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Lights, camera, action: high-throughput plant phenotyping is ready for a close-up

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Anticipated population growth, shifting demographics, and environmental variability over the next century are expected to threaten global food security. In the face of these challenges, crop yield for food and fuel must be maintained and improved using fewer input resources. In recent years, genetic tools for profiling crop germplasm has benefited from rapid advances in DNA sequencing, and now similar advances are needed to improve the throughput of plant phenotyping. We highlight recent developments in high-throughput plant phenotyping using robotic-assisted imaging platforms and computer vision-assisted analysis tools.

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Introduction

Modern techniques for crop improvement rely on both DNA sequencing and accurate quantification of plant traits to identify genes and germplasm of interest. With rapid advances in DNA sequencing technologies, plant phenotyping is now a bottleneck in advancing crop yields [1,2]. Furthermore, the environmental plasticity of plants creates an interesting interdisciplinary modeling challenge for plant scientists, computer scientists, and statisticians, and resulting models have the potential to guide adjustments in agricultural practices to optimize yield prior to genetic improvements. With increasing options in image capture and open-source analysis tools, the field of high-throughput plant phenomics is poised to enter a phase of exponential growth. The last few years have seen focus shift from platform development to biology, but have also revealed the necessary improvements to hardware, software, and community resources required to advance the field. This review highlights recent developments in high-throughput image-based plant phenotyping of above-ground plant tissues, and discusses the next steps and obstacles that the field will encounter.

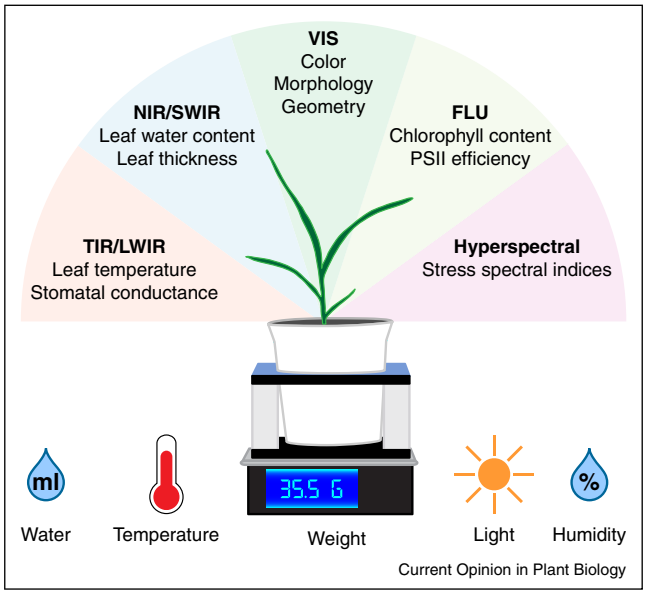
Phenotyping platform design

A key advance in high-throughput phenotyping platforms is the capability to non-destructively capture plant traits. This advance permits time-series measurements that are necessary to follow the progression of growth and stress on individual plants. Eliminating destructive measurements also increases the experimental capacity for genotypes, treatments, and biological replicates by reducing the required replicate sampling sets. 'High-throughput' is a classification that is relative to the effort associated with the measurement. Here high-throughput image-based phenotyping is defined as technology that can minimally image hundreds of plants per day. A population on the order of hundreds allows for analysis of mutant populations, detection of QTLs, and discovery of gene by environment associations.

Advances in plant science research have undeniably benefited from the use of model organisms in the development of molecular and genetic tools. But the impact of model-enabled knowledge on crops ultimately depends on successful translation to the field. Similarly, high-throughput phenomic advances will need to occur across scales of phenotyping platforms to sustain and improve crop yields. Field platforms have the advantage of growing crop-sized plants under natural settings, but are constrained to seasonal growing conditions. Growth-chamber and greenhouse-based phenotyping platforms have the advantage of increased experimental cycling and greater environmental control, but are often restricted by pot growth and the spectrum of environmental conditions that can be assayed. Controlled environments are also well suited for examination of root phenotypes, but further discussion is beyond the scope of this review (see, Bucksch *et al.* [3], Moore *et al.* [4], and Topp *et al.* [5], for details). For further information on high-throughput phenotyping platforms in field and controlled environment settings, see recent reviews by White *et al.* [6], Fabio *et al.* [7], and Araus *et al.* [8].

Regardless of the platform setting, the goal is to automate the acquisition of plant images, and other physical data that can be used to quantify genotype by phenotype by environment interactions (Figure 1). The choreographed movement of cameras or plants, and the logistics associated

Figure 1



High-throughput plant phenotyping platforms collect images, environmental (water supply, light intensity, temperature, humidity) and physical (e.g. plant weight) data to quantify genotype by phenotype by environment interactions.

with watering large populations are important platform design considerations. Automated watering systems, often integrated with high-throughput phenotyping platforms, are critical to experimentation, as manual application of water is imprecise, labor intensive, and reduces imaging time. The necessary frequency of watering, the amount of water, type of soil, and fertilizer type, should all be contemplated during platform design. Experimental questions should strongly influence platform design. For example, a phenotyping platform with a static-pot scale-watering system enabled Wallach *et al.*, 2010 to measure temporal oscillations of whole plant transpiration in tomato [9]. Conversely, Tisné *et al.*, found that continuous plant movement and frequent target weight watering created ideal conditions for controlled drought experiments on their Phenoscope platform [10]. In addition to platform design strategies that support experimentation, camera technologies are needed to capture the data related to agronomically important traits, such as water-use efficiency. The following section will review recent developments in high-throughput imaging technology.

Plant phenotyping using imaging and computer vision

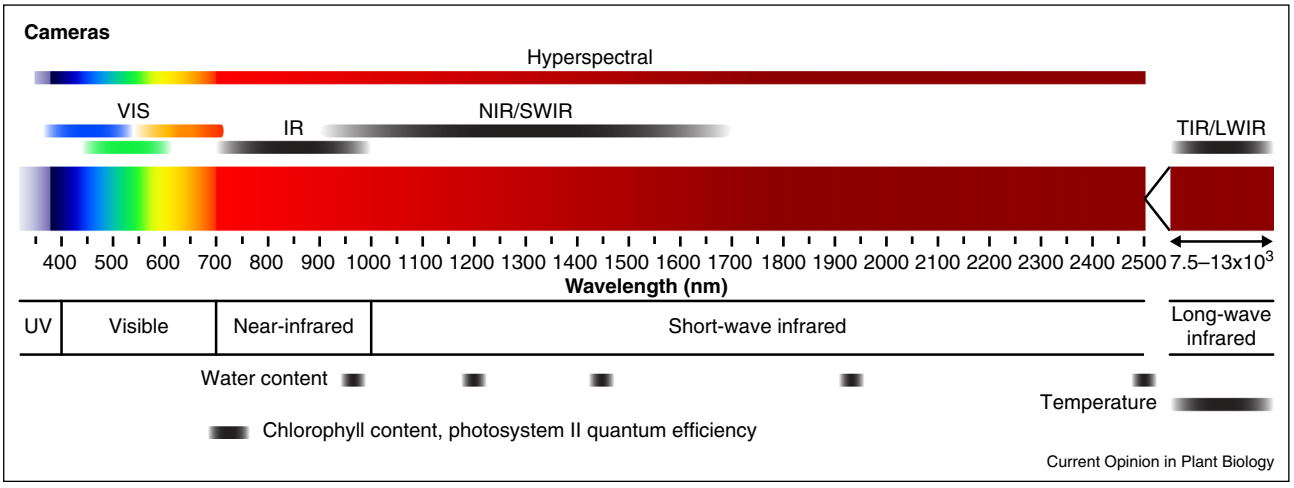
The goal of plant imaging and analysis is to measure the physiological, growth, development, and other phenotypic properties of plants through automated processes. Computer vision is an active area of research for navigation, industrial automation, medical diagnostics, and other technologies [11], and many of the techniques developed for image processing and analysis can be applied to plant phenotyping.

Digital camera technology has become relatively inexpensive and ubiquitous, leading to a recent surge in high-throughput phenotyping systems that utilize plant imaging to capture data. Standard consumer cameras use silicon-based sensors that are responsive to light wavelengths in the 400–1000 nm range. However, color cameras are further restricted to the 400–700 nm range visible to humans with the inclusion of an infrared-blocking filter (VIS camera, Figure 2). VIS camera sensor arrays have three color sensors (blue, green and red) that are used to estimate the true color of each pixel (Figure 2). High-throughput platforms have been developed that couple controlled-environment plant growth facilities with VIS imaging systems to measure plant growth, development, and responses to the environment [10,12–16,17••,18•].

For phenotyping platforms built for small rosette plants such as *Arabidopsis thaliana*, VIS cameras are positioned overhead (top-view) and a single image including one or more plants is captured per time point per camera [10,14,15]. For larger plants, an image stack is collected for individual plants per time point. Image stacks can include a top-view image and multiple images from a side-mounted camera (side-view) [16,17••,18•]. Image-processing algorithms are used to identify pixels that are plant-derived, and the identified object is used for measuring morphological (shape, structure), geometric (length, area), and color properties of each plant. VIS images have been particularly successful in estimating plant biomass. Several groups have demonstrated that plant pixel area from a single image stack can be used to calculate an approximate plant volume or total leaf area value that can accurately model fresh- and dry-weight above ground biomass of barley [16,17••,19], rice [18•,20], sorghum [21], and wheat [19]. In addition to single-point measurements, high-throughput phenotyping with VIS imaging is used to measure dynamic processes. For example, plant growth rates were modeled using logistic growth functions in *Arabidopsis* [15], barley [17••], and sorghum [21].

Additional camera systems can complement color imaging. The infrared spectrum spans wavelengths longer than the limit of red light perception in humans (~700 nm) to 1 mm. The interaction of infrared light with plant tissue has several properties that make infrared analysis potentially useful for phenotyping. First, wavelengths in the near-infrared (NIR) subrange are strongly reflected by plant tissue, relative to visible light [22]. Cameras with standard silicon-based sensor can be modified to detect infrared light sources up to 1000 nm and used for imaging in the absence of visible light by replacing the infrared blocking filter with a visible-light blocking filter (IR camera, Figure 2). Matos *et al.* used infrared

Figure 2



A variety of cameras are available to capture signal from the visible and infrared spectrum of light. VIS cameras detect light in the visible range from ~400 to 700 nm and are used to measure the morphological, geometric, and color properties of plants [10,14,15,16,17••,18•,21]. Infrared (IR) cameras detect near-infrared (NIR) light and are used for night imaging [23••]. NIR cameras detect NIR and short-wave infrared (SWIR) light in a region that is useful for detecting leaf water content [22]. Thermal infrared (TIR) cameras detect long-wave infrared (LWIR) light that is emitted by leaves in a temperature-dependent intensity [24]. Hyperspectral cameras detect hundreds of spectral bands with nm-level resolution between 350 and 2500 nm and are currently being developed to detect plant stress [26,34]. Specialized imaging systems measure chlorophyll fluorescence after excitation [17••,28].

imaging to measure the growth rate of *Brachypodium distachyon* under circadian and entrained conditions to show that temperature drives oscillations in growth rate [23••]. Second, the NIR and short-wave infrared (SWIR) subranges contains several major and minor water absorption troughs that can be used as an index of leaf relative water content (Figure 2) [22]. NIR/SWIR cameras contain indium gallium arsenide (InGaAs) sensors with spectral sensitivity in the range of 900–1700 nm that overlaps three of the five water absorption regions (Figure 2). Chen *et al.* observed a significant difference in NIR reflectance in barley plants under drought conditions followed by a return to control levels during recovery [17••]. However, NIR reflectance can be influenced by leaf thickness in addition to leaf water content [22]. Indeed, Neilson *et al.* found that leaf thickness was the primary factor behind the differences observed between two sorghum varieties tested in a drought experiment [21]. Finally, thermal infrared cameras can detect long-wave infrared (LWIR) radiation that is emitted by objects based on temperature (TIR/LWIR camera, Figure 2). Sirault *et al.* used thermal imaging to show that leaf temperature is negatively correlated with stomatal conductance and that the method can be used to measure response to salinity stress in barley [24].

A disadvantage of VIS and NIR cameras is that the detection sensors are sensitive to relatively broad ranges of electromagnetic spectrum and specific wavelength information is lost in the output image. In contrast, state-of-the-art hyperspectral cameras can measure hundreds of spectral bands between 350 and 2500 nm at nm-level resolution for each image pixel (Figure 2) [25]. Hyperspectral imaging is a promising technology for the detection of abiotic [26] and biotic stresses [25]. However, hyperspectral image acquisition is currently slower than other cameras, and analysis of hyperspectral images poses additional computational challenges due to higher data dimensionality.

Chlorophyll fluorescence imaging is another method to detect plant stress [27]. Chen *et al.* used steady-state chlorophyll fluorescence excited by blue light as a relative measure of photosynthetic health in a drought stress experiment in barley [17••]. The quantum efficiency of photosystem II (PSII) can also be measured by comparing chlorophyll fluorescence levels under minimal light induction to the maximum fluorescence after induction with a saturating pulse of red light [27]. Jansen *et al.* developed a high-throughput system to screen PSII efficiency in *Arabidopsis* and found that chilling stress can significantly reduce PSII efficiency, while only severe drought stress can reduced PSII efficiency measurements [28].

Finally, although three-dimensional (3D) traits can be effectively estimated using two-dimensional (2D) image data, 3D plant modeling can be particularly informative for measuring architectural features (e.g. the number of leaves or tillers). Pound *et al.* developed a computational

Box 1 Maker-made phenotyping

Installation and maintenance of large commercial phenotyping facilities is a significant financial commitment for both public and private research sectors. The growing Maker Movement and Community enable the design of low-cost custom phenotyping hardware with inexpensive 3D-printers, computers, microcontrollers, cameras, and a plethora of plug-and-play sensors. For a list of maker resources please visit http://maker.danforthcenter.org/. Recent studies demonstrate the ingenuity and utility of this approach in measuring plant growth [23••] and 3D architecture [32,51]. Simple imaging stations made of modified digital cameras capturing infrared light and small IR LED panels demonstrated the importance of temperature cycles in the circadian regulated growth of *Brachypodium distachyon* [23••]. Using the inexpensive Microsoft© Kinect depth camera to segment shoot biomass and describe 3D plant architecture, Chéné *et al.*, mapped regions of pathogen infection identified with a secondary thermal camera [32]. The Kinect© was recently compared to another low cost 3D imaging tool (a David Laser scanning platform [54] for accuracy in measuring plant features [51]. Additional low-cost tools, curated public webcameras [55], and applications ideal for crowd-sourced plant phenotyping experiments are also being actively developed and deployed for photosynthetic measurements (http://photosynq.org/) and temporal plant imaging (http://projectrephoto.com/; http://amos.cse.wustl.edu/).

approach to construct 3D models of plants by using multi-perspective 2D image stacks [29]. Alternatively, 3D structure can be directly measured using laser-scanning techniques [13,30,31] and depth/time-of-flight sensors [32]. Dornbusch *et al.* demonstrated the utility of 3D information by using point clouds of *Arabidopsis* rosettes to analyze rhythmic leaf growth and movement [33]. Ultimately, the implementation of these diverse camera systems on high-throughput phenotyping platforms results in images that require thoughtful plant-centric trait extraction tools (Box 1).

Image processing and trait extraction tools

The images produced from diverse high-throughput phenotyping platforms and camera types can present common image processing and trait extraction challenges. Plant Image Analysis (http://www.plant-image-analysis.org/) is a convenient database of commercial and open-source image analysis tools [35••]. The majority of open-source tools found in the Plant Image Analysis database [35••] focus on phenotyping of plants with rosette architecture (*Arabidopsis*), and specific plant organs such as excised leaves.

Phenotyping of whole shoots, as opposed to excised organs, allows for examination of architecture-dependent traits, such as tillering or flowering, in addition to architecture-independent traits such as biomass and color. Open-source tools for phenotyping whole shoots with either vertical or rosette architecture include, HTPheno [36], the Integrated Analysis Platform [37••], and PlantCV (http://plantcv.danforthcenter.org/). PlantCV is a newly developed suite of open-source, open-development, image processing and trait extraction tools written in Python. Canopy Reconstruction is a new open-source tool for 3D reconstruction from 2D images [29].

The greatest and most time-consuming challenge in plant phenomics is the correlation of image-extracted traits to empirically-measured and biologically relevant traits. Accordingly, current image-based phenotyping experiments are generally targeted at specific questions of interest. However, more data can be extracted from these image datasets with new algorithms, analogous to reanalyzing sequencing data. For example, research on progressive drought may be specifically interested in measurements associated with changes in water-use efficiency; initial trait-extraction algorithms may determine the amount of biomass accumulated in a plant per unit of water added, while further advanced algorithms may specify in what regions of the plant biomass is accumulated per unit of water added. To increase the population of researchers developing image processing and trait extraction tools it is imperative that large image datasets are publically available and well-curated.

Public datasets and metadata

Advances in computational tools for assembly and analysis of sequencing data have benefited significantly from the availability of public datasets. The field of plant phenomics is inherently interdisciplinary and demands concerted interaction between plant science, engineering, and computer science. In recent years there have been annual plant-focused image-processing challenges (http://www.plant-phenotyping.org/) that have successfully stirred the community and encouraged computer scientists to work on common plant datasets [38,39]. However, access to high-throughput phenotyping platforms is limited in comparison to access to DNA sequencing facilities. Accordingly, there are relatively few well-curated high-throughput image phenotyping datasets available to the public (Table 1).

A central depository for plant imaging data, such as GEO [40,41] for sequencing data, is critical for easily aggregating and accessing imaging data. Successful implementation of a central depository for high-throughput phenotyping will require community input on standard metadata, and the support of existing plant biology infrastructure, such as the Bisque Image Analysis Environment on iPlant [42], since there can be significant diversity in the size and structure of high-throughput imaging data. Easily accessible public data will attract new tool developers, and incite creative data usage. A recent study by Chitwood is a prime example of creative interdisciplinary usage of well-curated public image data [43••]. Chitwood, who previously used morphometrics to analyze tomato leaf shapes [44], applied similar analysis to the evolution of violin shape [43••]. Following this example, well-curated public high-throughput plant phenotyping data has the potential to generate standard

Table 1

Publically accessible high-throughput phenotyping image datasets available upon review. For a current list of image datasets available please visit http://plantcv.danforthcenter.org/.

Camera	Plant species	Experiment type	# of images	Data access link	Associated publication
IR	*A. thaliana*	Temporal Growth- Root	~1 200 000	http://phytomorph.wisc.edu/	[4,52]
IR	*B. distachyon*	Temporal Growth-Shoot	~1700	[23••]	[23••]
VIS	*O. sativa*	Architecture-Root	~58 000	http://www.danforthcenter.org/scientists-research/principal-investigators/chris-topp/resources	[5]
VIS	*O. sativa, V. unguiculata, Z. mays*	Architecture-Root	~3000	http://www.dirt.biology.gatech.edu/	[3,53]
PSII, VIS, NIR	*S. viridis, S. italica*	Progressive Drought-Shoot	~79 200	http://plantcv.danforthcenter.org/	–
FLU, NIR, VIS	*Z. mays*	Temporal Growth-Shoot	~33 700	http://iap.ipk-gatersleben.de/	[37••]

ontology and methods that can be applied across disciplines.

Conclusions and future prospects

Considerable effort and attention has been placed on predicting the strain that changing environmental conditions and population growth will have on food supplies [45–47]. The focus must shift from predicting deficits to improving and sustaining crop yield. Sustaining and increasing crop yields with the advantages afforded modern genetics tools now hinges on rapid advancement of phenomics [1,2]. Phenomics, therefore, demands financial investment in an era of diminishing agricultural research funding [48,49]. Despite funding deficiencies, long-term benefit–cost ratios of agricultural research are large (32–1) [48], suggesting that returns on investments to relieve agricultural bottlenecks, such as phenotyping, would well exceed their cost. In addition to the direct role of phenotyping in improving crops with genomics tools, the capability of high-throughput image-based phenotyping to rapidly generate environmentally nuanced temporal measurements of plant growth and development is exciting for its potential to contribute to predictive environmental response models [50].

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