

Tracking dynamic changes of leaves in response to nutrient availability using an open-source cloud-based phenotyping system (*OPEN Leaf*)

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ABSTRACT (250 words)

Micronutrients, such as iron, zinc, and sulfur, play a vital role in both plant and human development. Understanding how plants sense and allocate nutrients within their tissues may offer different venues to develop plants with high nutritional value. Despite decades of intensive research, more than 40% of genes in *Arabidopsis* remain uncharacterized or have no assigned function. While several resources such as mutant populations or diversity panels offer the possibility to identify genes critical for plant nutrition, the ability to consistently track and assess plant growth in an automated, unbiased way is still a major limitation. High-throughput phenotyping (HTP) is the new standard in plant biology but few HTP systems are open source and user friendly. Therefore, we developed *OPEN Leaf*, an open source HTP for hydroponic experiments. *OPEN Leaf* is capable of tracking changes in both size and color of the whole plant and specific regions of the rosette. We have also integrated communication platforms (Slack) and cloud services (CyVerse) to facilitate user communication, collaboration, data storage, and analysis in real time. As a proof-of-concept, we report the ability of *OPEN Leaf* to track changes in size and color when plants are growing hydroponically with different levels of nutrients. We expect that the availability of open source HTP platforms, together with standardized experimental conditions agreed by the scientific community, will advance the identification of genes and networks mediating nutrient uptake and allocation in plants.

Keywords: High-throughput phenotyping, Cloud-based, CyVerse, Plant Nutrition

1. INTRODUCTION

Predictive biology, or predicting biological outcomes from a known input, is central to understanding the genome-to-phenome relationship¹. However, the phenome is a complex combination of the result of environmental conditions and the ability of the organism to adapt to environmental changes^{2,3}. In the case of plants, predictive biology requires a deep understanding of its genetics and its response to environmental cues, such as light intensity, water availability, and other organisms^{4,5}. Therefore, the prediction of plant responses to changes in environmental conditions has lagged compared to other advances in the understanding of other plant behaviors. The consistent collection of reproducible

data at the “-omic” and environmental level are vital towards addressing this issue.

A drastic decline in cost has allowed for large amounts of genomic and environmental data to be collected, yet major bottlenecks still stand in integrating, sharing, and analyzing these datasets. For example, methods characterizing genomes, such as DNA sequencing, have advanced far more quickly than methods for phenomes^{6,7}. Plant phenotyping at scale is costly and inaccessible to the majority of plant researchers^{8,9,10}. However, many components for high-throughput phenotyping, such as sensors and computer vision, have become cheaper and more accessible for development of phenotyping platforms. These platforms can collect information on roots^{11,12} and shoots^{13,14} in greenhouses^{15,16} to whole fields^{17,18}. However data management and cost still stand as major limitations in reproducing abiotic stresses from water and nutrient deficiency^{7,10,19,20}.

In response, we propose OPEN Leaf [Open PhENotyper]. OPEN Leaf is designed to be an open-source plant phenotyping system that tracks rosette growth in Arabidopsis by color and area. This system was built using commercially available materials and uses a high resolution RGB camera and a track system with user-defined positions to capture dynamic changes in Arabidopsis rosette growth over time. Furthermore, complications due to the COVID-19 pandemic, spurred the integration of remote communication to observe data collection in real time with OPEN Leaf. Overall, OPEN Leaf is a modular, scalable, and cloud-based system that will enable researchers across the globe to share and process reproducible experiments in predictive biology.

1. MATERIALS AND METHODS

2.1 OPEN Leaf

OPEN Leaf’s hardware is inexpensive and can be acquired from several commercial sources. The frame was built using the open-source T-slot aluminum beams (80/20 LLC., Columbia City, IN, USA) and can be bought pre-cut or bought in bulk then cut down to size. The lead-screw track system is a commercial product called C-Beam™ Linear Actuator (openbuilds.com). The system includes the C-beam track, the gantry plate, and a NEMA23 stepper motor. The bundle comes with detailed instructions from OpenBuilds and designed for extended heavy-duty use. A bracket used to convert from the 80/20 beams to the OpenBuild’s track was 3D printed on a Prusa i3 MK3 (Prusa Research, Partyzánská, Czech Republic). The camera used was the Allied Vision Mako G-503 (Allied Vision, Stadtroda, Germany), a gigabit ethernet RGB camera that can capture high-resolution images.

An open-source C# desktop application is used to control the machine. The program has the ability to control several systems in parallel; however, since each system requires an ethernet port, this can be a bottleneck. The track system uses the open-source GRBL CNC mill g-code parser to drive the lead

screw system. The desktop application sends manual g-code through an Arduino (arduino.cc), a hobbyist microcontroller, to travel to capture images.

2.2 CyVerse and Slack Integration

CyVerse (CyVerse.org) is an NSF-funded cloud-based system designed to revolutionize science with data-driven discovery for Life Sciences research with training for scientists in mind. We provide the choice of integrating the image processing with CyVerse or allowing researchers to use another method. CyVerse offers many benefits for cloud storage, including data management and sharing. A desktop application named CyberDuck uses a command-line interface for communicating with CyVerse. A python script is set with user credentials to send command-line commands to automate the process of synchronization data in the remote directories of CyVerse.

Slack is a prominent communications platform that provides tool for connecting workspaces across the globe. We use a “bot” account to instantly communicate with the OPEN Leaf system. We can remotely determine if the system is functioning and see the most recently collected series of images.

2.3 Image Analysis

The image analysis is hosted on CyVerse to allow for automated processing. All images captured on the OPEN Leaf system are consistent. Therefore, the image processing script requires no human inputs, eliminating the possibility for human bias. The image processing script produces an excel sheet of results that include traits of whole rosette and leaves such as: area, major axis, minor axis, and color distribution clusters. The area is measured in pixels. Major and minor axis are the vertical and horizontal height of the plant in pixels. The four most dominant hex value colors extracted from the plant are provided as a percentage out of 100%.

1. RESULTS AND DISCUSSION

3.1 Overview

A multi-faceted approach for acquiring and processing data in a low-cost and accessible manner is paramount towards predictive biology. Understanding the phenome to genome relationship requires discovering new genes through uncharacterized phenotypes (Figure 1a). Using several open source and commercial products, such as Allied Vision cameras and the hobbyist microcontroller Arduino, OPEN Leaf characterizes phenotypes at a macro and micro scale by quantifying growth by area and color of the whole plant and specific leaves. OPEN Leaf was designed to be affordable, accessible, and open source from the beginning. Images can be synced to the cloud service *CyVerse* to perform image processing. The communication platform *Slack* has integration for remote communication with the machine (Figure 1b).

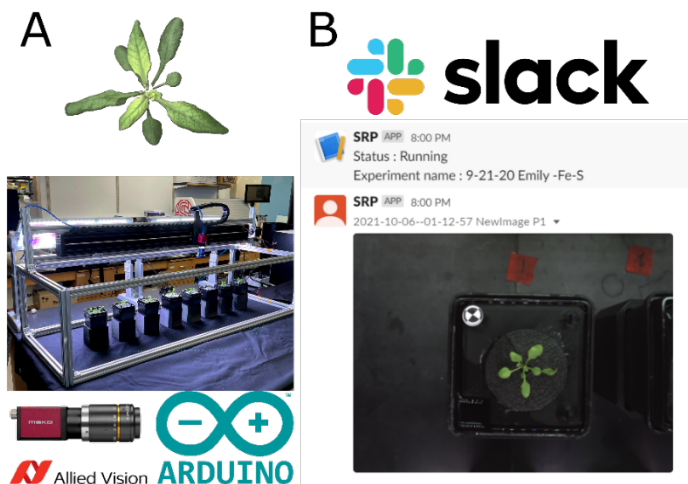


Figure 1. (a) Uncharacterized phenotypes can be characterized with OPEN Leaf through a high-resolution camera. (b) Slack integration allows for remote work with the system

3.2 Whole leaf analysis

OPEN Leaf captures whole images of plant to track the overall development of the plant dynamically through time. The two most important characteristics to observe in plant development, specifically for nutrient uptake, is the change in area and color¹⁴ (Figure 2). These approaches provide many valuable insights into plant development and the characterization of phenotypes. However, dynamic detail that occur on leaf level are often overlooked by only observing plant as a whole. For example, iron deficiency displays itself primary in younger leaves, and therefore, when observing the whole plant, area changes very little since older leaves are consistent in growth and color is minimally impacted²¹. Ergo, it is important to observe growth of leaves independently of the entire plant.

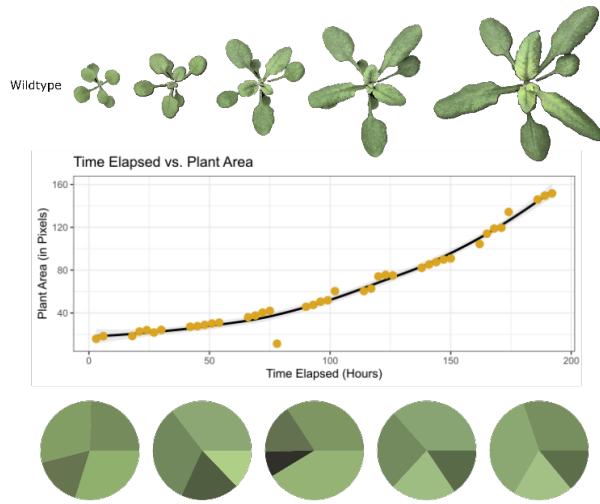


Figure 2. Whole plant analysis shows important macro trends of growth across the whole plant by area and color

3.3 Leaf specific analysis

Leaf development varies wildly throughout plant development depending on set of true leaves the leaf is (Figure 3). OPEN Leaf allows for the manual tracking of these leaves to observe the dynamic changes in leaves under nutrient stress across all age ranges. Moreover, color changes, such as the emergence of chlorosis, are much more pronounced at a leaf level. This resolution allows for a deep understanding of the complex relationship of mobile nutrients and growth.

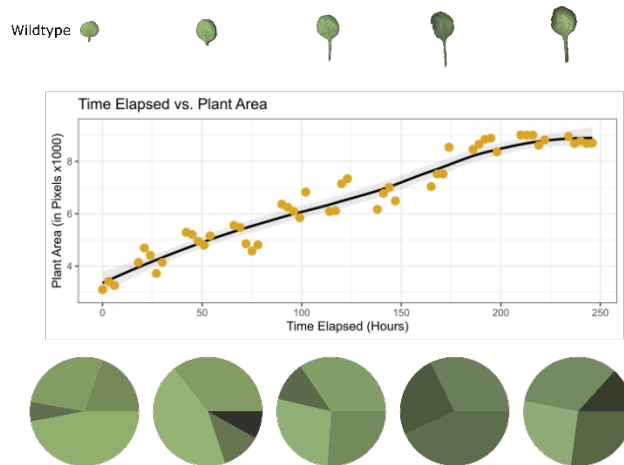


Figure 3. Leaf specific analysis provide new insights to nutrient

deficiency phenotypes by observing the changes of area and color one leaf at a time

1. CONCLUSION

OPEN Leaf addresses many of the current limitations and inconveniences of HTP systems in predictive biology. OPEN Leaf is open-source and accessible, characterizes abiotic stress responses within hydroponics, integrated data management through *CyVerse*, and automated image processing. Many high-resolution HTP systems are commercial and outside of budgets for most labs. OPEN Leaf is \$1000 and entirely open source. OPEN Leaf characterizes abiotic stress responses at a whole plant and leaf-specific to aid in understanding the genetically complex systems involved in stress response. Many other open-source HTP systems have no method for data management and processing. OPEN Leaf utilizes *CyVerse* to both manage and process data; allowing labs across the globe to participate in collaborative research. Future projects in the OPEN series includes increasing throughput of OPEN Leaf and a root phenotyper with similar OPEN principles.

DATA AVAILABILITY STATEMENT

Image data available at CyVerse link provided at the following link:

https://de.cyverse.org/data/ds/iplant/home/dgmendoz/DMC_phenotyping/DMC_photos/SRP/18Jan2021_

OPEN Leaf Controlling Software available at a Github repository: <https://github.com/LandonSwartz/Cyberbe> Row.

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REVIEWS

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