A simple and versatile 2-dimensional platform to study plant germination and growth under controlled humidity

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Published Version

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Published version at: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0096730
To link to this article DOI: http://dx.doi.org/10.1371/journal.pone.0096730

Publisher: Public Library of Science

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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 synergic 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
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, dwf1* [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 1c, 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
Results and Discussion

The performance of the germination and growth environment was assessed by (i) its control over relative humidity, and (ii) its ability to yield high germination rates. Figure 2a shows the relative humidity (RH) measured 5 cm above the surface of the growth sheet (the approximate height of the cotyledons of a *B. rapa* plant after the hypocotyl straightens), as a function of the super saturated salt water solution held in the external container. All measure-

Plants were grown in our growth chambers underneath an array of 225 white LEDs so that the plants would receive ~9000 lumens. Capable of supporting increasing levels of complexity. The support of the seed is filter paper. This choice was influenced by the recent reports of ‘lab-on-paper’ technologies that have been developed to provide fluid manipulation [41], chemical reactions [42], and environments for microorganisms and cell cultures [43] in paper substrates. The combination of the platform presented here with the tools of paper microfluidics is beyond the scope of this communication, and will be the focus of future publications.

**Figure 2. Performance of the germination and growth platform.** A) Relative humidity in the setup as a function of the salt used to form the supersaturated solution in the reservoir. Error bars are ± standard errors, n = 3. B) Plot of the maximum germination rates obtained for Fast Plants, Wheat and Corn in our platform, compared to optimal germination rates reported by our seed source. doi:10.1371/journal.pone.0096730.g002

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±8% (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 absorption of water by the saturated salt solution should increase the 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).

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References


Author Contributions

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

Acknowledgments

We thank Anthony Miller for designing and building the growth chambers depicted in Figure S13. We also thank Dr. Kuloth V. Shajesh for valuable discussions and William Rekemeyer for help in the laboratory.

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