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Engineering plants for tomorrow: how high-throughput phenotyping is contributing to the development of better crops

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Abstract High-throughput plant phenotyping has been advancing at an accelerated rate as a response to the need to fill the gap between genomic information and the plasticity of the plant phenome. During the past decade, North America has seen a stark increase in the number of phenotyping facilities, and these groups are actively contributing to the generation of high-dimensional, richly informative datasets about the phenotype of model and crop plants. As both phenomic datasets and analysis tools are made publicly available, the key to engineering more resilient crops to meet global demand is closer than ever. However, there are a number of bottlenecks that must yet be overcome before this can be achieved. In this paper, we present an overview of the most commonly used sensors that empower digital phenotyping and the information they provide. We also describe modern approaches to identify and characterize plants that are resilient to common abiotic and biotic stresses that limit growth and yield of crops. Of interest to researchers working in plant biochemistry, we also include a section discussing the potential of these high-throughput approaches in linking phenotypic data with chemical composition data. We conclude by discussing the main bottlenecks that still remain in the field and the importance of multidisciplinary teams and collaboration to overcome those challenges.

Keywords High-throughput plant phenotyping · Plant phenotypes · Phenomes · Phenomics · Abiotic stress tolerance

Introduction

Phenomics is an emergent research field that has recently moved into the spotlight within the scientific community. Plant phenomics relates an organism's phenotype, which is highly dependent on the environment, to the genotype through the collection of high-dimensional phenotypic data (Houle et al. 2010). High-throughput phenotyping systems, often defined as being able to image hundreds or thousands of plants a day, are paramount in furthering the understanding of "phenomes" and the underlying genetics behind them (Fahlgren et al. 2015b). Traditionally, plant phenotypes have been recorded manually, which is a very laborious and intensive process that often

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requires the destruction of the tissue for specific readouts on overall plant health and growth. With high-throughput technologies, on the other hand, plants are able to be measured in a non-destructive manner, providing useful temporal and spatial information with accuracy and precision that manual phenotyping cannot achieve and readouts that go beyond the resolution of the human eye. Experiments can be designed to span a number of hours, days, weeks, or months, and novel information about early germination, reproduction, and all developmental stages between can now be teased out using powerful sensors and commercial or open-source algorithms. The robust datasets generated by these experiments provide more information than ever before on plant performance, and they are instrumental in enabling the development of crops for the future.

A large portion of the published phenotyping research has been done on model organisms in laboratory settings, but as with any technology, applicability to real-world conditions is necessary (Fahlgren et al. 2015b).With the looming threat of climate change and a rapidly growing world population, traditional plant breeding techniques can no longer keep pace with global food and feed demand, and it is estimated that cereal grains alone must increase by 70% by 2050 to meet future needs (Furbank and Tester 2011). While sequencing technologies have grown exponentially allowing entire genomes to be sequenced at low cost in a short period of time, one of the current knowledge gaps in plant science lies in relating this wealth of genomic data with phenotypic data (Cobb et al. 2013). Therefore, it is more necessary than ever to support laboratory, greenhouse and field high-throughput phenotyping studies.

In this paper, we present an overview of the most commonly used sensors that empower digital phenotyping and the information they provide including the visualization of traits that interest breeders, such as increased biomass, yield, and tolerance to abiotic stresses/resistance to biotic stresses. We also describe modern approaches to identify and characterize plants that are resilient to common abiotic stresses that limit aforementioned growth and yield of crops. Of interest to researchers working in plant biochemistry, we also include a section discussing the potential of high-throughput approaches and hyperspectral sensors in linking phenotypic data with chemical composition data. We conclude by discussing the main bottlenecks that still remain in the field and the importance of multidisciplinary teams and collaborative research networks to overcome those challenges.

Available platforms that enable high-throughput plant phenotyping

With continuous advances in sensor technologies, high-throughput plant phenotyping (HTPP) has become widespread. The first commercial HTTP robots entered the market over 15 years ago, but since then, the number of providers of these platforms has grown significantly. Table 1 presents an up-to-date summary of the HTTP systems available, as well as recent papers describing their capabilities. These systems can range from semi-automated platforms, where users load and remove plants manually, to fully automated conveyor platforms that pull in plants growing in growth chambers, glass houses, and/or greenhouses. More recently, Spidercam-based and gantry-based systems have also become available for phenotyping plants in the field (Andrade-Sanchez et al. 2014; Kirchgessner et al. 2017).

Sensors and the information they can yield

There are numerous sensors to choose from when planning an HTPP experiment, and much of the selection of which ones to use depends on the goals of each experiment. Table 2 summarizes the most common sensors currently used in HTTP platforms, as well as the readouts and most useful information that can be extracted from the images and data they acquire. Some of these readouts on plant health include size, color (indicative of chlorosis/necrosis), architecture, chlorophyll fluorescence/photosystem II efficiency, water content, leaf/canopy temperature, and tolerance/resistance to abiotic/biotic stresses, respectively.

Figure 1 presents illustrative examples of images captured at the Plant Phenomics Facility at Arkansas State University (A-State). Using a commercial platform and the associated software, plants can be easily extracted from the background for measurements of size, color, and architecture using the RGB (a.k.a. visible) camera. Also illustrated are images acquired with a fluorescence (FLUO) camera that allows *in*

Table 1 Commercial and published platforms for high-throughput plant phenotyping

Name URL	Description	References
GROWSCREEN http://www.fz-juelich.de/ibg/ibg-2/EN/methods_jppc/GROWSCREEN	This platform was developed to study plant leaf growth fluorescence and root architecture from seedlings under controlled conditions for visual phenotyping of large plant populations	Walter et al. (2007) and Jansen et al. (2009)
HRPF NA	High-throughput rice phenotyping facility (HRPF) designed with two main sections: rice automatic phenotyping (RAP) and yield trait scorer (YTS). This high-throughput platform was developed for automatic screening of rice germplasm and populations throughout the growth period and after harvest	Yang et al. (2014)
PHENODYN http://bioweb.supagro.inra.fr/phenodyn	This platform monitors plant growth and transpiration rate with stressful environmental conditions	Rahaman et al. (2015)
PHENOPSIS http://bioweb.supagro.inra.fr/phenopsis	Represents specific setups for automated phenotyping, allowing a culture of approximately 200–500 Arabidopsis plants in individual pots with automatic watering and imaging system	Granier and Vile (2014)
PHENOSCOPE http://www.observatoirevegetal.inra.fr/observatoirevegetal_eng/Scientific-platforms/Phenoscope	This automated phenotyping platform is an integrated device, allowing simultaneous culture of 735 individual Arabidopsis plants and high-throughput acquisition, storage and analysis of quality phenotypes	Tisné et al. (2013)
PHENOSPEX https://phenospex.com/	Offers a range of systems, including multispectral 3D scanners, gravimetric weigh stations, and field/greenhouse high-throughput phenotyping options	Vadez et al. (2015)
PlantScan http://www.csiro.au/Outcomes/FoodandAgriculture/HRPPC/PlatScan.aspx	This is an automated high-resolution phenomic center which provides non-invasive analysis of plant structure, morphology and function by utilizing cutting-edge information technology including high-resolution cameras and 3D reconstruction software	Sirault et al. (2013)
Qubit Phenomics http://qubitphenomics.com	Integrated conveyor and robotic high-throughput plant imaging system for the laboratory, growth chamber, or field phenotyping	De Diego et al. (2017)
Scanalyzer PL, HT, 3D, Field http://www.lemnatec.com	Captures and analyzes 2D/3D non-destructive high-throughput images; monitor plant growth and behavior under entirely controlled conditions in a robotic greenhouse system. Growth chamber and field scale systems are also available	Arvidsson et al. (2011)
TraitMill http://www.cropdesign.com	High-throughput gene engineering platform developed by Crop Design. This is a versatile tool that enables large-scale transgenesis and automated high-resolution phenotypic plant evolution	Reuzeau et al. (2010)
WIWAM http://wiwam.be	Like PHENOPSIS, WIWAM is an automated imaging platform simultaneously handling a large number of plants and measuring a variety of plant growth parameters with automatic watering and imaging systems at regular time intervals	Skirycz et al. (2011)

Adapted from Rahaman et al. (2015). Manufacturers are constantly updating their websites to reflect newer models, sensors, and capabilities. For user ease, this table provides links to the manufacturer's websites, as well as references to papers describing some of their phenotyping capabilities

Table 2 Summary of the most common sensors used in HTPP experiments

Sensor	Phenotype parameters
RGB	Size, architecture, geometry, greenness and other colors
Quantum efficiency of photosystem II (PSII)	Photosynthetic activity, non-photochemical quenching
Fluorescence (FLUO)	Chlorophyll fluorescence, fluorescent proteins (used as signal markers, etc.)
Infrared (IR)	Canopy or leaf temperature, insect/pathogen infestations
Near infrared/short wave infrared (NIR/SWIR)	Water content
Thermal infrared/Long wave infrared (TIR/LWIR)	Canopy or leaf temperature
Light detection and ranging (LIDAR)	Location (GPS), plant height, aboveground biomass, canopy cover and leaf area index
Hyperspectral (HIS)	Leaf and canopy health, chemical profiling

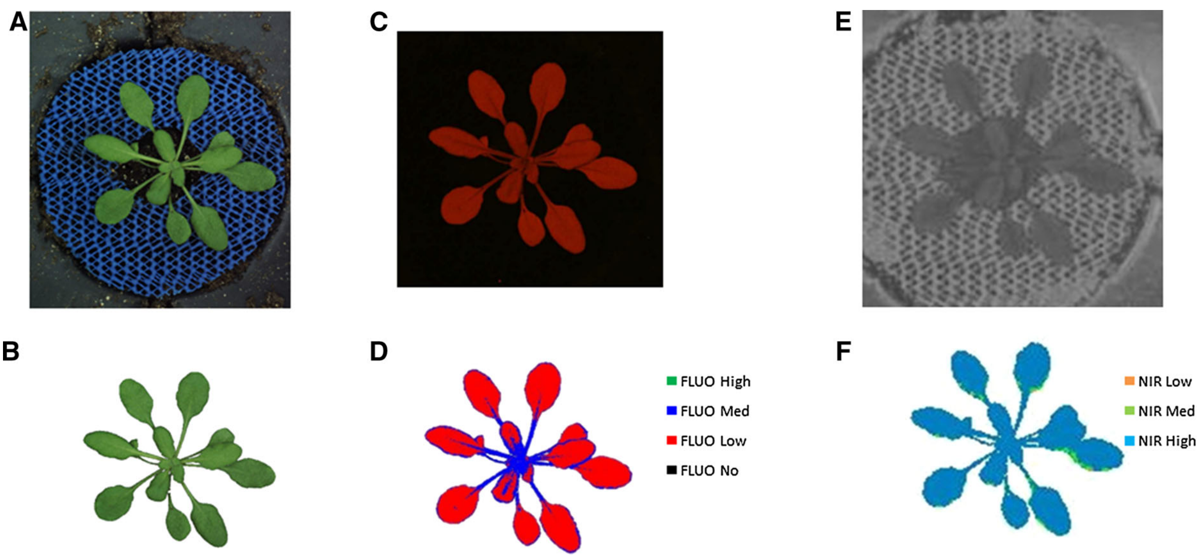

Fig. 1 Illustrative images captured with the visible, fluorescence, and near infrared sensors at the Arkansas State University Phenomics Facility. **a** shows an example of an *Arabidopsis thaliana* captured with a visible camera, and **b** shows the plant after using the LemnaGrid algorithm to extract the object from the background. **c** shows a fluorescence image of the same plant, and **d** a color classified analysis of the plant indicating areas of low, medium and high fluorescence. **e** shows an image captured with the near-infrared camera, and **f** shows the analysis of *in planta* water content

planta chlorophyll fluorescence assessment, as well as images acquired with a near-infrared camera (NIR) that allows monitoring *in planta* water content.

The maker movement

There is growing interest in the plant science community to develop cheaper and more flexible platforms for phenotyping. Dubbed the "maker movement", this recent trend has been focused on using open-source, homemade technology for plant phenotyping (Gehan and Kellogg 2017). Some examples of these devices are here described. The combination of a near-infrared LED panel and a Raspberry Pi NoIR camera has been shown to yield a 2D, automated imaging system that is affordable and useful in extracting plant information (Dobrescu et al. 2017). Cost-efficient, high-resolution phenotyping systems for plant roots have also been previously described as part of this movement (Slovak et al. 2014). A hand held device that allows assessing photosynthetic efficiency with results comparable to those obtained with the Li-Cor system has been recently developed. This sensor allows users to visualize the acquired data using an Android tablet or smartphone and to store this information in a portal in the cloud where users can visualize, graph, analyze and download the data (Kuhlgert et al. 2016).

Image acquisition is no longer the challenge, image analysis is the true bottleneck

Acquiring images is the easiest part of any HTTP experiment, the challenge is the analysis of the thousands of images that can be acquired in a short period of time. The Plant Image Analysis database is a very useful resource that was launched in 2013 and is continuously updated (Lobet et al. 2013). This database summarizes most available software for plant image analysis (http://www.plant-image-analysis.org/). Its current listings include over 150 algorithms that can be used for this purpose, including tools to analyze aerial tissue and also complex root systems (e.g. Symonova et al. 2015; Knecht et al. 2016; Pound et al. 2017).

One of the potential issues users may encounter with the analysis tools listed in the Plant Image Analysis database is that, once developed, most of these algorithms are never updated. An analysis tool that undergoes constant updates is PlantCV (Fahlgren et al. 2015b). The most current version, PlantCV2, that includes modules developed by at least six research teams was recently published (Gehan et al. 2017). The leaders of this effort anticipate to release a new version of this tool once a year.

Despite recent advances, the power of phenomics is still limited by data analysis, and data analysis is largely limited by ignorance of powerful resources (Houle et al. 2010). Phenotyping experiments are still undergoing massive amounts of standardization necessary for creating reproducible studies that can be publicly accessed, analyzed, and modeled. It is currently the burden of the scientific community to develop a more adaptable and less expensive framework for analyzing high-dimensional phenotype datasets (Rahaman et al. 2015).

Types of phenotyping assays

In plants, it is especially important to understand the plasticity of the phenome, or how the phenotype changes, when subjected to variable environmental conditions (Tardieu et al. 2017). As climate change and poor farming practices reduce arable land, agriculture faces more challenges than ever, since abiotic stresses are the biggest factor in crop loss (Mahajan and Tuteja 2005). For example, average yields usually range somewhere between 20 and 50% of record highs, with soil salinity and drought being cited as the major contributors. In fact, increased soil salinity is expected to reduce the amount of farming land available by 30% in the next 25 years and up to 50% by 2050 (Wang et al. 2003). Drought, on the other hand, is expected to reduce crop yields by 50% in 2050 and almost 90% by 2100 (Li et al. 2009). Heat and frost are also predicted to increase, and both events can lead to yield reductions in crops such as wheat (Barlow et al. 2015). Therefore, an increased knowledge of how these stresses affect plants through high-throughput phenotyping experiments must be obtained in order to further breeding and other genetic, and physiological tools to develop more resilient crops.

Although 2D and 3D platforms for above-ground plant phenotyping are empowering new discoveries, they provide only half of the story. A key aspect of plant health and development is the root system, and identifying the underlying root characteristics that make a stronger plant is crucial as well. Root architecture is key in a plant's ability to survive periods of water and nutrient deficit, as roots are responsible for collecting all the water and nutrients plants need from the soil (Malamy 2005). Phosphorous, an essential nutrient for plants, is largely immobile, and it is the limiting factor for crop yield in around 30% of arable land (Vance et al. 2003). Given the focus of this review, root phenotyping is outside of the scope of this paper, but we refer readers interested in advances in root phenotyping to a recent article (Tardieu et al. 2017).

Assessing size, architecture, and growth rate

With the utilization of HTTP platforms, assessing the growth of a plant across its life cycle has never been easier. While there is still room to grow and improve, it is now possible to monitor every step from seed, seedlings, early developmental stages, and beyond, and this can be done in environment controlled chambers, greenhouses, or field conditions. Figure 2 provides examples of the plant species that have been extensively studied at the Arkansas State University Plant Phenomics Facility.

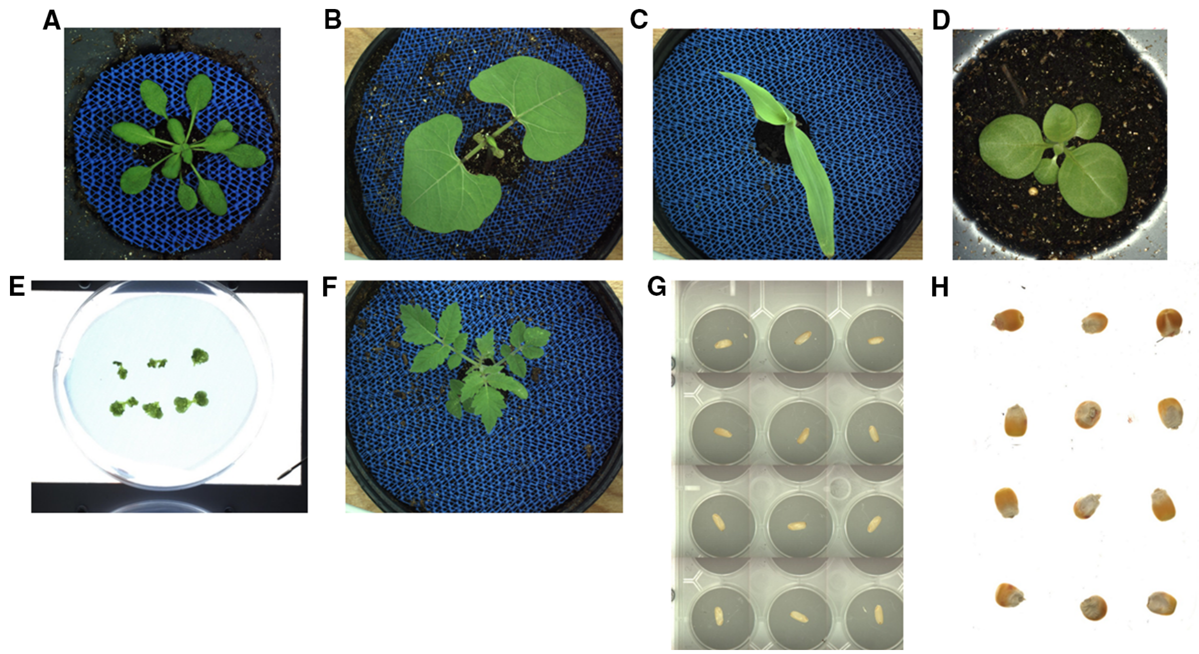

Fig. 2 Visible images of various plant species captured at the Arkansas State University Phenomics Facility **a** *Arabidopsis thaliana*, **b** common bean, **c** maize, **d** tobacco, **e** *Marchantia polymorpha,* **f** tomato, **g** rice seeds and **h** maize seeds. Assays on these plants that have been performed in the facility include water limitation stress, heat stress, cold stress, light stress, assessment of seed chalkiness (rice), and comparison of embryo/seed ratios (corn). Additionally, growth comparison assays have been performed on several transgenic lines (Arabidopsis/tobacco) with elevated ascorbic acid content, revealing differences in biomass, yield, and senescence

Abiotic stress tolerance assessment

As previously mentioned, understanding abiotic stress tolerance is paramount in furthering the movement to engineer heartier crops. Figure 3 provides illustrative examples of how the A-State Phenomics Facility has been using HTTP approaches to empower the screening of diversity panels and mutant collections to identify and characterize plant varieties/cultivars that display tolerance to key abiotic stresses. Many groups have extensively screened valuable germplasm in an effort to identify novel mechanisms and strategies to develop crops better adapted to withstand harsh environmental conditions. A list of key protocols and platforms that have been used to study common abiotic stresses in both model and crop plants using HTPP approaches is presented in Table 3.

Biotic stress resistance assessment

Biotic stresses are another cause that limit growth and yield of crops. Plants show evidence of the infective agent(s) affecting them, and those symptoms can include fungal growth, bacterial ooze, nematode cysts, and presence of mites or insects (Flynn 2003). A vast quantity of crops is lost every year due to pests. The financial losses caused by just herbivores ranges from 5 to 30% globally (Thurau et al. 2009; Masler and Chitwood 2016). These stress responses lead to physiological, molecular, and cellular adaptation, ultimately affecting phenotypic plasticity of plants (Pandey et al. 2015).

The host plant's resistance to biotic stressors is the ability of the plant to reduce the growth, reproduction, and development of biotic stressors. Tolerance refers to ability of the plant to grow, develop, and produce seed/fruit in the presence of biotic stressors. Herbivore infestation directly impact plants. Among the effects of herbivore infestation in plants are defoliation, cell content feeding, leaf mining, oviposition scars, and stem boring to name a few. On the other hand, herbivores systematically damage plants, causing signs of chlorophyll loss, discoloration, premature senescence, and distortion of new growth (Smith and Clement 2012; Goggin et al. 2015). Tolerance and

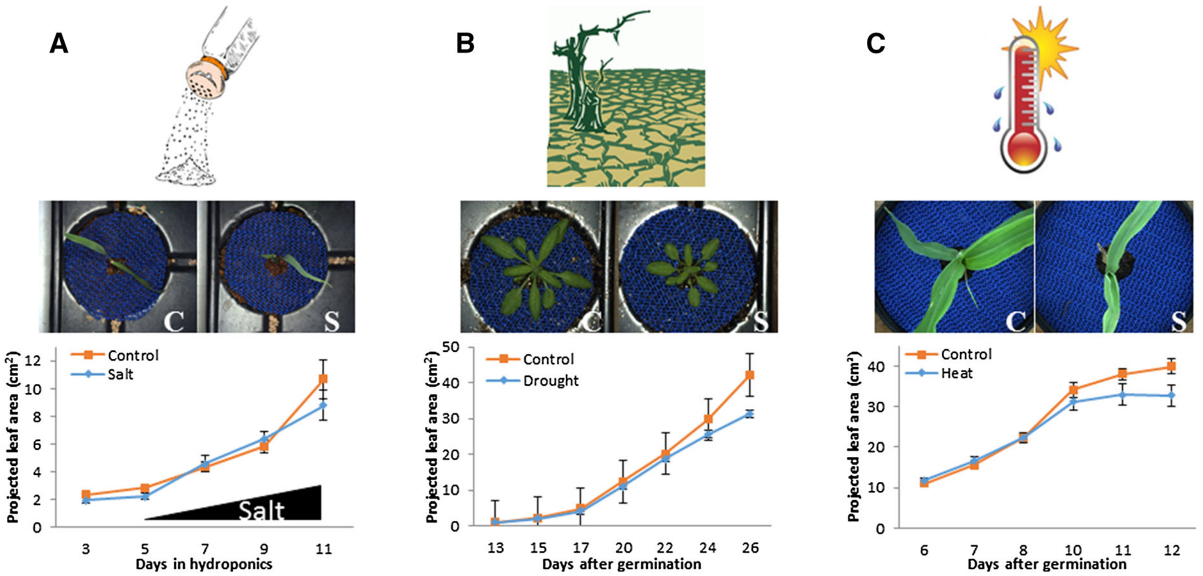

Fig. 3 Abiotic stress conditions regularly applied to plants for study in HTPP experiments. Whether its salinity (pictured in rice) (**a**), drought (pictured in *Arabidopsis thaliana*) (**b**), cold or heat (heat stress pictured in maize) (**c**), HTPP can greatly improve our knowledge of how plants respond to abiotic stresses, especially in the early developmental stages. Due to speed and accuracy, HTTP empowers the screening of large collections of lines (either diversity panels or mutant collections) to identify those with tolerance to stresses allowing to close the gap between genotype and phenotype. *C* control and *S* stress

resistance to biotic stresses have been assessed manually for many decades. However, more and more research teams are incorporating high-throughput approaches to do this type of assessment. For example, intracellular water level of plants, plant water balance, photosynthetic efficiency, chlorophyll content, hyperspectral camera plant reflectance and fungal infection lesion diameter have been used to assess tolerance and resistance to biotic stresses (Nabity et al. 2009; Backoulou et al. 2011; Kerchev et al. 2012; Nabity et al. 2013; Angulo et al. 2015). Additionally, the effect of biotic agents on plant health and growth have been used to assess the fitness of mutant plants comparing with wild type controls for biotic stress effects (Avila et al. 2012). Similarly, tolerance to lepidopteran infestation has been assessed using traits such as projected leaf surface area, photosynthetic efficiency, and seed yield (Chen et al. 2007).

Figure 4 illustrates the power of HTPP to assess tolerance to biotic stresses in crops. Hyperspectral images have been used for identification of blotch disease, rust disease, and powdery mildew disease in Barley (*Hordeum vulgare* L.), sugar beet rust disease; *Cercospora* leaf spot disease and powdery mildew disease in sugar beet, and *Alternaria alternata*, *Alternaria brassicae*, and *Alternaria brassicicola* in oilseed rape (*Brassica napus* L.) (Rumpf et al. 2010; Baranowski et al. 2015; Wahabzada et al. 2015). RGB images, on the other hand, have been used for identification of apple scab disease in apple (*Malus domestica* Borkh), southern green stink bug infestation, *Ascochyta* blight disease and insect infestation in cotton (*Gossypium hirsutum* L.), tomato yellow leaf curl disease in tomato, bacterial soft rot disease, black rot disease, and brown spot disease in Orchid (*Phalaenopsis*), wheat streak mosaic virus disease in wheat (*Triticum aestivum* L.), powdery mildew disease in tomato, and yellow vein virus disease in chili pepper (Huang 2007; Camargo and Smith 2009; González-Pérez et al. 2013; Casanova et al. 2014; Hernández-Rabadán et al. 2014; Mokhtar et al. 2015). A combination of RGB and multispectral images have been used for identification of *Uromyces betae* and *Cercospora beticola* disease in sugar beet (Bauer et al. 2011), while damage caused by leaf miner has been identified using RGB images and spectral reflectance

Table 3 Common stress assays in HTPP experiments

Stress tested	Species	Sensors platforms utilized	Scale	Main focus	References
Salinity	Wheat	LemnaTec Scanalyzer 3D	Hydroponics Greenhouse	Salinity tolerance in cereals	Rajendran et al. (2009)
	Wheat, barley	Infrared camera	Growth chamber	Osmotic component of salinity tolerance in cereals	Sirault et al. (2009)
	Wheat	LemnaTec Scanalyzer 3D	Greenhouse	Shoot biomass in cereals	Golzarian et al. (2011)
	Rice	LemnaTec Scanalyzer 3D	Greenhouse	Salinity tolerance in rice	Hairmansis et al. (2014)
	Rice	LemnaTec Scanalyzer 3D	Greenhouse	Imaging and genetics of salinity responses in rice	Campbell et al. (2015)
	Rice	LemnaTec Scanalyzer 3D	Greenhouse	Salinity tolerance loci revealed in rice	Al-Tamimi et al. (2016)
	Arabidopsis thaliana	RGB, Fluorescence	Growth chamber	Salinity tolerance in *Arabidopsis thaliana*	Awlia et al. (2016)
Drought	*Arabidopsis thaliana*	PHENOPSIS	Growth chamber	Soil water deficit in *Arabidopsis thaliana*	Granier et al. (2006)
	Barley	LemnaTec Scanalyzer 3D	Greenhouse	Drought tolerance in wild barley introgression lines	Honsdorf et al. (2014)
	Cotton	Sonar proximity sensor, infrared radiometer, multispectral crop canopy sensor	Field	Drought tolerance in cotton	Andrade-Sanchez et al. (2014)
	Barley	LemnaTec Scanalyzer 3D	Greenhouse	Drought based responses in barley	Chen et al. (2014)
	Setaria	LemnaTec Scanalyzer 3D	Greenhouse	Responses to water limitation in *Setaria*	Fahlgren et al. (2015a)
	Arabidopsis thaliana	LemnaTec Scanalyzer HTS	Growth chamber	Effects on the phenome and ionome of *Arabidopsis thaliana*	Acosta-Gamboa et al. (2017)
Heat	Wheat	Phenocart	Field	Stress-adaptive traits in wheat	Crain et al. (2017)
Cold	Pea	RGB, Fluorescence	Growth chamber	Cold tolerance in peas	Humplík et al. (2015)

in tomato (Wu and Xie 2008). Fluorescence images have been also used for identification of huanglongbing disease in *Citrus* (*Citrus sinesis* (L.) Osbeck) (Wetterich et al. 2013). For comprehensive reviews of how HTTP approaches can be used to develop crops that are resistant to biotic stresses, we refer readers to other review papers (Goggin et al. 2015).

Hyperspectral sensors allow tracking of chemicals

As discussed in previous sections the employment of new sensors to assess plant dynamics in a fast and non-destructive way is increasing. Hyperspectral imaging (HSI) has become an alternative technology that has been applied in a wide variety of research areas, including microbiology (Gowen et al. 2015), the food industry (Mishra et al. 2016), pharmaceutical sciences (Gendrin et al. 2008), remote sensing (Blackburn 2007), and plant sciences.

Hyperspectral imaging involves a spectrograph that accounts for the reflectance over a large range of the light spectrum into a digital sensor (Bock et al. 2010). This system consists of an integration of two modalities: point spectroscopy and imaging technology. Information about plant physiology is gathered by the first modality, while the imaging technology is then used to understand structural dynamics. The data

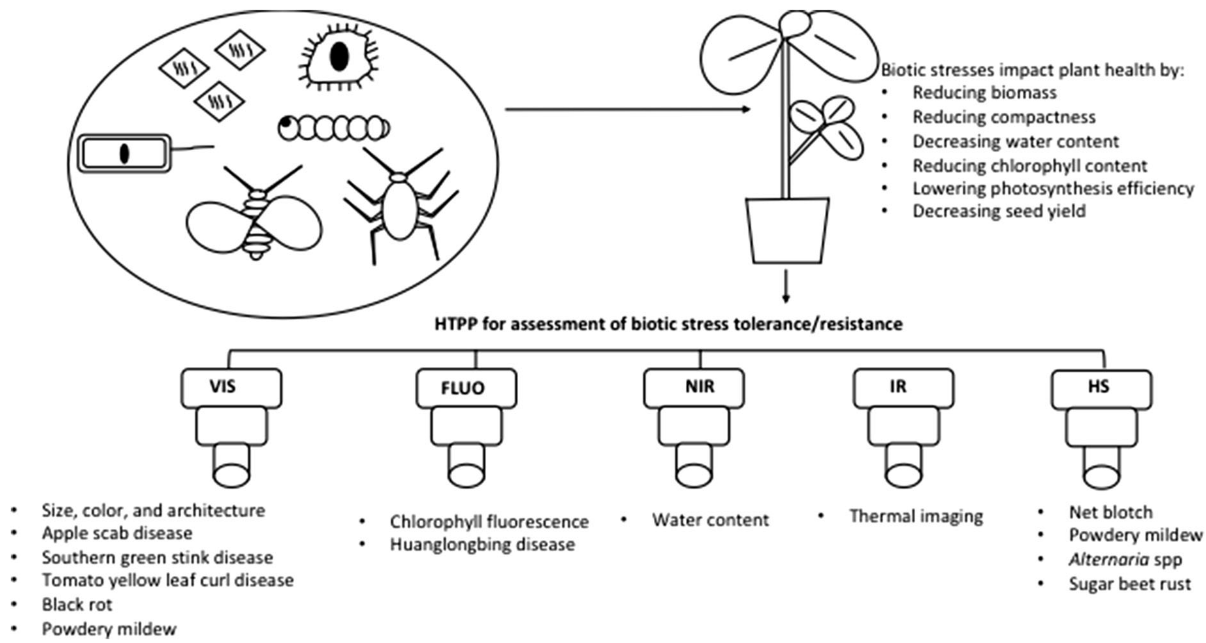

Fig. 4 Assessment of biotic stresses tolerance and resistance using phenomic approaches. Visible (VIS, RGB) cameras can be used to assess reduction in biomass, growth rate, and color changes. Fluorescence (FLUO) cameras can be used to assess chlorophyll fluorescence. Near-infrared (NIR) cameras can be used to measure changes in water content. Infrared (IR) cameras provide data on leaf temperature changes, while hyperspectral (HS) sensors can provide information about chemical composition changes in plant tissues. Visible, fluorescence, thermal, and hyperspectral images can also be used to identify symptoms of disease and insect invasion

generated by this type of imaging comes out as a 3D spatial map of spatial variation also called a 3D hypercube, where the first two dimensions give the spatial information and the third dimension corresponds to the spectral information (Mishra et al. 2017).

Recent applications of HSI in plant sciences are related with foliar chemical content estimation, disease detection, variety identification, stress studies, and plant phenotyping. The estimation of the foliar biochemistry allows a better understanding of the overall plant health. With the use of HSI, it is possible to link phenotypic data with biochemical processes. One example would be looking into chemicals present in plant leaves during photosynthesis, such as water, nitrogen, lignin, chlorophyll, and cellulose (Mishra et al. 2017). Most of the HSI systems use a regression method called Partial Least Squares Regression (PLSR) to estimate the concentration of these compounds. This method uses selected spectra with known values for the biochemicals of interest to estimate the model parameters. These parameters are then used to generate "maps" of the compound of interest and obtained its distribution either in the leaf or the plant (Pandey et al. 2017). PLSR has high collinearity when independent variables are numerous. This regression algorithm has strong predicting ability, it can prevent over-fitting and it can be used to process multivariate data in one test. However, there are some disadvantages to this algorithm such as inability to predict the distribution characteristics of unknown parameters, low computational speed, and complex calculations (Pan et al. 2016).

Some examples of the application of this new technology include the use of HSI to asses leaf nitrogen content in wheat leaves in field conditions (Vigneau et al. 2011), analysis of plant pigments such as chlorophyll *a* and *b*, carotenoids, and anthocyanins (Blackburn 2007), and analysis of characteristic symptoms of *Cercospora* leaf spot, powdery mildew, and sugar beet rust in sugar beet leaves (Mahlein et al. 2012).

This new non-destructive methodology can also be used to determine concentration of macronutrients such as nitrogen, phosphorus, potassium, magnesium,

Table 4 High-throughput phenotyping facilities available in North America

Location	Facility name	Available platforms Manufacturer Scale	Sensors	Facility director or manager	Contact Website
Canada					
McGill University	McGill Plant Phenomics Platform (MP3)	Scanalyzer HTS LemnaTec Growth chamber	RGB, IR, FLUO, NIR, laser scanner	M.Sc. Emilio Vello	emilio.vello@mcgill.ca http://mp3.biol.mcgill.ca/mcgill_mp3_summary.html
	McGill Plant Phenomics Platform (MP3)	Scanalyzer 3D LemnaTec Greenhouse	RGB top and side views, IR, NIR	M.Sc. Emilio Vello	emilio.vello@mcgill.ca http://mp3.biol.mcgill.ca/mcgill_mp3_summary.html
University of Saskatchewan	Plant Phenotyping and Imaging Research Centre (P^2IRC)	Various vendors	PET, PCI, FLUO, K-Edge Subtraction imaging	Dr. Maurice Moloney	gifs.director@gifs.ca http://p2irc.usask.ca/
USA					
Arkansas State University	Plant Phenomics Facility	Scanalyzer HTS LemnaTec Growth chamber	RGB, IR, FLUO, NIR	Dr. Argelia Lorence	alorence@astate.edu http://plantimaging.cast.uark.edu
Donald Danforth Plant Science Center, St. Louis	Bellwether Phenotyping Facility	Scanalyzer 3D LemnaTec and Conviron Growth House	RGB top and side views, NIR	Mindy Darnell	mdarnell@danforthcenter.org https://www.danforthcenter.org/scientists-research/core-technologies/phenotyping
	Bellwether Phenotyping Facility	Fluorescence PhenoVation	PSII	Mindy Darnell	mdarnell@danforthcenter.org
	PhenoPiSight	Camera array for 3D reconstructions Raspberry Pi Greenhouse	RGB	Dr. Nadia Shakoor	nshakoor@danforthcenter.org, https://github.com/calizarr/PhenoPiSight
Iowa State University	ENVIRATRON	Rover In-house design Growth chamber	RGB, IR, NIR, FLUO, holographic, hyperspectral, Raman Scattering Spectrometer	Dr. Steven Whitham	swhitham@iastate.edu https://enviratron.iastate.edu/
Purdue University	Controlled Environment Phenotyping Facility	Aris, PhenoKey, Bosman van Zaal, AgriNomix, Conviron, in house design Greenhouse	RGB top and side views, PSII, hyperspectral, RGB hybrid	Dr. Yang Yang	yang1527@purdue.edu https://ag.purdue.edu/cepf/
Texas A&M	AgriLife Research Facility, Dallas	DroughtSpotter Phenospex Greenhouse	Gravimetric transpiration monitoring	Dr. Jeanmarie Verchot	jm.verchot@ag.tamu.edu https://agriliferesearch.tamu.edu/

Table 4 continued

Location	Facility name	Available platforms Manufacturer Scale	Sensors	Facility director or manager	Contact Website
University of Arizona-Maricopa	Maricopa Agricultural Center and USDA Arid Land Research Station	Field Scanalyzer LemnaTec Field	RGB, IR, PSII hyperspectral, multispectral radiometer, PRI, PAR, spectrometer	Dr. Nadia Shakoor	nshakoor@danforthcenter.org http://terraref.org/#phenotyping-field-scanner-system
University of Nebraska-Lincoln	Beadle Center	Scanalyzer HTS LemnaTec Growth chamber	RGB, IR, FLUO, NIR	Mr. Richard Perk	rperk1@unl.edu https://ard.unl.edu/phenotyping/beadle-hts-chamber
	Greenhouse Innovation Complex	Scanalyzer 3D LemnaTec Greenhouse	RGB top and side views, IR, FLUO, hyperspectral	Dr. Vincent Stoerger	vstoerger2@unl.edu https://ard.unl.edu/phenotyping/nebraska-innovation-campus-greenhouse
	Agricultural Research and Development Center	Spidercam Spidercam Field	RGB, NIR, LIDAR, multispectral	Dr. Geng “Frank” Bai	gbai2@unl.edu https://ard.unl.edu/phenotyping/field-phenotyping-facility
USDA ARS	USDA South Carolina	PlantEye F500 Phenospex Growth chamber	3D multispectral scanner	Phillip Wadl	phillip.wadl@ars.usda.gov https://www.ars.usda.gov/southeast-area/charleston-sc/vegetable-research/
Washington State University	WSU Pullman Phenomics Center	FluorCam Photon Systems Instruments Greenhouse	PSII	Dr. Helmut Kirchhoff	kirchhh@wsu.edu http://phenomics.cahnrs.wsu.edu
	WSU Pullman Phenomics Center	PlantEye F400 Phenospex Greenhouse	3D laser scanner	Dr. Arron Carter	ahcarter@wsu.edu
Washington University in St. Louis	Radiological Chemistry and Imaging Laboratory	Plant PET system In house design Growth chamber	PET	Dr. Yuan Chuan Tai	taiy@mir.wustl.edu
Mexico					
Colegio de Postgraduados	Padilla-Chacón Laboratory	Scanalyzer PL LemnaTec Growth chamber	RGB top and side views	Dr. Daniel Padilla-Chacón	daniel.padilla@colpos.mx http://danielpadillachaco.wixsite.com/fotosintatos

IR Infrared, *LIDAR* light detection and ranging, *NIR* near-infrared, *PAR* photosynthetically active radiation, *PCI* phase-contrast X-ray imaging, *PET* positron emission tomography, *PRI* Photochemical Reflectance Index, *PSII* Photosystem II fluorescence, *RGB* visible camera

calcium, and sulfur; and micronutrients such as sodium, iron, manganese, boron, copper, and zinc. Pandey et al. 2017 for example recently showed how HSI can be used in maize and soybean plants subjected to detect different levels of water deficiencies and nutrient limitation and determined the variation in the chemical properties (macro–micro nutrients) of plant leaves.

Table 5 International plant phenotyping networks currently operating

Acronym	Name	Website
APPF	Australian Plant Phenomics Facility	https://www.plantphenomics.org.au/about-us/
CPPN	China Plant Phenotyping Network	http://www.appp-con.org/
EPPN^{2020}	European Plant Phenotyping Network 2020	https://eppn2020.plant-phenotyping.eu/EPPN2020_home
IPPN	International Plant Phenotyping Network	https://www.plant-phenotyping.org/
LatPPN	Latin American Plant Phenomics Network	Not available; Camargo and Lobos (2016)
NAPPN	North American Plant Phenotyping Network	http://nappn.plant-phenotyping.org/

The importance of collaboration and coordination

Collaboration is paramount when considering the advancement of high-throughput phenotyping technologies, especially when factoring in what a truly interdisciplinary area of research this is. The expertise involved in HTTP research ranges from the actual engineering and manufacturing of the sensors and platforms, to the design and implementation of images analysis schemes by computer scientists, data aggregation, visualization, statistical analysis, and modeling, as well as deep knowledge of plant biology, genetics and biochemistry needed to interpret the meaning of all these data.

Established in 2011 the A-State Phenomics Facility currently functions as an academic research facility as well as a cost recovery-center. A summary of the HTTP facilities available in North America is presented in Table 4. We include relevant information about each platform including manufacturer(s), available sensors, website, and contact information of director and/or manager of each facility.

Recognizing the multidisciplinary needed for the success of HTTP approaches multiple countries have established national facilities that serve a large group of users, these include Australia, France, the UK, India, and China. To foster further interaction, collaboration, and coordination phenotyping networks have formed. Table 5 presents a summary of the regional and international plant phenotyping networks currently operating.

Conclusions and perspectives

Recent advances in high-throughput phenotyping technologies have offered a much more detailed look into plant growth and health. As these technologies improve, the link between genotypes and phenotypes will be further solidified, allowing researchers to engineer crops that can sustain our increasingly populated and over changing world. However, there are still many issues that need to be addressed.

With the advent of the high-throughput phenotyping movement, there remains a need for standardizing publication guidelines. Minimum requirements for published works and for experimental designs are still being fleshed out by North American and European Phenotyping Networks. Additionally, while there has been a great deal of advancement in the public sector regarding data repositories and open-source software, the bulk of phenotyping data accumulation is being done by corporate entities and is therefore not publically accessible. It is important to expand the amount of publically available data in searchable depositories in order to avoid redundancy and improve collaborative efforts.

As has also been discussed, there is a need to further reduce the entry cost into the phenotyping market to help fledgling laboratories and startups begin to make their own contributions.

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