and contact with both nutrients and atmosphere. The flat and horizontal geometry of the surface supporting the roots eliminates the gravitropic bias on their development and facilitates the imaging of the entire root system. Experiments can be set up under sterile conditions and then transferred to a non-sterile environment. The system can be assembled in 1-2 minutes, costs approximately 8.78$ per plant, is almost entirely reusable (0.438$ per experiment in disposables), and is easily scalable to a variety of plants. We demonstrate the performance of the system by germinating, growing, and imaging Wheat (Triticum aestivum), Corn (Zea mays), and Wisconsin Fast Plants (Brassica rapa). Germination rates were close to those expected for optimal conditions.

Introduction

Approximately 97% of the calories consumed by humans originate from plants [1]. Recent estimates indicate that the food supply will have to increase by approximately 70% by 2050 to match demand [1]. However, even optimistic estimates predict only a 50% increase in crop yield by 2050 [2].

Improving our understanding of seed germination and root growth could be necessary to ensure our food security in the future, since the germination, emergence, and early establishment of seedlings have a large effect on agricultural yields, especially if below a critical level [3]. Low germination rates reduce crop density, which results in indirect yield loss. Late emergence can result in poor plant performance and a direct yield loss [4], because roots are inadequately established and have less access to water and nutrients during later stages of vegetative and reproductive growth.

Tests of seed viability and vigor typically employ paper to act as a support and to supply moisture: seeds are placed over moist germination paper (and often covered with a second sheet) and incubated. A germination table (also known as a Copenhagen table or Jakobson apparatus) can germinate several seeds simultaneously under one set of conditions [5,6]: filter paper wicks moisture from a temperature-controlled water tank and provides a flat, horizontal surface on which germination can be observed. However, germination tables are expensive, not universally available, and do not provide control of conditions to individual replicates. Furthermore, they are not ideally compatible with – and never used for – the study of plant root growth. Most plants grown for research purposes are transplanted at least once after germination.

Roots are responsible for the vast majority of the water and nutrient supply to the plant [7], they establish synergetic interactions with soil biota [8,9], and they anchor the plant to the soil [10]. By these functions, the roots influence the growth of the plant and its resilience against environmental stresses such as drought. Root architecture (i.e. its size and structure) plays a fundamental role in plant productivity and crop yield [11]. Nonetheless, roots and their development are one of the most complex and relatively unexplored aspects of the food supply problem [12].

Seedlings are grown in granular media (e.g., soil, sand, perlite, vermiculite) or homogeneous media, such as water (hydroponics), air (aeroponics), or gels (e.g., agar, gelatine, gellan gum). Gels provide a 3D growth environment for the roots, but they otherwise poorly represent the mechanical and structural properties of soils [13], and may expose plants to anoxic conditions [14]. Analysis of the size and structure of a 3D root system requires relatively sophisticated equipment and cumbersome image analysis [15]. Granular media (e.g., soil, sand, vermiculite) is structurally closer to soil, but is opaque to most forms of radiation. The imaging of root systems in those environments requires expensive equipment (X-ray computed tomography or magnetic resonance imaging [16,17]) that is not widely available, currently has low throughput (individual scans can take hours), and cannot routinely or...
accurately distinguish live roots from dead organic matter [18]. Roots can be imaged growing against a transparent surface in soil-filled 2D root-boxes called rhizotrons [19]. However, even when tilted at a 45° angle to encourage the roots to grow against the transparent surface, less than half of the total root length is visible [20], root density is overestimated [21], root development is affected by gravitropism, and the soil/glass interface is unlikely to be representative of real soil structure.

The study of both germination and root development in the same environment is experimentally and logistically difficult, because of the lack of convenient and yet highly controlled and capable environments in which to study these processes [22]. Conditions for experimental plant germination and growth must be precise and uniform, because plants are highly sensitive to environmental conditions, and exhibit phenotypic plasticity to a vast array of abiotic stimuli [23]. Some of these conditions (e.g. temperature, light, humidity and CO₂ concentration) can be controlled using growth chambers [24]. Growth chambers are not ideal environments to study the effect of humidity on plant development because (i) they cannot control humidity of individual replicates, (ii) they expose the plant to the atmosphere and potential contamination, and (iii) they are expensive and not universally available. Therefore, laboratory studies of plant germination and growth under controlled humidity conditions typically require a large upfront investment. These barriers are bound to inhibit or prohibit investigators from other disciplines or developing nations from entering into this area of science.

We describe in this paper an experimental setup for the study of germination and root development of a variety of plants (as shown here, *Brassica rapa*; Wisconsin Fast Plants; *Astroplants*, *daffil* [25], *Triticum aestivum*; Wheat, and *Zea mays*; Corn). The platform displays the following capabilities and characteristics: (i) It constantly exposes the plant to a nutrient solution and to a controlled humidity (ranging between ~56% and ~91% in each setup), (ii) It can be used on any laboratory bench, as long as uniform illumination and temperature are provided. (iii) It is composed of reusable or inexpensive parts. (iv) It is scalable to virtually any plant size. (v) It allows imaging of the shoot and root. (vi) It eliminates the gravitational bias on root development by growing the roots on an horizontal and flat 2D surface, which facilitates the imaging and analysis of the entire root system architecture.

### System Design

The assembly of the platform is shown in Figure 1a. It consists of 8 steps that can be completed in approximately 1 to 2 minutes (see Supporting Information S1 for a detailed description and Movie S1 and Movie S2 for a video demonstration) and result in the self-contained plant growth environment shown in Figure 1b.

The design of the platform was constrained by a stringent set of conditions. **Delivery of nutrients and moisture to the seed / plant.** In our setup, the seed (*B. rapa*, *T. aestivum*, or *Z. mays*) is supported on a flat sheet (the “growth sheet”) of filter paper (Whatman #1). The growth sheet lies on top of a larger sheet (the “pump sheet”) of filter paper (Whatman #1) that wicks nutrient solution from an underlying reservoir. The pump sheet imbibes the growth sheet with the nutrient solution. Coating the newly sown seed with a hydrogel droplet (50 μl of gellan gum) improves germination rates: the hydrogel draws water from the filter paper and ensures the seed is moist without eliminating the access to oxygen. **Compatibility with both germination and growth.** The setup is easily scalable. Figure 1b-d show that three plants with different seed size can be germinated in our platform. The overall scale of the experiment can be controlled to match the size of the plant after the intended growth period (see Supporting Information S1). Plant roots anchor to the filter paper. As shown in Figure 1e, plants grown for 2-3 weeks can be turned sideways without toppling over. **Control of humidity.** Supersaturated salt solutions in a closed environment establish an atmosphere of known relative humidity [26]. Different salts controlled the relative humidity of the air 5 cm above the growth sheet between ~56% and ~91% at 25°C (Figure 2a). The external container (containing the salt solution) is never in contact with the nutrient solution, so neither the salt nor the container can contaminate the paper on which the plant is grown. **Exclude the influence of gravity on the direction of plant root growth.** Gravity affects root growth by creating a gradient of auxin across the root tip. Auxin is a hormone that inhibits the expansion of root cells. A gradient of auxin across the root cross-section causes the root to bend due to differential expansion of the tissue [27,28]. The

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**Figure 1. Schematics and picture of plant germination platform.**

(a) Scheme of the assembly of the plant germination and growth platform. Pictures of the assembled setup growing (b) *Brassica rapa*; Wisconsin Fast Plants; *Astroplants*, *daffil*, (c) *Triticum aestivum* (Wheat), and (d) *Zea mays* (Corn). (e) Picture of a Corn seedling held at 90 degrees on paper, demonstrating the anchoring of the roots to the filter paper support.

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The plants, and prevent the establishment of thermal gradients. The purpose of the chambers was to provide uniform illumination of plants growing requirement to consider the cost of science from the beginning [34]. Our setups cost 8.78 $ per plant, of which only 0.43 $ is for disposable items. The setup does not require any equipment unless sterilization is required (in which case a class II biosafety cabinet is sufficient). High throughput. High throughput plant experiments are typically conducted on gel in Petri dishes that (i) are capable of processing thousands of individual seeds/plants per week or month [35,36,37], (ii) can be setup in less than 1 minute, and (iii) can be stacked so that up to 5000 per m² [38] can be fit in growth chambers. Our experimental units can be assembled in ~1 to 2 minutes (see Movie S2 for a demonstration) and each of the parts can be prepared (i.e., autoclaved, cut, dissolved in batches (we can assemble from scratch approximately 100 setups per person, per day). Each setup has a footprint of 60 cm², or ~167 units per m². Although our setup does not have the throughput of Petri dishes, it provides control over humidity and is compatible with much larger plant sizes. Sterile. Plant science research requires the growth and development of plants under sterile conditions [38]. All components are easily sterilized (the LEGO bricks, plastic cup, glass slide, MAGENTA box, gelatin gum, salt and nutrient solutions are autoclaved, while the paper and plant seeds are soaked with 70% ethanol). After our setup is assembled in a sterile environment and sealed within the MAGENTA box, it can be transferred to a non-sterile environment without contamination. Suitable for any laboratory bench. The advancement of life science in the 21st century will require contributions from other disciplines [39] and developing world laboratories [40]. Facilitating these collaborations will require methods compatible with any laboratory bench in the world, regardless of discipline or resources. Our experiments were performed in handmade chambers (see Supporting Information S1) constructed from a wooden frame and aluminum foil. The purpose of the chambers was to provide uniform illumination of the plants, and prevent the establishment of thermal gradients.
ments were performed at 20°C: the measurements were made on a laboratory bench where the temperature was not stabilized (we estimate the error on the temperature to be ±2°C). The RH can be controlled between 90.6 ± 0.9% (with KSO₄, error is three standard errors, n = 3) and 56±5% (with LiCl, error is three standard errors, n = 3). The difference between these measured RH values and those expected from the respective saturated solutions – a super saturated LiCl solution in water should establish a RH of 12% – probably results from the fact that the atmosphere within the enclosed setup is exposed to both the saturated salt solution and the nutrient solution. Thereby, while the saturated salt solution is absorbing water from the atmosphere, reducing the RH, the nutrient solution is evaporating, increasing the RH. The steady state results in the observed RH. Of course, the above explanation implies that the observed RH will not only depend on the salt solution chosen to reduce RH, but also on the ratios between the areas of the exposed surfaces of the saturated salt solution and the nutrient solution in the setup. Broader ranges of RH should be accessible by changing the ratios of the exposed surfaces. The evaporation of the nutrient solution and the absorption of water by the saturated salt solution should increase the concentration of the nutrient solution over time. Our measurements indicate that the change is not detectable over the course of 15 days, at least when using NaCl as the saturated salt solution (see Supporting Information S1).

Figure 2b shows the germination rates for B. rapa, T. aestivum, and Z. mays, in our platform, compared to the germination rates reported by our seed source. The rates we obtained are remarkably close to the expected ones, especially considering that minimal effort was put into optimizing standard seed handling protocols for our platform (see Supporting Information S1 for details).

The ability to visualize whole root systems will be increasingly important for understanding the responses of roots to stimuli, and breeding plants with desirable traits. Figure 3 demonstrates the use of our setup for the quantitative analysis of the whole root system of a T. aestivum seedling. The root system was photographed from above after the shoot is removed (Figure 3a). We increased the contrast of the image (details in Supporting Information S1) and removed the seed from consideration by superposing a white colored circle over it (Figure 3b). The resulting image was then analyzed with standard root-analysis software (in our case WinRhizo) yielding phenotypic data for the whole root system (Figure 3c).

Conclusions

We addressed in this communication the challenge of providing a simple, inexpensive, and yet reproducible and capable apparatus for the observation of germination and seedling growth in sterile environments with controlled humidity.

The system we designed combines tools that are commonly used by plant scientists (e.g., filter paper, MAGENTA boxes) and others that are not (e.g., LEGO bricks) to fulfill a number of strict design requirements which include low cost, simplicity, structural precision, control over humidity, scalability to any plant size, and high throughput. Specifically, we demonstrated that the setup, as it is designed, (i) can grow plants for weeks, despite its planar geometry (the plants do not topple over but balance and anchor themselves with their roots), (ii) provides a constant supply of water, to the seed and root system, (iii) maintains a constant relative humidity between 91% and 56%, (iv) is capable of growing plants with desirable traits. Figure 3 demonstrates the use of our platform (see Supporting Information S1) to buy and 1-

Figure 3. Example of root image analysis performed on roots grown on our experimental setup. a) Top-view photograph of a Wheat (Triticum aestivum) seedling grown for 7 days after the shoot has been removed. b) Modified version of the photograph in panel a) after the seed has been digitally removed, the color has been made black and white, the contrast has been maximised. c) Table of selected root parameters obtained by the analysis of the image in panel b) by WinRhizo. doi:10.1371/journal.pone.0096730.g003

Supporting Information

Supporting Information S1 Figures S1-S16. Figure S1. Construction of LEGO support. Figure S2. MAGENTA box containing LEGO support and glass slide. Figure S3. Preparation of nutrient solution and saturated sodium chloride solution. Figure S4. Preparation of cylinders and tweezers for sterilization. Figure S5. Form and dimensions of pump sheet (Whatman no. 1 filter paper). Figure S6. Form, dimensions and wax pattern of growth sheet (Whatman no. 1 filter paper). Figure S7. All consumables needed for the experiment. Figure S8. MAGENTA box.
containing 50 ml of super saturated sodium chloride, solution (liquid + powder NaCl). Figure S9. Mini cup (reservoir) box containing 25 ml of nutrient solution (x0.5 MS). Figure S10. Steps of MAGENTA box assembly with nutrient reservoir and pump sheet. Figure S11. Addition of the growth sheet paper. Figure S12. Gel and seed added to experimental setup, fully assembled setup. Figure S13. Growth chamber containing 18 setups. Figure S14. Photographs of a corn plant growing on a scaled-up version of the setup described in the manuscript. Larger sizes are possible. Figure S15. Measure of relative humidity (%), control without pump and growth paper and the experiment with pump and growth paper. Figure S16. The concentration of nitrates and ammonium ions measured in the pump sheet does not significantly change over time.

(PDF)

References


Movie S1 Assembly of LEGO support setup. (M4V)

Movie S2 Assembly of plant growth setup. (M4V)

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Author Contributions

Conceived and designed the experiments: LC TS. Performed the experiments: KKL TS SB HV. Analyzed the data: LC TS KKL SB. Wrote the paper: LC TS.

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