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High throughput phenotyping to accelerate crop breeding and monitoring of diseases in the field

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Effective implementation of technology that facilitates accurate and high-throughput screening of thousands of field-grown lines is critical for accelerating crop improvement and breeding strategies for higher yield and disease tolerance. Progress in the development of field-based high throughput phenotyping methods has advanced considerably in the last 10 years through technological progress in sensor development and high-performance computing. Here, we review recent advances in high throughput field phenotyping technologies designed to inform the genetics of quantitative traits, including crop yield and disease tolerance. Successful application of phenotyping platforms to advance crop breeding and identify and monitor disease requires: (1) high resolution of imaging and environmental sensors; (2) quality data products that facilitate computer vision, machine learning and GIS; (3) capacity infrastructure for data management and analysis; and (4) automated environmental data collection. Accelerated breeding for agriculturally relevant crop traits is key to the development of improved varieties and is critically dependent on high-resolution, high-throughput field-scale phenotyping technologies that can efficiently discriminate better performing lines within a larger population and across multiple environments.

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Introduction

High throughput phenotyping (HTP) in the field

Rapidly rising demand in global food production requires a doubling of crop production yields by 2050 [1]. Advances in agronomic and breeding efforts to increase the rate of genetic improvement and enhance crop yield and stress tolerance has been limited by the process and costs of high-throughput phenotyping (HTP) methods. More recently, high-throughput, high-resolution phenotyping has emerged as a rapidly advancing discipline that successfully integrates plant science, engineering and computation to identify and assess both simple and complex plant traits that are key breeding targets for crop improvement including, but not limited to, plant height, biomass, flowering time and grain yield [2•]. Field deployment of various sensor technologies enhances the capacity and impact of agricultural studies by increasing the number and variety of germplasm tested by automating data collection and analysis. Phenotyping technologies that increase the throughput of plant screening can be used to generate complete sets of field data, speeding up the breeding process and increasing the rate of genetic gain and disease tolerance in field crops [3].

HTP platforms

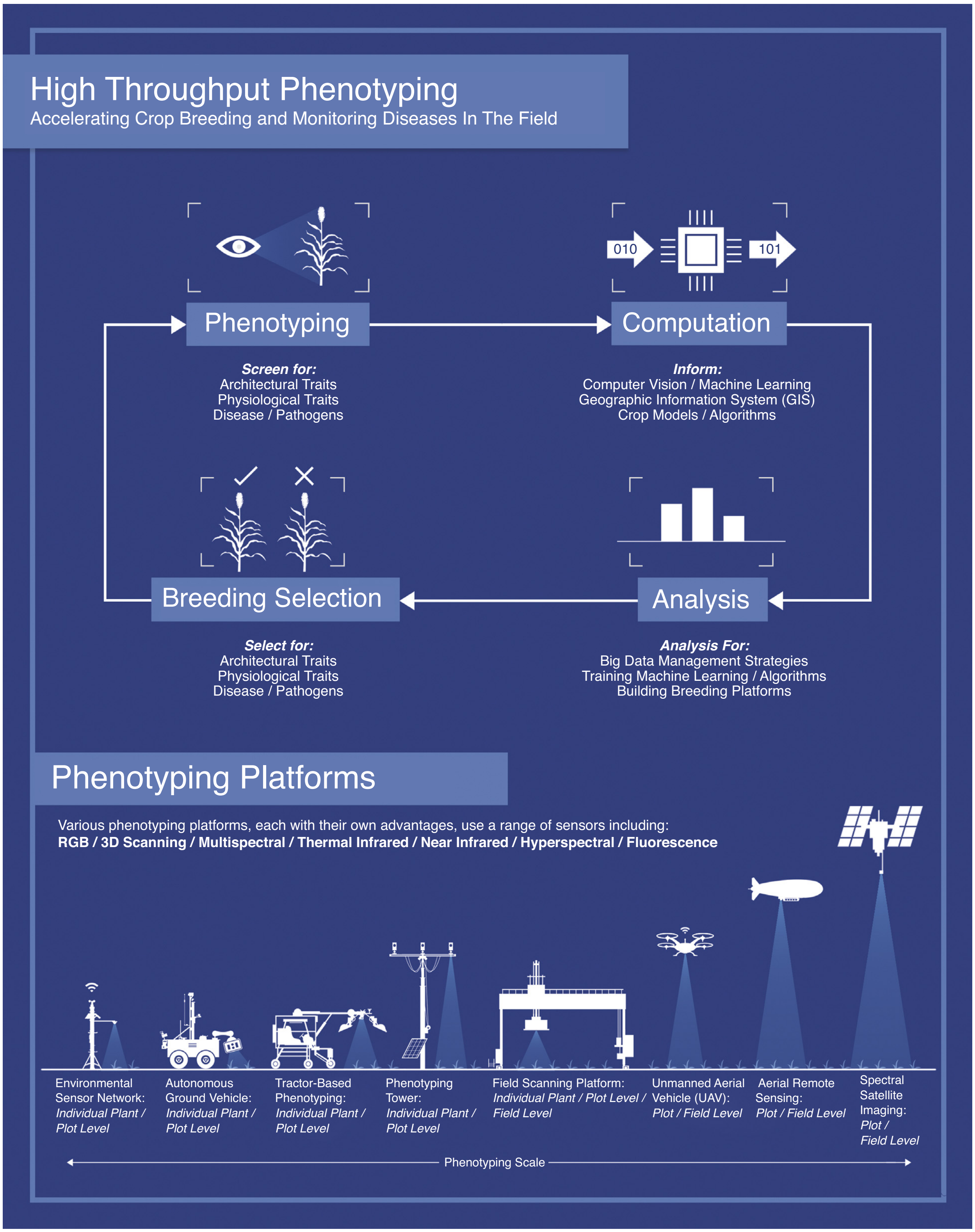
Varying the scale of phenotyping platforms from proximal to remote sensing allows for precise and consistent monitoring of single leaves/plant organs, individual plants, field plots and full fields as required (Figure 1). These platforms include, but are not limited to, environmental sensor networks [4], autonomous ground vehicles/rovers (www.terra-boost.com) [5], phenomobiles/tractors/buggies [6], phenotyping towers [5], field scanning platforms [7] (terraref.org), unmanned aerial vehicles (UAVs), aircraft, zeppelins and satellites [8•] (Table 1).

Yet new solutions yield new challenges, as high quality sensor systems can exceed the payload limitations of traditional field UAVs, necessitating the use of field scanning platforms and larger aerial aircraft such as planes and zeppelins. Field scale monitoring and identification of crop diseases over large growing regions are of practical importance in guiding differential field management and treatment regimes. Several high-resolution multi-spectral satellite-based sensors can also provide routine observations and remote monitoring of field crop health [9]. Satellite platforms are particularly promising for the identification and monitoring of crop diseases over large growing areas. Recently, satellite platforms equipped with multi and hyperspectral sensors, including NASA's Ecosystem Spaceborne Thermal Radiometer Experiment on Space Station (ECOSTRESS), Soil Moisture Active Passive (SMAP), and Hyperspectral InfraRed Imager (HyspIRI) aim to collect high-resolution spectral and environmental data and provide applications for drought mitigation and water use efficiency [10–12].

HTP to accelerate crop breeding and monitor disease

HTP techniques in crop breeding are generally used to screen for architectural traits and early detection of

Figure 1



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High throughput phenotyping in the crop improvement cycle and scales of phenotyping platforms.

Table 1

Attributes	Environmental Sensor Network	Autonomous Ground Vehicle (AGV)	Tractor-Based Phenotyping	Phenotyping Tower	Field Scanning Platforms	Unmanned Aerial Vehicles (UAVs)	Aerial Sensing (Zeppelin/ Plane)	Spectral Satellite Imaging
Phenotyping Scale	Individual Plant / Plot	Individual Plant / Plot	Individual Plant / Plot	Individual Plant / Plot	Individual Plant / Plot / Field	Plot / Field	Plot / Field	Plot / Field
Sensor Payload Size	Small / Medium	Medium	Medium / Large	Small / Medium	Large	Small	Large	Large
Can Be Autonomous?	Yes	Yes	Yes	Yes	Yes	No	No	Yes
Data Post-Processing	Light	Light	Moderate	Light / Moderate	Significant	Moderate	Moderate	Significant
Data Collection Interval	Continuous	Daily*	Daily*	Continuous	Continuous / Daily	Daily*	Daily*	Daily*
Platform Accessibility	High	Low	Medium / High	Medium	Low	High	Low	Low / Medium

*Dependent on environmental conditions

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Phenotyping platforms and their attributes.

desirable genotypes. HTP allows for accurate, automated, and repeatable measurements for traits such as seedling vigor, flower counts, biomass and grain yield, height, leaf erectness, and canopy structure. HTP can also be applied for screening physiological traits, including photosynthesis, transpiration, disease and stress tolerance. Accurate and early season detection of plant diseases are key to reducing crop yield losses. Plant disease diagnosis traditionally relies on symptom recognition through visual observation and ratings. HTP, specifically methods such as RGB imaging, 3D scanning, thermal and near-infrared sensing, multi-spectral and hyperspectral sensing, and fluorescence imaging have successfully been used to identify, quantify and monitor plant diseases. A variety of sensors technologies have been used for different plant pathogen systems [3] (Table 2). Careful consideration and understanding of appropriate sensors and HTP time-points for field traits of interest is critical for manageable data collection and analysis. For example, low cost and low maintenance sensing technologies such as RGB camera networks can be deployed to collect images continuously over days and weeks to assess traits including growth rate, biomass, and disease progression. Further, high-resolution sensor technologies such as hyperspectral imaging are time-intensive, and field scans may be technically feasible only a few times in a growing season. Thoughtful experimental design and a clear definition of the scientific questions will determine the HTP platform and timescale necessary for the most informative data collection strategy.

Sensing technologies for HTP

RGB/stereo RGB

Suitable for use under natural illumination outdoors, RGB cameras are key optical sensors for non-invasive field phenotyping [13]. Providing high-resolution data with fast acquisition rates, RGB cameras allow for rapid and objective assessment of plant growth, architecture and disease screening [14–18]. The use of single RGB images for effective field phenotyping however is limited by inherent size distortions in the 2D image plane caused by areas of the plant or field plot being closer to the camera than others. 3D information generated by stereo RGB imaging can increase the precision of phenotypic data. For example, a recent study in grapevine used stereo RGB imaging to calculate fruit to leaf ratios and assess the growth habits of new breeding lines compared to known cultivars [19].

3D laser scanning

3D laser scanning techniques, including LIDAR, have been used extensively in the last 10 years due to the

Table 2

Sensor	Examples of Potential Applications	Disease/Pathogen Assessment	References for Disease/Pathogen Assessment
RGB / Stereo RGB	biomass, morphology, height, leaf area and surface normal angles, disease symptoms, growth dynamics, yield traits, panicle traits, root architecture, germination rates, flowering time	**Cotton**: Bacterial angular (Xanthomonas campestris), Ascochyta blight (Ascochyta gossypii); **Sugar beet**: Cercospora leaf spot (Cercospora beticola), Sugar beet rust (Uromyces betae), Ramularia leaf spot (Ramularia beticola), Phoma leaf spot (Phoma betae), Bacterial leaf spot (Pseudomonas syringae pv. Aptata); **Grapefruit**: Citrus canker (Xanthomonas axonopodis); **Tobacco:** Anthracnose (Colletotrichum destructivum); **Apple**: Apple scab (Venturia inaequalis); **Canadian golden-rod**: Rust (Coleosporium asterum); **Potato**: Late blight (Phytophthora infestans)	Camargo and Smith (2009); Neumann et al. (2014); Bock et al. (2008); Wijekoon et al. (2008); Sugiura et al. (2016)
3D Laser Scanner	plant architecture: height, leaf area, leaf angle distributions, canopy structure	**Sugar beet**: Cercospora leaf spot (Cercospora beticola); **Oil palm**: Basal stem rot (Ganoderma lucidum)	Roscher et al. (2016); Khosrokhani et al. (2016)
Multispectral	senescence evaluation, nutrient status, pigment degradation, photosynthetic efficiency, water content	**Avocado**: Laurel wilt (Raffaelea lauricola) **Citrus**: Citrus black spot (Guignardia citricarpa) **Cassava**: Cassava mosaic virus	de Castro et al. (2015); Pourreza et al. (2016);
Thermal Infrared (IR)	transpiration, heat stress, leaf senescence, leaf/canopy temperature, water stress, disease, and pathogen detection, evaluating fruit/vegetable maturity, and bruise detection	**Sugar beet**: Cercospora leaf spot (Cercospora beti-cola); **Cucumber**: Downy mildew (Pseudoperonospo-ra cubensis), Powdery mildew (Podosphaera xanthii); **Apple**: Apple scab (Venturia inaequalis) **Olive**: Verticillium wilt (Verticillium dahliae)	Chaerle et al. (2004); Berdugo et al. (2014); Oerke et al. (2006); Oerke et al. (2011); Calderón et al. (2015)
Near Infrared (NIR) 700-1100nm	transpiration, water content heat stress, NDVI, leaf area index	**Barley**: Powdery mildew (Blumeria graminis hordei) **Wheat**: Powdery mildew (Blumeria graminis f. sp. Tritici)	Kuska et al. (2015); Cao et al. (2015)
Visual-Near IR (VNIR) 380-1000nm	vegetation indices, leaf structure, lignin/flavonoid contents, leaf and canopy water status, leaf senescence, chlorophyll fluorescence, vegetation indices	**Barley:** Net blotch (Pyrenophora teres), Brown rust (Puccinia hordei), Powdery mildew (Blumeria graminis hordei); **Sugarcane**: Orange rust (Puccinia kuehnii); **Wheat**: Head blight (Fusarium graminearum); **Almond**: Red leaf blotch (Polystigma amygdalinum)	Wahabzada et al. (2015); Apan et al. (2004); Bauriegel et al. (2011); López-López et al. (2016)
ShortWave IR (SWIR) 900-2500nm	water, lignin, cellulose contents, fluorescence, vegetation indices	**Barley**: Net blotch (Pyrenophora teres), Brown rust (Puccinia hordei), Powdery mildew (Blumeria graminis hordei); **Apple**: Apple scab (Venturia inaequalis); **Maize**: Phaeosphaeria leaf spot (Phaeospharia maydis)	Wahabzada et al. (2015); Delalieux et al. (2007); Adam et. Al (2017)
Fluorescence	photosynthetic status, quantum yield, non-photochemical quenching Fv/Fm, heat or drought stress, architecture leaf/plant health	**Wheat:** Leaf rust (Puccinia triticina), Powdery mildew (Blumeria graminis f. sp. tritici); **Sugar beet**: Cercospora leaf spot (Cercospora beticola); **Bean**: Common bacterial blight (Xanthomonas fuscans subsp. fuscans); **Lettuce**: Downy mildew (Bremia lactucae); **Rice**: Leaf scald (Monographella albescens)	Bürling et al. (2011); Chaerle et al. (2004, 2007); Konanz et al. (2014); Rousseau et al. (2013); Bauriegel et al. (2014); Brabandt et al. (2014); Tatagiba et al. (2015)

Source: Updated from Mahlein (2016). Reference list for Disease/Pathogen Assessment is not comprehensive

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Disease assessment by HTP sensor technologies.

robustness and resolution of the sensors to create accurate and detailed 3D models by precision light projection and scanning. Laser scanners can be used to rapidly calculate leaf area index (LAI) [20] and, in a number of recent crop phenotyping studies, allow for measurements that can differentiate genotypic variation in plant architecture, canopy traits, and growth rates. Frequent 3D scanning has successfully been used to assess field-level growth fluctuations in response to rapidly changing environmental conditions [7,21,22]. Custom-designed, multisensor 3D laser scanning platforms (e.g. LeasyScan) combine 3D laser scanning with lysimetric measurements to assess canopy traits affecting water use (e.g. leaf area, leaf area index, transpiration) [23]. Recent studies combining 3D data with additional optical sensor data (e.g. hyperspectral images) indicate a marked gain in accuracy of disease detection with applied data fusion methods [24•,25].

Thermal IR

Thermal infrared (IR) cameras are used to visualize differences in temperature and are reliable and scalable crop phenotyping instruments for assessing canopy temperature in large field experiments. Radiometrically calibrated thermal cameras allow for airborne thermography from UAVs and manned aircraft. Thermal image data can be gathered from large field experiments in seconds for essentially simultaneous measurement of canopy temperature on hundreds to thousands of field plots [26]. Thermal IR cameras can be used to monitor disease outbreaks [27–31] and provide quantification of plant responses to water stress in the field. For example, a recent study identified significantly higher canopy temperatures in water-stressed apple trees compared to well-irrigated ones. By using thermal IR data to characterize individual plant responses to drought, the authors were able to analyze and link the genotypic variation of individual apple trees with differential phenotypic responses to water limitation [32].

Multi and hyperspectral

Multi and hyperspectral imaging techniques divides each pixel of an image into wavelength bands and capture electromagnetic radiation reflected from vegetation in the visible (400–700 nm), near infrared (700–1300 nm) and short-wavelength infrared (1400–3000 nm) regions, which may contain information about leaf physiological status, water content, pigments, and structural components of biomass [33•]. The reflected spectra of crop plants convey information about plant architecture and health and can be used to evaluate plant growth characteristics. For example, healthy green plants reflect NIR light from 700 to 1500 nm, whereas plants under stress have a reduced red absorption in the chlorophyll active band and reflect more red wavelength light compared to healthy plants [34]. Vegetation spectra can be captured in the order of seconds, and numerous indices have been utilized to estimate leaf foliar traits, including chlorophyll content, nitrogen (N) content and photosynthetic radiation use efficiency [33]. The high spectral resolution of hyperspectral technology also makes it a promising method for detecting the severity of damage caused by disease [35,36•,37–45]. For example, sequential combinations of multiple hyperspectral indices have been used to quantify levels of stripe rust infection and N deficiency in wheat crops [46•].

Fluorescence imaging

Fluorescence imaging quantifies the light reemitted from the chlorophyll *a* photosynthetic pigment and can provide information about the photosynthetic apparatus under abiotic and biotic stresses. Useful for early disease detection, perturbations in fluorescence readings can often precede the appearance of visible symptoms [27,45,47–52]. For example, fluorescence spectroscopy was used to distinguish citrus plants experiencing bacterial infection underlying citrus greening [53]. A combination of fluorescence imaging with thermal image analysis techniques was useful for identification and quantification of fungal infections in avocado [54]. Research has been directed at extracting fluorescence parameters from sun-induced reflectance in the field, which has potential for plant disease assessment at both the canopy and field level. The use of solar-induced chlorophyll fluorescence was recently implemented successfully for the early detection of cassava mosaic disease [55]. Fluorescence imaging is continually improving, however it is subject to the same limitations as other spectral imaging techniques, including inconsistent illumination and environmental disruptions (e.g. wind) under field conditions [56].

HTP to inform machine learning (ML)

Machine learning (ML) employs a variety of statistical and probabilistic tools to 'learn' from massive collections of crop phenotypes and environmental datasets to classify unique data, identify new patterns and predict novel trends. Following initial data collection, the use of ML methods relies on several key aspects of data preprocessing, including identification, classification, and quantification. Data preprocessing for crop yield traits and plant disease detection is a pivotal step before ML approaches are applied and involves curating images based on quality, resolution, contrast, segmentation, thresholding, image format, and feature extraction. Numerous open source tools, packages, and programs have been developed to assist with or implement computer vision or image preprocessing, and they are implemented in a variety of programming languages including Python and ImageJ [57–60].

For identification/classification in phenotyping, numerous ML methods have been utilized, and they divide into several categories including supervised versus unsupervised learning and generative versus discriminative modeling. In supervised ML, training datasets are labeled with classifications before implementation (e.g. healthy leaves versus diseased leaves). In unsupervised learning, no initial classifications are made and the data are clustered based on pattern recognition into discrete classes. Discriminative ML models learn and classify data based on two distinct data patterns and learn to separate the distinct groups whereas as generative approaches attempt to capture overall data patterns to form more general classification models. Discriminative classification models tend to be more suitable for discrete problem spaces such as classifying maize plants versus Arabidopsis plants. Generative classification models tend to be more general and useful at analyzing more complex, less well-defined datasets. In terms of phenotyping for plant disease, discriminative models tend to perform better for classification, as generative models tend to be prone to overfitting data and perform poorly when applied to new datasets.

Two separate studies focused on automating disease detection in tomato utilized a support vector machine (SVM) classifier to isolate plant tissue followed by feature extraction and achieved accuracy above 90% in predicting disease onset [61,62]. One group utilized a robust image database in conjunction with an established deep convolutional neural network (CNN), known as Caffe, and could achieve, on average, 96.3% precision in measuring plant disease through leaf image classification [63,64]. Another group also utilized a convolutional neural network approach and public image database, consisting of over 50 000 images of diseased and healthy plants, and achieved greater than 99% accuracy in identification of 14 species and 26 disease states [65•]. However, one study concluded that spectral vegetation indices, a method that utilizes spectral measurements to estimate plant health, had higher accuracy and sensitivity for measuring varying disease symptoms when compared to several machine learning approaches [66•]. ML approaches, while promising, are not without flaws and require careful consideration in terms of image curation and processing, as well as appropriate selection of ML methods and model choice. Yet, as image datasets increase in size, scope and complexity, ML still represents the most viable approach for generating meaningful insights and analysis from exponentially growing field crop image datasets.

HTP infrastructure for data analysis/big data management

Effective use of HTP requires data management strategies for handling raw data, metadata, derived data, derived data provenance, and standardized processing workflows. To guide crop breeding decisions, reducing the sheer volume and dimensionality of plant sensor data with high spatial, temporal and spectral resolution will require advanced methods of data management, analysis, and interpretation. Public [67] and private efforts (CropOS: www.bensonhillbio.com/technology) to utilize big data analytics and machine learning capabilities to improve crop performance are dependent on successful integration of multiple layers of data, including pedigrees, sequence data, genotypes and phenotypes.

Leveraging field HTP with environmental sensors and geographic information system (GIS)

Environmental conditions throughout a growing season, including temperature, rainfall, radiation intensity, soil moisture, relative humidity and day length are all key contributors to predicting crop yield and disease tolerance. Accurate environmental characterization can be achieved using geographic information system (GIS) for crop monitoring. GIS allows users to search and link traits to spatial data by combining geographic data to generate maps and reports, enabling users to collect, manage, and interpret location-based information. The sources of such crop data include satellite imagery, aerial photos, maps, ground surveys, and global positioning systems (GPS).

Robust computational models for crop yield and disease traits require the incorporation of genotypic, phenotypic, environmental and crop management data. The concept of 'envirotyping' encompasses the measurement of all environmental factors that affect plant growth and production at the resolution of field plots and individual plants. Ensuring that envirotypic data are collected concurrently with specific genotypic and phenotypic data effectively inform crop modeling and phenotype prediction [68]. Guiding current crop improvement and breeding strategies, reliable estimates of early season phenotypic traits and robust crop models could dramatically reduce the experiment duration, number of plots and locations needed for crop improvement trials. For example, ML and algorithms for predicting late-season phenotypes such as total biomass from early season phenotypes could be used to rapidly make multiple rounds of line selections within one growing season.

Conclusions and prospects

With the rapid advancement of robust and high quality genetic and genomic technologies, the functional analysis of crop genomes is currently limited by the quality and speed of high throughput phenotyping. Perpetual advances in genomics and HTP creates multiple layers of valuable information that can be exploited to rapidly advance crop breeding and monitoring of diseases. In recent years, major contributions from government and private organizations have been invested in the creation and use of HTP tools to speed the development and deployment of phenotyping and breeding technologies to benefit researchers and farmers. Projects funded by the Department of Energy (terraref.org) and organizations such as the Bill & Melinda Gates Foundation aim to use HTP to increase the productivity and resilience of crops that can reduce hunger and poverty and make communities economically stronger and more stable. International and U.S.-based organizations and universities are employing cutting-edge technologies to sequence and analyze crop genomes, along with capturing millions of phenotypic observations across growing seasons and accelerating crop breeding efforts by connecting those phenotypes to genotypes.

Integrating heterogeneous information from reliable, automated, multifunctional, and high-throughput phenotyping platforms will require the continued development of novel technologies, with more effort dedicated towards developing low cost and high performance HTP technologies. With multifunctional phenotyping platforms obtaining large quantities of sensor data and images, data-storage, management and analysis will continue to be a challenge for crop HTP. Data volume is dependent on the resolution of the sensors/imagers and the numbers of acquired readings. To further promote the application of HTP in crop improvement programs, less expensive and more accessible data analysis infrastructures will need to be developed.

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