Roadmap to High Throughput Phenotyping for Plant Breeding

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ORIGINAL ARTICLE

Roadmap to High Throughput Phenotyping for Plant Breeding

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Abstract

Background Population growth and climate uncertainty increase the demand for more food to feed the world. Finding a new variety and discovering novel genetic traits that confer stress tolerance will be a game changer in future crop production for sustainable agriculture. Developing and implementing high throughput phenotyping (HTP) technologies and skills become increasingly important to those involved with applying genomics in breeding and biotechnology research. Many institutes and private companies are initiating HTP programs to improve the quality and speed of plant breeding and biotechnology. Breeders and plant scientists need to secure solid data pipelines of phenotypes throughout years-long breeding programs, but they lack an engineering background on sensors, image processing, data management, and cloud-running architecture.

Purpose There are a series of components and steps that must be considered for HTP programs to meet their needs and goals. Those components include sensors, platforms, analytics, and data management. The aim of this paper is to address key information of what plant breeding is, why HTP is important, and how the HTP system is designed.

Review The paper describes background of plant science to engineering to enlighten the need of HTP and provides a review of the current HTP systems and future strategies. Discussion includes specifics of each HTP component to consider and a roadmap to HTP for plant breeding throughout genetics, phenotypic metrics, algorithm development, data standardization, and scale-up.

Keywords Breeding · Data Management · Image processing · Phenotyping · Sensors

Introduction

The advent of DNA sequencing technology combined with genomic data analysis has revolutionized plant biology in the past two decades (Phillips 2010; Jiao and Schneeberger 2017) and may allow us to predict the performance of crops based on their genetic constitution (Pieruschka and Schurr 2019). However, it has been difficult to record phenotypes on the scale needed for genetics studies and challenging to quantify plant interactions with the environment. The development of remote sensing and imaging technologies has provided new opportunities to phenotype the large diversity of plants populations from leaf to field levels and led to a substantial increase of plant phenotyping capacity.

Phenotyping is the measurement of aspects of plant growth, development, and physiology and arises from interactions between genotypes and environment, including fluorescence properties of the photosynthetic machinery, rates of growth, disease resistance, abiotic stress tolerance, gross morphology, phenology, and, ultimately, yield components and yield (Hickey et al. 2019). The plant research community needs accurate phenotyping to help plants to adapt to resource-limiting environments and low-input agricultural systems (Pieruschka and Schurr 2019). Unstructured high throughput phenotyping (HTP) data acquired in spatial and temporal dimensions requires standardization in data acquisition, sensor calibration, and metrics extraction to share the data and analyses in plant breeding community. Expedited phenotyping is essential for the advancement of plant breeding and crop management to measure diverse traits from an increasingly large number of plants.

To replace the laborious and inconsistent method of conventional manual phenotyping, breeding industry and public institutes are highly motivated to deploy image-based automated high throughput phenotyping (HTP). However, it is challenging to achieve a reliable imaging solution due to the variability of images affected by lighting intensity and angle. In addition, quality control of image acquisition and throughput processing must be solved. With the validation of data collection and development of image analytic toolbox, a novel

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method delivers image acquisition and processing to expedite advancement decision and enhance breeding programs. Benefits are highly estimated to breeding programs in government and industry.

Changes in Agricultural Industry

There are three main challenges in current agriculture: climate change, resource depletion, and population growth. According to U.N. report of Department of The Social and Economic Bureau, the current 7 billion (B) people are expected to be 10B by 2050 (United Nations 2019). In other words, we must provide approximately 45% more food in the next three decades. The current population has been already doubled since 1970 from 4B to 7B. During the past 4 decades, agricultural mechanization has been playing a major role to feed the world by automating the field machinery and increasing the yield. Can the agricultural mechanization continue the yield increase to meet the food demand in the next three decades? Can the precision agriculture provide 45% more yield? The agricultural mechanization has mostly reached the saturation and precision agriculture gets more toward environmental benefits by minimizing inputs while maintaining the yield. Biotech and breeding bring a new paradigm to meet the future food demand by increasing the yield doubles and triples. Average corn yield in the USA is about 8200 kg/ha (130 bu/acre) and precision agriculture may bring up a yield gain of 630~1260 kg/ha (10~20 bu/acre) by site-specific field management, while using biotech and breeding technology a corn grower in Virginia in 2017 had a world record of 34,021 kg/ha (542 bu/acre) (Agdaily 2018).

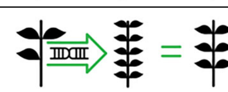
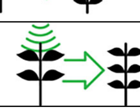
Plant Breeding

Plant breeding has been studied to improve yields, enhance taste, and extend growing seasons. All major crop plants, which provide 90% of the globe's food and energy intake, have been extensively manipulated, hybridized, inter-bred, and modified over the millennia by countless generations of farmers' intent on producing crops in the most efficient ways (Monsanto 2019). Table 1 summarizes the types of breeding and the mechanism of each type. Conventional breeding approaches have so far produced nutritious crops with high yields that can be harvested mechanically to meet the food needs of growing populations. But the current pace of yield increase for major crops such as corn, wheat, and rice is not sufficient to meet the future demand. Breeders and plant scientists are under pressure to improve existing crops and develop new crops that are higher yielding, more nutritious, pest- and disease-resistant, and climate-smart (Hickey et al. 2019).

Novel technologies have been developed in breeding industry for efficient breeding process. Doubled haploid (DH) technology revolutionized corn breeding expedited by inbreeding completely homozygous lines through haploid identification and chromosome doubling (Blakeslee and Avery 1937; Laurie and Bennett 1988). This saved a significant amount of breeding cost and time by reducing six generations taken in the conventional method to one generation. Today, all corn breeding companies use haploids to shorten the time required to produce parent lines by several years (Syngenta 2019).

For plants with larger seeds, seed chipping is another significant advancement in breeding technology and facilitated high throughput marker-assisted breeding. It is a process of sampling seeds with desired genotypes by extracting DNA makeup before planting, which avoids waiting for growing into tissue analysis and allows to determine and make seeds with undesired genetic makeup never planted. Automated chipping has been applied on corn, soy, cotton, wheat, melon, cucumber, etc. Traditional breeding for 5 traits needs 1 million plants sown, but with chipping 5 mg of thousands of seeds per day, space is no longer a limitation, with more stacked traits into the genotype, e.g., some lines with 8 genes, up to 20 genes in the near future.

Table 1 Types and descriptions of breeding (Folger 2019)

Traditional Breeding	Desired traits are identified in separate individuals of the same species, which are then bred to combine those traits in a new hybrid variety	
Interspecies Crosses	Desired traits are identified in separate individuals of the same species, which are then bred to combine those traits in a new hybrid variety	
Marker-Assisted Selection	When genes for a trait aren't precisely known, targeting a DNA marker near them can speed up breeding: It identifies plants with the trait even before they mature	
Genetic Modification	Genes identified in one species can be transferred directly to an unrelated species, giving it an entirely new trait—resistance to a pest, say, or to a weed killer	
Mutation Breeding	Seeds are irradiated to promote random mutations in their DNA. If a mutation happens to produce a desirable trait, the plant is selected for further breeding	

Genetically engineered crops can achieve highly specific tasks such as containing a bacterium that kills a certain pest. Such breeding takes $100 million and 10 years for regulatory approval to create one variety. Marker-assisted selection (MAS) is another breeding method and was first introduced in 2009 on a rice variety in India, taking 3 years to create a new rice variety once a genetic marker was identified. Molecular breeding strategies have placed greater focus on selection based on genotypic information, but they still require phenotypic data to train a prediction model in genomic selection and identify markers for subsequent selection throughout generations (Li et al. 2014). It is a powerful tool to assemble an array of desirable traits in a plant and has become alluring (Higgins 2014). Because the new technology focuses on finding desired genes and can lead to a long-term concern of the decreased genetic diversity, there needs to be a balanced approach between going for a particular trait and maintaining a diverse genetic population.

Plant Biotechnology

Traditional genetic breeding is used to find a new variety that contains desired genes by transferring many mixed (desired and undesired) genes from a compatible donor plant to a commercial variety, whereas modern plant biotechnology is used to create a genetic enhancement in a new variety by adding only one or a few desired genes selected from a donor plant to DNA strands of a commercial variety (Fig. 1). Plant biotechnology takes the breeding enhancement a step further, going directly to the plants' DNA to make the enhancement more precise and easier to control (Monsanto 2019). Examples of the gene coding technology are insect-protected cotton and maize and herbicide-resistant crops (soy, maize, and cotton) that have been commercially introduced. Given increasing demand of food, feed, and fuel, plant biotechnology provides a way for farmers to produce more yield on the same area of land with fewer inputs by improving crop protection from insects and diseases and increasing tolerance to heat, drought, and other stresses. Value-added biotech traits can provide consumer benefits such as increased protein or oil, improved fatty-acid balance, or carbohydrate enhancement (Monsanto 2019).

RNA interference (RNAi) is another approach for crop protection that leverages a naturally occurring process that occurs in plants and animals to protect plants from pests, weeds, and viruses and improving the health of beneficial insects such as bees and butterflies in a sustainable way. Pollination is also an important aspect in plant biotech, as agriculture and biodiversity depend on pollinations. Honey bees cover 1/3 of pollination and their economic values are $20B to value of U.S. crop production (KoreaDaily 2019). U.S. hives have been decreased from 6 M in 1947 to 2.4 M in 2008 due to pesticides and parasites (Papenfuss 2019). Fewer bees mean less pollinations and thus decrease in grains and fruits. There has been a controversy on herbicides that may impair bees' ability to return to the hive and decrease butterfly population by killing milkweed that is a primary food source of monarch butterflies (Balter 2019; Donley 2018; Tosi et al. 2017). There is a need to develop pesticides discriminating bees and butterflies from other insects. A potential solution is to provide more milkweed and other habitats in farms, ranches, and roadsides across the entire range of monarch's annual migration pattern from Canada to Mexico.

Phenotyping

Phenotyping is the measurement of plant's physiological and metabolic responses developed from the interaction of genotypes with the environment to identify key genes/alleles and associated molecular markers conditioning yield, abiotic stress tolerance, and agronomic traits. Plant breeding and biotechnology promote the development of new cultivars for sustainable agriculture. In order to enhance the selection process, robust phenotyping is critical because it is a basic tool to determine line selection at each stage of the years-long breeding pipeline. Traditional plant phenotyping for breeders includes walking through their trial fields and scoring plots based on how they look, taste, and/or feel. Improvements in phenotyping methods are highly desired and must address the

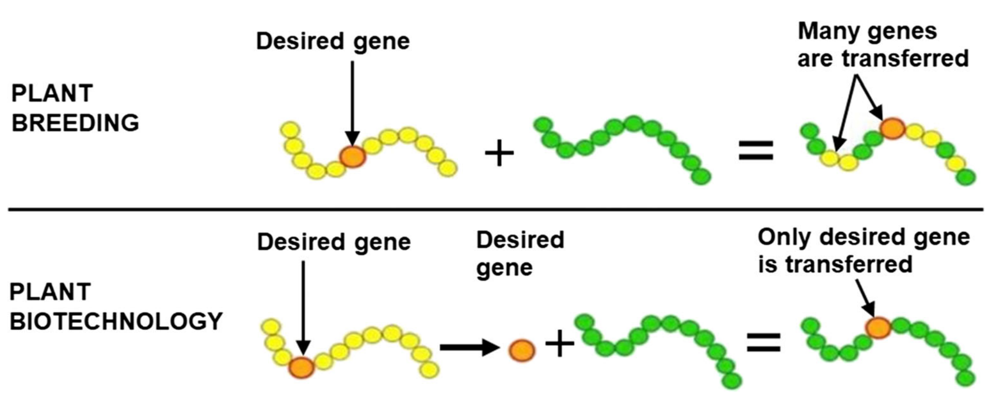


Fig. 1 Gene transfer of plant breeding and plant biotechnology (Katic 2015)

balance of accuracy, speed, and cost. Engineered phenotyping can augment what the breeders can see and offer better phenotype-based choices.

There are many private and public sectors pursuing plant phenotyping, with the goal of developing and implementing new HTP approaches that accelerate plant breeding for food, fiber, and bioenergy crops in certain environments. The technology promises to reduce costs and speed the development of better adapted crops for growers at a faster pace. Field-based selections are subject to limited resources and precision, but still highly effective for characterizing crop responses to environment for many traits that are more complex than those discerned in a lab.

A series of components and steps must be considered for HTP system design which includes sensors, platforms, analytics, and data management. The objective of this paper is to address key information of what plant breeding is, why HTP is important, and how the HTP system is designed so that the readers can build and implement their own HTP system in an effective way to meet their needs and goals.

HTP System

The development of automated HTP systems can enable evaluation of larger populations, which increases selection intensity and improves selection accuracy (Araus et al. 2018). When designing an HTP system for breeding or crop protection programs, the developers need to consider and understand components of the HTP system suitable for their needs. As shown in Fig. 2, a typical HTP system consists of four components including the sensor, platform, analysis, and data, so-called SPAD. These four components are interrelated and technically connected for seamless integration.

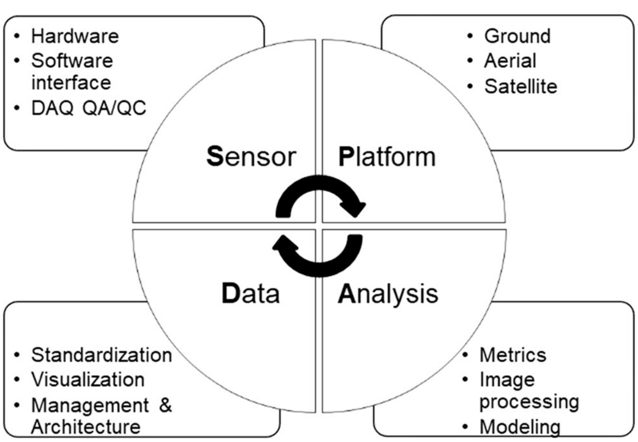


Fig. 2 HTP system components and roles: (1) sensor: hardware, software, data acquisition (DAQ) quality assurance and quality check (QA/QC), (2) platform: ground, aerial, and satellite, (3) analysis: metrics, image processing, and modeling, and (4) data: standardization, visualization, and management & architecture

Sensor

The sensor is the first component to consider when answering questions of what plant traits are of interest and what phenotypic metrics can be delivered. Typical phenotypic metrics include plant vigor, biomass, canopy temperature, plant height, stand count, etc. There are several types of sensors that can be used for phenotyping: multispectral, hyperspectral, thermal, and light detection and ranging (LiDAR). Spectral and morphological features are commonly measured by multispectral cameras that can estimate vegetation indexes, a leaf area index, stand count, yield, and growth rate. Plant height can be measured by LiDAR (Bai et al. 2019; Beauchêne et al. 2019; Burnette et al. 2018; Busemeyer et al. 2013; Thompson et al. 2019; Virlet et al. 2017), ultrasonic sensors (Andrade-Sanchez et al. 2014; Barker et al. 2016; Thompson et al. 2018a, 2018b; Thompson et al. 2019; Wang et al. 2016), or stereo camera. Canopy temperature can be measured by IR thermometer (Andrade-Sanchez et al. 2014; Barker et al. 2016; Crain et al. 2016; Thompson et al. 2018a, 2018b; Wang et al. 2016; White and Conley 2013) or thermal imager (Bai et al. 2019; Burnette et al. 2018; Drew et al. 2019; Park et al. 2017; Sagan et al. 2019; Virlet et al. 2017; Walter et al. 2015) and has been commonly used for plant stress detection and phenotyping. Other metrics such as CO_2 and chlorophyll are also measured by a gas analyzer and chlorophyll sensors, respectively. Among the variety of choices of sensors and metrics, the common consideration is to find cost-effective and robust sensors.

Spectral reflectance of plant canopy facilitates the functional mapping for genetic architecture of dynamic complex traits in a non-destructive mode. Multispectral imaging sensor has been widely used for plant sensing (Bai et al. 2019; Blancon et al. 2019; Burnette et al. 2018; Huang et al. 2010; Ostos-Garrido et al. 2019; Svensgaard et al. 2014; Walter et al. 2015). Multispectral sensor can deliver broadband indexes such as normalized difference vegetation index (NDVI) using (R800–R680)/(R800+R680) and normalized difference red edge (NDRE) using (R750–R705)/(R750+R705), whereas hyperspectral sensor offers narrowband indexes such as photochemical reflectance index (PRI) using (R531–R570)/(R531 + R570) and red edge index (REI) using R740/R720. Hyperspectral spectrometer and camera were also deployed for filed-based plant phenotyping by many researchers (Bai et al. 2019; Beauchêne et al. 2019; Burnette et al. 2018; Busemeyer et al. 2013; Comar et al. 2012; Virlet et al. 2017). For outdoor application, it is noted that the hyperspectral camera must be carefully calibrated as frequently as needed to accommodate illumination changes to acquire a valid spectral cube image. HTP system developers need to take a comprehensive decision for sensor selection by leveraging the sensor performance and operational cost. The sensor selection also must be coordinated with the platform selection to fit its size, weight, mounting, and data rate on the platform in height and speed.

The canopy pigment change is the most direct indicator of the plant response to abiotic stresses and commonly measured by spectral sensors in two types: point or image. Active spectral sensors such as Crop Circle (Holland Scientific, Lincoln, NE, USA) or GreenSeeker (Trimble, Sunnyvale, CA, USA) are point sensors that deliver an average point data per scan by producing active light and receiving the light reflected off the target surface. The active spectral point sensors were used for HTP (Andrade-Sanchez et al. 2014; Barker et al. 2016; Crain et al. 2016; Freeman et al. 2007; Hugie et al. 2018; Thompson et al. 2018b; Wang et al. 2016) but needs attention on sensor's linear field of view (FOV) on the full canopy coverage and spectral deflection along the solar zenith angles (Kim et al. 2012).

Multispectral cameras capture a spectral image and can deliver both spectral and morphological features through image analysis. Four commercially available models of the multispectral sensors are illustrated in Fig. 3 with specifications. One thing to notice is a frame rate: three models are 1 fps and one in 30 fps. If the camera is designed on the ground platform that needs to capture multiple images per second, the camera must be selected for more than 1 fps. Those 1-fps cameras can be used if slow-driven (< 4 km/h) ground platform or mostly for unmanned aerial vehicle (UAV) platforms where the image is captured per second or longer. Camera interface is another factor to be considered with acquisition software. Some models come with a built-in acquisition software (e.g., TetraCam, Chatsworth, CA, USA) and others come with an application program interface (API) (e.g., JAI, San Jose, CA, USA; MicaSense, Seattle, WA, USA) or pulse width modulation (PWM) interface for camera control (e.g., MAPIR, San Diego, CA, USA).

In developing in-house software for image acquisition, it is suggested to check in what program languages the API offered for the compatibility and integration of other HTP sensors. For ground/drone imaging, image acquisition requires a method to automatically collect images without missing area between images and with minimum overlap by georeferenced triggering algorithm and must be advanced to automatically trigger images only within the pre-defined field boundary.

For aerial HTP platforms, most aerial vendors use the same camera system family of Ultracam (Vexcel, Graz, Austria) in 4 bands (RGBN) with different resolutions: Eagle Mark 1 (20,100 × 13,080), Falcon Prime (17,310 × 11,310), X (14,430 × 9420), and Lp (11,704 × 7920). Many companies are flying the Falcon which has a resolution of 196 MP. Like a hyperspectral camera, a lines-scan multispectral camera (ADS100, Leica, Norcross, GA, USA) is another sensor for aerial imaging and ADS100 has a swath of 20,000 pixels in 4 bands (RGBN) with 120-mm focal length and 0.5-ms scan interval. Each airplane is equipped with global positioning system (GPS) receivers, inertial measurement unit (IMU), and an azimuth-stabilized camera mount. Once the airborne images are acquired by the multispectral camera, they are processed through a georeferencing tool such as Trimble (Sunnyvale, CA, USA) Digital Sensor System (DSS) to produce highly accurate, high-resolution digital orthophotos and orthomosaics.

Platform

HTP system equipped with various sensors can be mounted on three different HTP platforms: ground, aerial, and satellite. Each platform has its own pros and cons, and the developers need to know what outcomes and limitations are expected from each platform. Although there are still positive aspects on each platform, in general, the HTP research trend is shifting from the ground to the aerial/drone platform with several reasons such as increased coverage and mobility due to shortened acquisition window that minimizes the solar radiation effects. The future HTP platform design must accommodate the two most important factors: the field coverage in acquisition and turnaround time in data analytics. Decentralized platform along with plug-n-play sensors and development of a robust

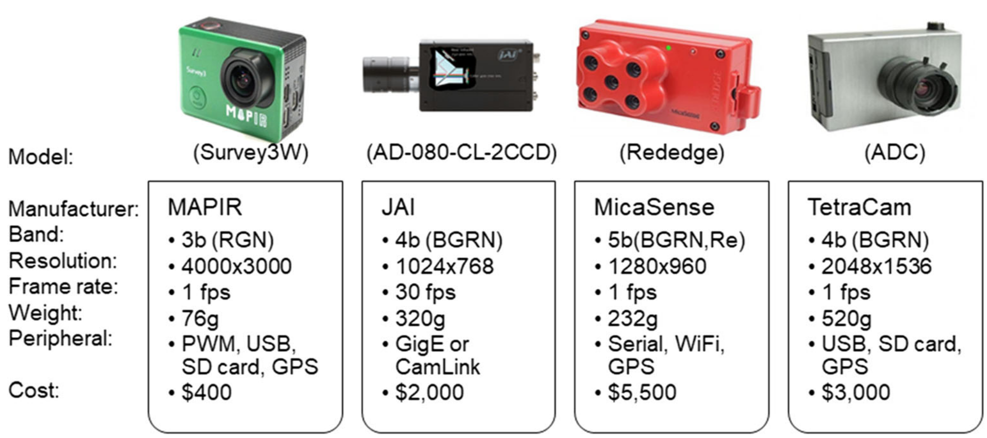


Fig. 3 Multispectral sensors commercially available for HTP systems: MAPIR (Survey3W, San Diego, CA, USA), JAI (JAI-AD-080-CL-2CCD, San Jose, CA, USA), MicaSense (Rededge, Seattle, WA, USA), and TetraCam (ADC, Chatsworth, CA, USA)

analytic toolbox to process raw data to plot-level metrics and visualization would be ideal for the required field coverage (e.g., 50 ha/h) and turnaround time (< 1 h).

Ground Platform

The ground platform is the most commonly used for plant phenotyping under two different environmental settings: indoors and outdoors. The most commonly used HTP platform is an outdoor ground platform operated by a cart or a tractor. Manual push-behind carts (Crain et al. 2016; Freeman et al. 2007; Thompson et al. 2018b; White and Conley 2013) were upgraded to motor-driven carts (Thompson et al. 2018a). The cart needs frames with adjustable height and width to accommodate different crop height and row space, and its design must consider the size and weight of the sensors, accessories, and battery to optimize the frame materials to avoid excessive loads on tires in turning and slopes. A motorized cart can be equipped with chain-driven electric motors and needs gear reduction to increase torque enough to smoothly start from a stop. Built-in geared hub motors are another option to simplify the drive and gear design and avoid chain maintenance. The motorized cart is controlled by a remoter controller through PWM signals and can be programed with GPS readings and path planning for upgrading to autonomous navigation. The current limited speed (< 1 m/s) of most HTP carts covering 2~4 rows per path needs to be faster (> 4 m/s) to increase the field coverage with technical upgrades on the FOV and data frequency of the sensors and interpolated georeferencing between GPS readings. There are some design concerns and recommendations: (1) use bigger tires (> 66 cm-dia.) to avoid mechanical displacement artifacts on the rough surface, (2) use a motor with enough power (at least 36VDC motor with 500 W), and (3) use light material for the cart frame to minimize cart flex and strain and support ease adjustment.

Tractor-based ground platforms are also widely studied (Andrade-Sanchez et al. 2014; Barker et al. 2016; Busemeyer et al. 2013; Comar et al. 2012; Higgs et al. 2019; Hugie et al. 2018; Montes et al. 2007, 2011; Peshlov et al. 2017; Svensgaard et al. 2014; Thompson et al. 2019; Wang et al. 2016, 2019). These ground platforms can measure great details of plant morphological and spectral features using proximal and spectral sensors. The major concern on the ground platforms is slow acquisition (e.g., < 3 km/h (1.9 mph)) that can be severely affected by ambient illumination changes, resulting in the narrow field coverage, and complexity of postprocessing of the hundreds of images for as well as plot-level georeferencing and noise filtering.

There are two types of indoor HTP platforms depending on stationary or mobile plants. If plants are fed into a scanning chamber on a conveyer belt, all sensors are mounted in the sensor package in different angles. The mobile plant-based HTP platform takes advantage of detailed observation under the controlled environment using various sensors such as VIS, NIR, fluorescence, stereo cameras, thermometer, and LiDAR. Fahlgren et al. (2015) used Bellwether Phenotyping Platform to study the temporal responses to water stress with automated watering and image capturing by daily feeding 1140 plants to the sensor station. The disadvantage of this method is a long operational time to feed all plants to the scanning chamber (e.g., 30 h for 30,000 images of 1140 plants (Fahlgren et al. 2015)) and frequently occurring maintenance issues in software with the sensors and hardware with the conveyer machine. The stationary plant-based HTP platform requires mobile sensors in a gantry mode to move to a target plant (Bai et al. 2019; Burnette et al. 2018; Virlet et al. 2017; Wang et al. 2018) using commercially available devices, Scanalyzer (LemnaTec, Aachen, Germany) or SpiderCam (Spidercam GmbH, Austria), respectively. Alternatively, the sensors can also be stationary using a grid of sensors permanently mounted over the entire area of the target plants, which relieves the operational issues with time delay and allows a localized troubleshooting of an individual sensor instead of the entire system stop caused by a single issue of the moving machine or sensor on the mobile plant-based platform.

Aerial Platform

Aerial phenotyping has two platforms: a manned aerial vehicle (MAV) and an unmanned aerial vehicle (UAV). MAV has been well established in urban and forest industry and increasingly adopted for agricultural applications (Huang et al. 2010; Pinter Jr. et al. 2003; Yang et al. 2013; Yang and Hoffmann 2015). The current MAV platform offers high-resolution multispectral imagery (> 200 MP). The aerial image quality is exceptional, and its ground sampling distance (GSD) can be as small as 2 cm at the lowest altitude of 305 m (1,000 ft) using Ultracam Eagle (Vexcel, Graz, Austria) covering 260 × 400-m footprint. The usage of MAV is expanded with additional sensors such as LiDAR, thermal, and hyperspectral sensors (Cornerstone Mapping 2019).

Sensors are mounted on a small airplane, popularly on Piper Saratoga (Piper Aircraft, Vero Beach, FL, USA), Air Tractor 402B (Air Tractor, Inc., Olney, TX, USA) (Huang et al. 2010; Yang and Hoffmann 2015), and Cessna 182, 210, or 320 (Cessna Aircraft Company, Wichita, Kansa, USA) (Huang et al. 2010).

MAV platform is mostly operated by aerial mapping vendors and their service charges for aerial imaging currently varies from \$2000 to \$8000 per flight based on mobility distance, GSD, additional sensors, ground control point (GCP) attachment, and post processing. The cost is high, but the quality is proved with no hassle of poor stitching and blurry pixels. The ground attachment of GCPs is optional to achieve high ground accuracy (< 10 cm) but can be skipped if relative accuracy of the field in the image is of interest. The final image captured on the aerial platform could be a spot image or an orthomosaiced image depending on the field size and GSD. The GSD for aerial HTP platform is recommended to 5~10 cm for plot-level accuracy resulting in 14~7-pixel swath per 30-in. row crop and 2 cm or less for plant-level accuracy offering 38-pixel swath per plant. Once the image is delivered from the vendor, the image needs to be further processed for plot-level metrics extraction.

UAV has been broadly adopted for agricultural applications: crop water use (Thorp et al. 2018), cotton boll detection (Yeom et al. 2018), maize green leaf index (Blancon et al. 2019), bioenthaol in cereals (Ostos-Garrido et al. 2019), and plant phenotyping (Sagan et al. 2019). UAV flying is currently regulated by FAA to be operated by a licensed person in line of sight with maximum limit of 122-m (400-ft) altitude, 25-kg (55-lb) payload, and 161-km/h (100-mph) speed. The tile images collected by UAV are orthomosaiced by commercial software, such as Pix4Dmapper (Pix4D, Prilly, Switzerland) or PhotoScan (Agisoft, St. Petersburg, Russia). The stitching is a time-consuming process and its quality is affected by the number of tile images, overlapping percentage, crop stage, and weather conditions, and thus consistency of stitching quality remains challenging. UAV platform is capable of carrying additional sensors of LiDAR, thermal, and/or hyperspectral sensors and expected to become more popular with decreasing costs and improvements in quality.

Satellite Platform

A satellite platform has been used by several government agencies to estimate weather conditions, crop areas, and yield. Private firms are investing in satellite remote sensing technology for decision support on crop production and developing models from high throughput satellite imagery for crop health monitoring. The most common satellite imagery has been provided in 11 spectral bands from Landsat operated by USGS and in 12 bands from Sentinel operated by European Aerospace Agency, but their image resolutions were limited to 30 m and 10 m with image update every 6 days and 5 days from Landsat 8 and Sentinel 2, respectively. High-resolution satellite imagery became commercially available in 3 × 3-m/pixel 4 bands (RGBN) from 120+ microsatellites (PlanetScope, Planet Lab. San Francisco, CA, USA). With over a hundred satellites in sun-synchronous orbit, PlantScope provides daily images anywhere on Earth at 3-m resolution (Planet 2019) and has been used in agriculture mostly on large scale or field-level crop health monitoring.

Though their latest satellites of 14 SkySats improved the image resolution to 1 × 1 m 4-band imagery, the current satellite imagery does not provide image resolution and frequency enough for phenotypic trait identification. In addition to improvements in image resolution and update frequency, there needs to be increases in plot-level geometric accuracy and spectral consistency of surface reflectance against solar and atmospheric cloud effects. As satellite and sensing technology grows, however, it is still possible for satellite as a future HTP platform when those limiting factors are addressed.

Roadmap

Platform selection needs to consider image processing methods. Main differences in image processing among the three platforms are clipping and stitching. When a ground platform is selected, image stitching is required to mosaic hundreds of tile images into a single master image of the field that is further processed for plot-level metrics extraction. UAV platforms also require image stitching from excessively (~90%) overlapped tile images to generate an orthomosaiced field image. On the other hand, MAV and satellite platforms generate images that need to be clipped to crop the oversized image into a field boundary. An image captured by MAV or satellite commonly contains multiple fields in a single shot and may require multiple rounds of clipping to separate into each field boundary image. Image clipping is straightforward to execute but can be more advanced by employing multi-gridding algorithms that process multiple fields at once to extract plot-level metrics per field. Accuracy of the stitching process is an ongoing challenge and still has room for improvement. These two processing tools must be considered when selecting and designing the analytic software.

With numerous commercial and custom-built image-based phenotyping platforms, it is likely that standard hardware will be used to capture image-based phenomics data in the near future (Fahlgren et al. 2015). Considering all pros and cons of ground and aerial platforms, MAV is currently the most reliable, accurate platform, but with consideration of the cost and accessibility, UAV will likely be the more common platform in the next decade. Acquisition software along with hardware platform has been commercially offered for user-friendly interface to easily adjust configuration parameters. The core part of image-based HTP is image processing of big data and a seamless data processing pipeline of plot-level metrics extraction validated for global consistency. Engineering decision of the sensor and platform selection needs to be (1) achievable for analytic methods to properly acquire and process the sensory data, (2) actionable for plant breeders with data delivered, and (3) scalable for farmers and plant breeders working at any scale to contribute to shared results.

Analysis

HTP has high potential to improve genetic modeling and expedite the identification of germplasm that increases the yield and productivity of crop plants. Several analytic toolboxes have been published: PlantCV (Fahlgren et al. 2015), LemnaGrid (Berger et al. 2010; Golzarian et al. 2011; Honsdorf et al. 2014; Munns and Tester 2008), HTPheno (Hartmann et al. 2011), and integrated analysis platform (Klukas et al. 2014). For instance, HTPheno (Hartmann et al. 2011) is implemented as a plugin for ImageJ and automates image analysis of barley plant in top and side views to extract height, width, and shoot area, but was applied only for images of a single plant captured in a chamber by LamnaTec system (LemnaTec, Aachen, Germany). Lack of standardized analytic tool delays data processing from different platforms and sensors and is a major hurdle in the HTP processing pipeline. Despite different sensors and platforms in existence, image analysis and trait extraction are common challenges for image-based phenotyping platforms, and thus open-source trait extraction software with a mechanism for community development will help to alleviate the phenotyping bottleneck on crop improvement (Fahlgren et al. 2015). The main aspects for HTP image analytics include image formation, radiometric calibration, stitching, and image analysis.

Image Formation

Image brightness is significantly affected in image formation by changes of ambient light condition and determined by light intensity and the camera settings of aperture, exposure, gain, and ISO. When an image is formed by receiving light energy through the lens, the camera captures the reflected light in the wavelengths that the camera's sensor is sensitive to. Most multispectral cameras use silicon that is sensitive in the visible to near-infrared spectrum from 400 to 1200 nm and captures the reflectance of objects in spectral bands through band-pass filters or prisms. Pixels have a value that ranges from a minimum to a maximum based on the image bit-depth. For instance, 8-bit images range from 0 to 255 and are displayed on a computer screen, while 16-bit images are formed with pixels ranging from 0 to 65,535 and requires GIS software to properly display on the screen by stretching to min and max values of pixels per band. When pixels reach the min (too dark) or max (too bright) values, image saturation occurs and must be avoided by adjusting camera settings. Another preparation step before image acquisition is to conduct white balance to properly assign the pixel scale from dark current to white reference in each band. If the image looks bluish or yellowish, the camera needs white balance to achieve normal color and brightness on plant image, which spaces out the pixels within a full range of spectral signal in each band and delivers correct pixel ratios among image bands. For plant phenotyping, the fixed camera settings are recommended for all channels during image acquisition to achieve true pixels on the target plant to avoid the independent pixel changes of each image band caused by automatic exposure (Kim et al. 2016).

Radiometric Calibration

Radiometric calibration is to obtain actual reflectance of the objects against illumination changes by reading pixels on a known reflectance reference and applying a calculated calibration factor to all pixels, which transforms an image to a calibrated image. This procedure repeats by applying the calibration factor to all other images captured under the same lighting condition or by calculating an individual calibration factor for the image if each image contains its own reference target. Each pixel in the calibrated image represents a percentage of reflectance and range from black (0% reflectance) to white (100% reflectance) in each spectral band. Once images are calibrated, there are two options for processing the images for plot-level metrics extraction: tile-based and stitch-based (Kim and French 2015). The tile-based process is to extract the metrics directly from tile images based on the georeferenced plot boundaries, while for the stitch-based process, the calibrated tile images are stitched into an orthomosaiced master image using in-house or commercial software and then plot boundaries are applied on the master image for metrics calculations. In the stitch-based method, the radiometric calibration is recommended before stitching due to the possible issues with unmatched features in stitching the uncalibrated images. If stitching software has difficulty in finding tie points between the calibrated images, however, the user can choose to stitch the raw images and then apply the calibration on the master image.

Stitching

Stitching is to create a master mosaiced image containing many tile images taken in the field. The results vary based on the capabilities of the software. GPS points tagged in each tile image are used to locate tile images on a master map and overlapped area between adjacent images are either smoothed or trimmed by a selection condition such as a ratio of foreground to background. Fine-tuning of stitching uses features of the images and its accuracy varies on contents of the images and decreases with complex matter such as plant canopy in the agricultural field where the images look very similar to each other.

The tile images can be stitched by cloud-based services and stand-alone programs running on a local computer. Cloud-based packages such as DroneDeploy (DroneDeploy, San Francisco, CA, USA) and MapsMadeEasy (Maps Made Easy, Bend, OR, USA) require tile images uploaded to their website and complete stitching on their cloud server, providing orthomosaiced images, digital terrain models, 3D model, and NDVI data. These cloud-based services do not support aligning the 1-band cameras with each other, so the stitched images cannot be layered into a composite image. Other software packages like Pix4Dmapper (Pix4D, Prilly, Switzerland)

and PhotoScan (Agisoft, St. Petersburg, Russia) support aligning the image datasets and allow the user to process the stitched layers in a raster for various band math. These programs use structure from motion (SfM) by looking at each pixel to match up the images and can stitch some images without georeference, but georeferenced images are stitched faster and better (MAPIR 2019). Some cloud-based solutions are also beginning to support SfM, but typically still not supporting process of multi-datasets with each other.

Image stitching algorithms have been studied in the last decades using color fusion in gradient domain (Chuang et al. 2009; Fattal et al. 2002; Levin et al. 2004) and image features such as scale invariant feature transform (SIFT) (Lowe 2004; Reddy and Chatterji 1996; Yang and Guo 2008), speeded up robust features (SURF) (Bay et al. 2006), and principal component analysis (PCA) (Ke and Sukthankar 2004). Juan and Gwun (2009) reported SIFT for stability and SURF for speed and concluded that PCA-SIFT is the best choice but still in need of improvement of blur performance. UAV image stitching has been studied using SIFT (Chen et al. 2019; Li et al. 2012; Zhao et al. 2019), SURF (Xiong et al. 2013) and custom-developed algorithm (Liu et al. 2011). Though many successful studies were carried out, fine-tuning for further improvement is required to meet the processing time, the geometric accuracy, and radiometric consistency for plot-level HTP data processing.

Ground Truthing

Ground truthing is a necessary step in developing an HTP system to validate the performance of sensors and analytic methods. Manual methods typically conducted to measure plant vegetation, biomass, height, canopy temperature, and stand count by using a handheld sensor equipped with a datalogger and built-in GPS or by taking samples for lab analysis. It is a tedious task but a key step to prove the performance of the HTP system by collecting valid data in a proper way throughout the season for at least two consecutive years. For the validation purpose, the wide field variation would enhance the validation of the capacity of HTP system compared with ground truthing.

Image Processing

In order to automatically transform images into meaningful phenotypic measurements, image processing plays a key role in feature extraction and data analysis. Throughput processing power and advance algorithm to handle vast amounts of images enabled image-based phenotyping as the most promising HTP tool for past few years. Various image processing pipelines were developed for growth rate by measuring leaf area (Leister et al. 1999), plant vigor by NDVI and thermal signature (Walter et al. 2015) and chlorophyll by fluorescence (Campbell et al. 2015), and shoot phenotypes by image classification (De Vylder et al. 2012). Machine learning has been also applied for data analysis such as object detection, segmentation, and classification (He et al. 2016; Tsaftaris et al. 2016; Ubbens and Stavness 2017), and leaf counting (Ubbens and Stavness 2017), plant disease detection and diagnosis (Mohanty et al. 2016), and fruit and flower classification (Pawara et al. 2017). In many image processing programs, parameters are manually tuned for a certain condition, but they can be invalid in changes of imaging conditions of lighting intensity, bidirectional effect, and atmospheric effect. The key success to the technology transfer and production is to make image processing pipeline automated by deploying a standardized method of radiometric and geometric calibrations, segmentation, and plot-level metrics extraction, regardless of image types and platforms.

Data

HTP data management is increasingly important as the big data are built up for spatial and temporal phenotypes from multiple fields in various regions under different weather conditions. HTP study generates huge volume of raw data in diverse formats and undergo data abundance with high-resolution, redundancy, and invalid or unnecessary data, and thus HTP data are heavily involved in big data management. Collecting phenotypic data by automated HTP machine like TERRA-REF, the world's largest robotic field scanner, can produce up to 10 TB data per day (Binder 2018) and is estimated to reach 10 PB over 3 years (Kooper et al. 2017). Many institutes similarly collect huge volume of phenotypic data and face a challenge as data piles, and thus the percentage of data that can be processed is decreasing. The metadata are essential to integrate the heterogeneous HTP data, but generating metadata for geospatial data is challenging due to the data's intrinsic characteristics of high-dimensionality and complexity such as space-time correction and dependency (Yang et al. 2017). Big data visualization uncovers patterns and discovers unknown correlations to improve decision-making (Nasser and Tariq 2015) and must provide interactive pictorial and graphical representation.

Phenotypic data collected in the field are transferred to cloud database (DB) through a local storage, accessed via cloud for data processing and analysis using an analytic toolbox, and cataloged for visualization (Fig. 4). Standardization for the data format and field layout is necessary to achieve seamless data processing and integration from the different platforms. Standard procedure and formats would allow transformative research and accelerated discovery through integration of data across species, time, and location (Shakoor et al. 2019). Once data is processed, visualization is implemented to allow the end-users to access field images and understand the results. In order to make easy access and quickly visualize the

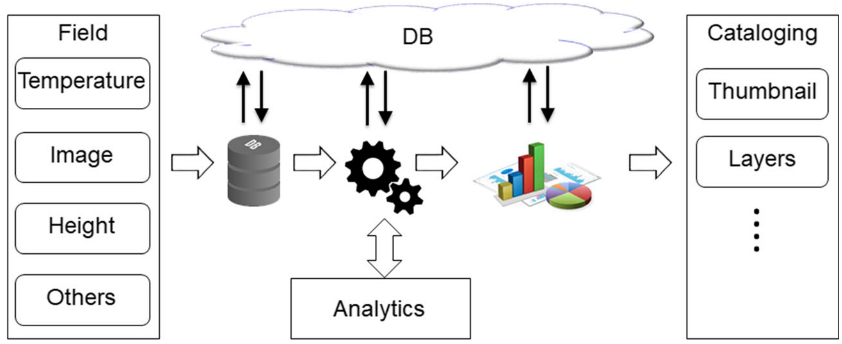


Fig. 4 Data management for HTP system: phenotypic data collected in the field are transferred to cloud database through a local storage, accessed for data processing and analysis, and cataloged for visualization

analysis, data architecture must be designed and implemented to organize where the data come and go, how they processed and visualized, and where they stored and accessed.

HTP research has a challenge to close a gap between the plant science and data analytics to ensure for plant science communities know how to use the HTP data and for data analytics communities understand how to deliver actionable results for scientists and farmers. Thus, coordination and integration among key discipline of breeding, genetics, agronomy, engineering, computer science, and statistics are needed so that these HTP tools are accessible for broad and applied agricultural use (Shakoor et al. 2019).

High-resolution proximal dataset has great details for plant-level phenotypes but is limited by a narrow spatial coverage in elongated paths due to the low speed and narrow FOV. In many cases, the resolution of proximal images on the ground platform is oversized and commonly downsized to save the processing time and memory without losing data accuracy. Remote sensory dataset from aerial/satellite platforms has a great spatial coverage in a short time window with high temporal resolution but has less performance in spatial resolution and ground accuracy. HTP data management needs to integrate these multiple layers of datasets from proximal ground platform and remote aerial/satellite datasets to improve phenotyping models field monitoring and coordinate them for cross validation and data enhancement.

One of the concerns in big data driven agricultural community is lack of data quality. There is a consensus that "garbage in" in terms of primary data quality results in "garbage out" of final data quality (Shakoor et al. 2019). For instance, poor quality of the positive images used for machine learning models would mislead to a poor quality of prediction results. Quality assurance and check become more important for sensor precision and consistency, and quality protocols need to be developed and standardized for the future HTP research programs.

Conclusions

Breeding industry and public institutes are highly motivated to image-based automated HTP to replace the laborious and inconsistent methods of conventional manual phenotyping. However, it is challenging to achieve a reliable imaging solution due to the variability of images affected by environmental uncertainty and limited analytics. This paper provides fundamental knowledge of the importance and consideration of the HTP system to help properly design and implement the applications of HTP research. Authors also addressed potentials and limitations of the current HTP systems and included suggestions to connect HTP research to production. Key to the success for HTP in the breeding industry is to meet the field coverage and turnaround time, i.e., speed of data collection and throughput processing. The development of standardized, automated analytic methods of calibrations, segmentation, plot-level extraction, and data management will bring the strong impact to breeding communities. Validation of global consistency takes an important role in the development of an analytic toolbox. A cost-effective, scalable HTP solution will be a promising tool to make benefits high at any scale in breeding programs. The HTP development needs joint efforts from interdisciplinary working group from plant scientists to engineers and further advancement to international community network.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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