

Crop Phenomics and High-Throughput Phenotyping: Past Decades, Current Challenges, and Future Perspectives

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ABSTRACT

Since whole-genome sequencing of many crops has been achieved, crop functional genomics studies have stepped into the big-data and high-throughput era. However, acquisition of large-scale phenotypic data has become one of the major bottlenecks hindering crop breeding and functional genomics studies. Nevertheless, recent technological advances provide us potential solutions to relieve this bottleneck and to explore advanced methods for large-scale phenotyping data acquisition and processing in the coming years. In this article, we review the major progress on high-throughput phenotyping in controlled environments and field conditions as well as its use for post-harvest yield and quality assessment in the past decades. We then discuss the latest multi-omics research combining high-throughput phenotyping with genetic studies. Finally, we propose some conceptual challenges and provide our perspectives on how to bridge the phenotype–genotype gap. It is no double that accurate high-throughput phenotyping will accelerate plant genetic improvements and promote the next green revolution in crop breeding.

Key words: crop phenomics, high-throughput, field phenotyping, root system architecture, yield and quality, genetic studies

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INTRODUCTION

The term “phenotype”, which comes from the Greek *phainein* and *typos* (meaning show and type, respectively), was characterized by Wilhelm Johannsen in 1911: “all types of organisms can be distinguished by direct inspection or with finer methods of measurement or description” (Johannsen, 1911). In 1949, as genome was defined as the material basis of the genotype, the term “phenome” was first defined as the sum total of extragenic, non-autoreproductive portions of the cell and represented the set of phenotypes (Davis, 1949). In the 1990s, in the context of the study of complex human disease and as the key complement of genomics, a new discipline, phenomics, emerged with the aim of bridging the gap between genes and clinical endpoints (Schork, 1997). In 2010, Houle et al. (2010) defined phenomics as the acquisition of high-

dimensional phenotypic data on an organism-wide scale. In the field of plant science, Fiorani and Schurr (2013) referred to plant phenotyping as the set of methodologies and protocols used to accurately measure plant growth, architecture, and composition at different scales. In addition, we prefer to define crop phenomics as the multidisciplinary study of high-throughput accurate acquisition and analysis of multidimensional phenotypes on an organism-wide scale through crop development.

Next-generation sequencing technology has greatly accelerated progress in functional genomics (Huang et al., 2010; Werner,

2010; Li et al., 2018), allowing quantitative trait locus (QTL) mapping and genome-wide association studies (GWASs) (Xiao et al., 2017) to become powerful tools for elucidating the genetic architecture of complex traits (Atwell et al., 2010; Huang et al., 2010; Tian et al., 2011; Wang et al., 2018a; Zhang et al., 2019a), and many genes governing important agronomic traits have been identified (Zuo and Li, 2014; Yao et al., 2018; Fernie and Yan, 2019; Shi et al., 2019). For example, before 2000, only approximately 130 genes had been cloned in rice. By the end of 2017, more than 3000 genes were well characterized through various traditional phenotypic approaches (Yao et al., 2018). However, phenotypic data acquisition is still a bottleneck restricting crop breeding and functional genomics studies (Deery et al., 2016). Traditional crop phenotyping methods are labor intensive, time consuming, subjective, and frequently destructive to plants (Furbank and Tester, 2011; Chen et al., 2014). The technological advances have, in general, lagged far behind the developments of other “omic” technologies and tend to be fragmentary.

In recent years, advanced sensor, machine vision, and automation technology has been widely adopted across the agri-food industry to augment automation and promote efficiency, with applications ranging from product quality assessment to sorting and packaging (Ruiz-Garcia et al., 2009). In medicine, noninvasive clinical screening and assessment methods have proven their value over many years and are increasingly being developed for physiological measurements. The attractiveness of this package of technologies is its potential for scaling and the promise that it could begin to match the other omics. The fact of adapting such technologies in farming is that the production-grade processes are often satisfied with binary decisions, delivered rapidly, and discarded immediately after use, although vision-guided robotics is developing rapidly and should allow the technology to be applied in less constrained environments, including on the farm (Pieruschka and Schurr, 2019). In addition, biomedical procedures can tolerate a substantially higher cost threshold than agriculture and already implement diverse highly sophisticated sensors, ranging from computed tomography (CT) imaging to targeted metabolic sensors.

Advances in a range of technologies, from sensors to information technology (IT) and data extraction, combined with systems integration and decreasing costs, means that morphology and physiology can be assessed non-destructively and repeatedly across whole populations and throughout development. However, the technologies are still under active development. Over the past decade, the question of why and how to measure all of genomics has been investigated in depth, and now technological advances will allow us to answer the question of why and how to measure whole-plant phenotypes in the coming decades (Houle et al., 2010). In this article, we review the major progress on high-throughput phenotyping in controlled environments and field conditions as well as its use for post-harvest yield and quality assessment in past decades (Figure 1). We analyze and discuss the latest multiomics studies combining high-throughput phenotyping with genetic studies. Finally, with reference to the survey and database of the International Plant Phenotyping Network (IPPN), we discuss the current challenges in crop phenomics and provide our perspectives on crop phenotyping-related research.

DYNAMIC PLANT PHENOTYPING

Multiple genes interact with the multiple environments (G×E) across the life cycle to affect performance (Orgogozo et al., 2015). The effect of the environment on plants can be particularly pronounced because their static lifestyle precludes the use of the avoidance strategies commonly used by animals. Sensor technologies now enable the detailed recording of the environmental history of plants as well as the dynamic response of plants or crops. Since the whole-genome sequencing of *Arabidopsis* and many other crops (https://genomevolution.org/wiki/index.php/Sequenced_plant_genomes) has been achieved (Kersey, 2019), the next goal is to describe the whole phenotypes of important crops and to dissect the key functional loci (genes or QTLs) and better understand the crop genetic architectures. High-throughput phenotyping platforms will play a key role in achieving this goal. In this section, we discuss high-throughput shoot phenotyping for *Arabidopsis* and larger crops and root phenotyping in the laboratory environment (Table 1).

High-Throughput and Integrated Shoot Phenotyping: Unlimited Expansion in Model Plants

Over the past few decades, many intelligent and high-throughput phenotyping platforms have been developed for screening small plants (such as *Arabidopsis* and rosette species) and larger plants (such as rice and maize). *Arabidopsis thaliana* is widely used as a model plant because of its rapid life cycle, relatively small genome, and wide geographic range (Kaul et al., 2000), as well as its close relationship to crops in the Brassicaceae family. Automated and large-scale phenotyping under controlled environmental conditions is particularly attractive in such a species for forward genetics, reverse genetics, and quantitative genetics.

One of the first automated red-green-blue (RGB) imaging and weighing systems, PHENOPSIS (2003) was developed to understand *Arabidopsis* responses to water deficit (Granier et al., 2006), but it lacked integrated data management and had relatively simple environmental controls. In 2011, the PHENOPSIS platforms and growth chambers were extended, and a database, PHENOPSIS DB, was developed to store hundreds of gigabytes of images and metadata and to provide data and image analysis modules; this database served as a template for other groups to develop a similar data management system (Fabre et al., 2011). GROWSCREEN was established to quantify the dynamics of seedling growth acclimation (total leaf area, relative growth rate, and root area) under altered light conditions (Walter et al., 2007). In addition, based on the linear displacement stages, the color imaging device of GROWSCREEN could be replaced with a chlorophyll fluorescence imaging system, GROWSCREEN FLUORO, which allowed the simultaneous phenotyping of leaf growth and chlorophyll fluorescence in *Arabidopsis thaliana* and *Nicotiana tabacum* with a throughput of approximately 60 plants per hour (Jansen et al., 2009). The relatively limited capacity of GROWSCREEN, 200–500 plants, combined with the micro-environmental heterogeneity across the platform, can reduce the mapping power for QTL analyses or GWAS.

A large-scale phenotyping platform, Phenoscope, was designed to obtain shoot growth and water content information

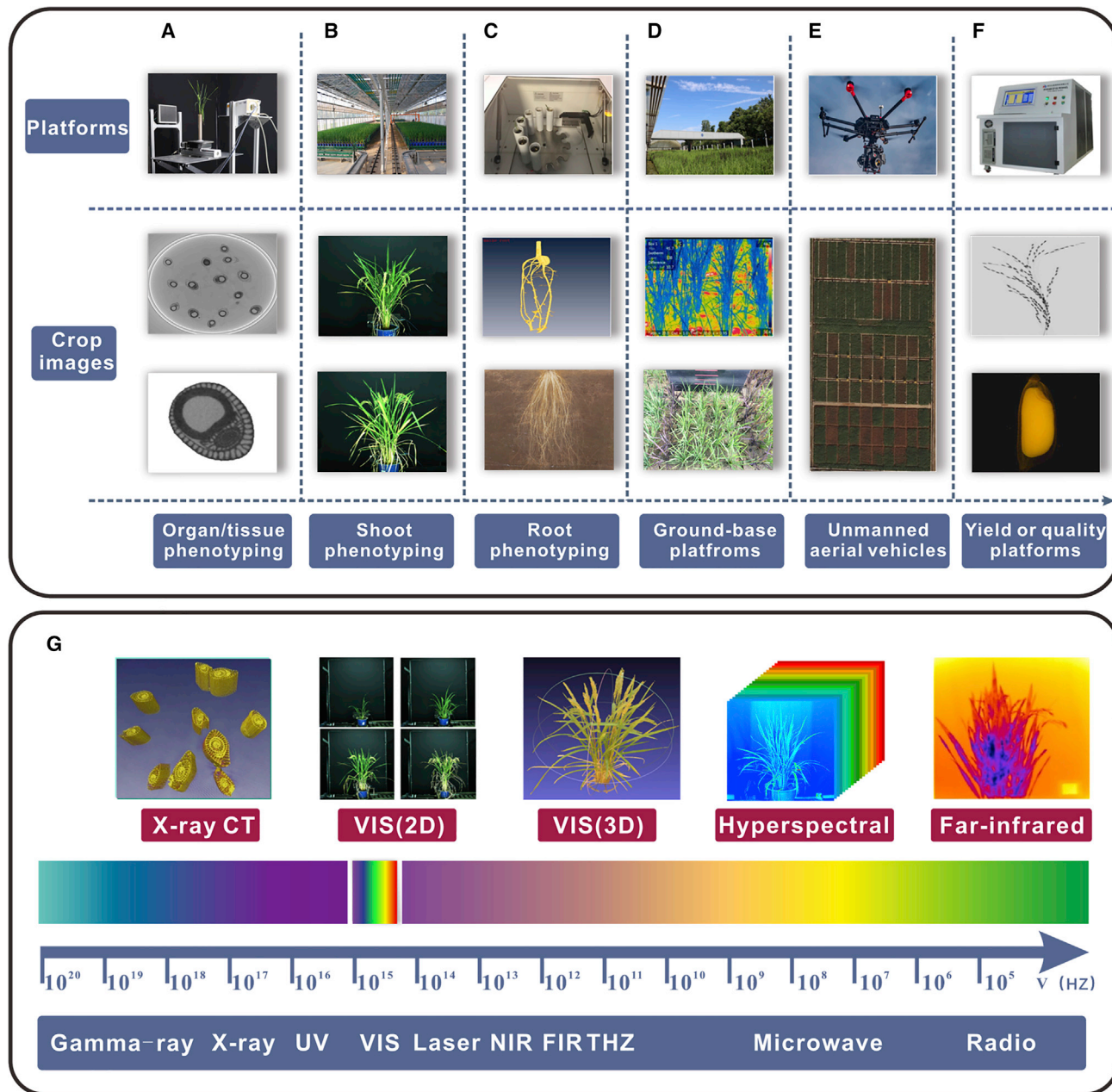


Figure 1. Schematic Overview of Phenotyping Platforms and across Different Scales.

Organ/tissue (A), shoot (B), root (C), ground-base (D), and UAV (E) to yield/quality (F) phenotyping and exemplification of different spectra used in crop phenotyping (G).

for 735 pots, compensating for environmental heterogeneity by continuously rotating the 735 pots across the platform (Tisné et al., 2013). Phenovator utilized a high-speed x-y moving rail system to move a monochrome camera with eight specific band-pass filters above a platform carrying up to 1440 plants to obtain data on photosynthesis (Φ_{PSII} , light-use efficiency of photosystem II electron transport) and plant growth traits (projected leaf area accumulation) (Flood et al., 2016). This configuration had a high acquisition speed, with the collection of leaf area in 20 min and PSII efficiency in less than an hour. Instead of moving sensors, the commercially available system PlantScreen transported trays (20 pots per tray) on conveyor

belts from the growing area to a dark acclimation chamber and chlorophyll fluorescence (ChlF) and RGB imaging cabinets, with automatic weighing and watering (Awlia et al., 2016). The results showed that some photosynthetic parameters, such as F_v/F_m , were robust in reflecting severe salt stress, while other parameters (Φ_{NPQ} , qP , and Φ_P) reflected early salt stress. However, the influence of the movement of potted plants and environmental variation (e.g., wind, temperature, and other microclimate; Brien et al., 2013) in a greenhouse may affect chlorophyll fluorescence parameter measurements (Haworth et al., 2018) and should be further investigated.

| Applications | Platforms | Advantages | Limitations | References |
|--|---|--|--|---|
| Shoot phenotyping for <i>Arabidopsis</i> in the laboratory | PHENOPSIS (DB); GROWSCREEN (FLUORO); Phenoscope; Phenovator; PlantScreen | Relatively affordable, rapid, and automatic measurements for large populations | Not suitable for larger crops | Fabre et al., 2011 ; Jansen et al., 2009 ; Tisné et al., 2013 ; Flood et al., 2016 ; Awlia et al., 2016 |
| Shoot phenotyping for crops in the laboratory | TraitMill; Scanalyzer3D; PHENOARCH; HRPF | Dynamic and automatic obtain shoot growth, biomass, and lots of information for large populations | Costly; requires multidisciplinary experts to maintain and update | Reuzeau et al., 2005 ; Hairmansis et al., 2014 ; Brichet et al., 2017 ; Yang et al., 2014 |
| | PhenoBox | Affordable; easy to maintain | Labor intensive for large screening | Czedik-Eysenberg et al., 2018 |
| Root phenotyping in the laboratory | PlaRoM; Rhizoslides; Rhizoponics; RADIX; RhizoTubes | Affordable; obtain 2D root system architectures | Root growth in transparent media | Yazdanbakhsh and Fisahn, 2009 ; Le Marie et al., 2014 ; Mathieu et al., 2015 ; Le Marie et al., 2016 ; Jeudy et al., 2016 |
| | GiARoots RootReader3D | Affordable; obtain 3D root system architectures | Root growth in transparent media | Galkovskiy et al., 2012 ; Clark et al., 2011 |
| | GROWSCREEN-Rhizo | High-throughput to obtain shoot and root traits in soil-filled rhizotrons | Root growth is limited in 2D rhizotrons (rhizobox) | Nagel et al., 2012 |
| | MRI-PET; PET-CT; MRI-CT | Obtain 3D root system architectures in soil-filled tubes | Costly; time consuming; lack of specialized prototype for crop study | Jahnke et al., 2009 ; Garbout et al., 2011 ; Metzner et al., 2015 |
| Ground-based phenotyping in the field | CPRS, a fixed phenotyping tower | Easy to install and maintain | Limited crop information in fixed areas was obtained | Fukatsu et al., 2012 |
| | Field Scanalyzer, a rail-based gantry phenotyping system | Integration of various optical sensors; high image resolution | Costly; limited image area; variable ambient light | Sadeghi-Tehran et al., 2017 |
| | BreedVision, a self-propelled tractor equipped with multiple sensors mounted in a mobile dark chamber | Integration of various optical sensors; stable imaging conditions; non-restriction of image area | Restricted by wet soil and weather conditions (rainy and strong breeze) | Busemeyer et al., 2013a |
| Remote sensing in the field | Drones or UAVs equipped with multiple sensors | Integration of various sensors; non-restriction of imaging area; rapid measurements (crop growth, yield, stress response, etc.); flexible to install and use | Cannot obtain information below the canopy; strict operating and local flight laws should be noted to ensure flight safety | Maes and Steppe, 2019 |

Table 1. Examples of High-Throughput Phenotyping Applied in *Arabidopsis* or Crops.

(Continued on next page)

| Applications | Platforms | Advantages | Limitations | References |
|--------------------------|---|--|---|---|
| Pocket phenotyping | PocketPlant3D, a smartphone equipped with APP to measure maize canopy and leaf traits | Affordable; flexible to use; easy to popularize | Limited traits and single function; lack of robust models to face complex conditions in field | Confalonieri et al., 2017 |
| Post-harvest phenotyping | Seed Evaluation Accelerator (SEA) | Automatic threshing rice panicles and rapid measurements of yield-related traits | Cannot obtain 3D grain traits and panicle traits | Duan et al., 2011 |
| | P-TRAP; PANorama | Quantify both rice panicle traits and grain traits; no need for threshing | Need to manually separate panicle branches; cannot obtain 3D grain traits | AL-Tam et al., 2013; Crowell et al., 2014 |
| | PhenoSeeder | Extract 3D traits from individual seeds with high accuracy | Low measuring speed; need for threshing | Jahnke et al., 2016 |
| | X-ray CT | Extract 3D cereal grain traits and spike traits without threshing | Costly; time consuming; need to develop a bespoke image analysis pipeline for new species | Hughes et al., 2017 Hughes et al., 2019 |
| | Hyperspectral imaging | Infer protein content, and other physiological or biochemical information | Costly; need bespoke image analysis and update model for new species, new physiological or biochemical indicators | Sun et al., 2019 |

Table 1. Continued

High-Throughput Phenotyping in Crop Science: Multidisciplinary Teamwork

Some of the first developments were aimed at the industrial-scale assessment of genetically modified crops. CropDesign (Belgium) developed TraitMill, comprising proprietary bioinformatics tools, a high-throughput gene engineering system, plant transformation methods, and a high-throughput phenotyping platform, which was used to determine morphometric traits (aboveground biomass, plant morphology, plant color) that might be related to yield (Reuzeau et al., 2005). Unfortunately, the details of TraitMill, the experimental design, and outcomes were proprietary. LemnaTec GmbH (Germany) developed the Scanalyzer3D platform and installed versions around the world. The Plant Accelerator (Australian Plant Phenomics Facility, University of Adelaide, Adelaide, Australia), fitted with computer-controlled conveyor systems, automated weighing–watering devices, and imaging stations (RGB, near-infrared [NIR], fluorescence, and hyperspectral imaging), has a capacity of 2400 plants and has been used to assess the salinity tolerance of rice (Hairmansis et al., 2014), nitrogen/water deficiency in crops (Neilson et al., 2015), and the salinity tolerance of chickpea (Atieno et al., 2017). In addition, the total plant biomass is estimated from RGB images using the projected shoot area in three different view images and linear models, and the predication accuracy of the model can be improved by adding additional factors such as the growth date (Golzarian et al., 2011), or more structural or physiological features in a more sophisticated model (Chen et al., 2018). However, the commercial software of Scanalyzer3D, LenmanGrid, with its built-in algorithms, is not open source and cannot be easily modified to extract more traits. Therefore, its application to new species or experimental scenarios is often unsatisfactory (Hartmann et al., 2011).

To alleviate this problem, the Leibniz Institute of Plant Genetics and Crop Plant Research developed HTPPheno, a plugin for ImageJ, and other open-source image-processing software to extract standard traits from color images, such as plant height, width, and projected area (Hartmann et al., 2011). The Java-based open-source Integrated Analysis Platform (IAP) with broader functionalities and the flexibility to integrate third-party algorithms (Klukas et al., 2014), supports additional sensor types (fluorescence, near-infrared, infrared) and a broader range of species (maize and *Arabidopsis*) and facilitates data management. IAP was used to extract features from images and model-derived parameters to determine the drought response (DR) of barley (Chen et al., 2014) and screen the genetic variation of growth dynamics in maize in combination with GWASs (Muraya et al., 2017).

The Bellwether Phenotyping Platform installed a Scanalyzer3D system within a climate-controlled growth facility at the Donald Danforth Plant Science Center (St. Louis, MO, USA). They developed PlantCV, platform-independent software based on open-source libraries (OpenCV, NumPy, and Matplotlib) to process RGB, fluorescent, and NIR images and quantify different temporal responses to water availability in *Setaria* (Fahlgren et al., 2015).

Interestingly, the INRA group (PhenoArch Phenotyping Platform, INRA, Montpellier, France) developed an automatic procedure to

monitor silk growth dynamics in maize, by modifying two successive imaging cabins—one cabin to determine the spatial coordinates of potential ears and the second to inform a robotic camera—thereby providing clearer images of maize ears and calculating silk traits across time (Brichet et al., 2017). This illustrates the need for continued multidisciplinary development of hardware and software according to user requirements.

A multidisciplinary team from Huazhong Agricultural University and Huazhong University of Science and Technology (Wuhan, China) developed the High-throughput Rice Phenotyping Facility (HRPF, Figure 1), incorporating color imaging, X-ray CT, automatic controls, and an image analysis pipeline that can monitor at least 15 agronomic traits in populations of up to 1920 rice plants, with a total greenhouse capacity of 5472 (Yang et al., 2014). Moreover, some image-based traits (i-traits) that are difficult to assess manually, such as leaf rolling and stay-green properties of drought-tolerant rice, can also be quantified (Duan et al., 2018). Together with GWASs and 51 i-traits, the HRPF can dissect the complex DR traits into heritable and simple i-traits and discover new genes for DR (Guo et al., 2018a). In addition, with minor adjustments of the image analysis pipeline, the HRPF can be extended to phenotype other species, including 3D wheat plant architecture (Fang et al., 2016), the leaf traits of rape seedlings (Xiong et al., 2017), and the genetic architecture of variation in maize growth (Zhang et al., 2017). However, the establishment of high-throughput conveyor-based phenotyping systems is costly and time consuming and requires in-depth knowledge of engineering and computational sciences to maintain function and flexibility. The unit cost depends on the throughput, so the implementation of such systems can only be justified at major research centers or companies.

To provide cheaper phenotyping solutions, Czedik-Eysenberg et al. (2018) developed PhenoBox, a flexible open-hardware and open-source phenotyping system to extract shoot traits. Because of the low cost of materials (€3000), PhenoBox was widely applied to study infection of model grass, salt stress, and observation of other crops with additional sensors, such as hyperspectral imaging sensors. To benefit more labs, it is important to decrease the unit cost associated with high-throughput phenotyping not only in the lab but also in the field (Hawkesford and Lorence, 2017). Another trend is to increase the number and quality of quantified traits, especially novel traits or those requiring labor-intensive and destructive measurement; i.e., tiller growth (Wu et al., 2019) or panicle development (Jhala and Thaker, 2015) by X-ray CT.

Choosing the appropriate imaging sensors and proper imaging-transfer mode are both vital in designing phenotyping facilities, which depend on the different experimental objectives. In comparison, RGB, fluorescent, thermal, hyperspectral, and 3D imaging all have their pros and cons: (1) RGB imaging (also called visible light imaging) is cost-effective and is the most widely used to measure plant or organ morphological traits, biomass, and plant growth (Yang et al., 2014), but it cannot provide physiological information. (2) Equipped with specific excitation illumination, fluorescent imaging can reflect the physiological signal, such as photosynthetic function and reactive oxygen species signal (Fichman et al., 2019). (3) Thermal imaging (also called far-infrared thermal imaging) can obtain the plant or leaf

temperature, which is also affected by environmental conditions (Zarco-Tejada et al., 2012). Moreover, both fluorescent and thermal imaging cannot provide sufficient spectral information. (4) Hyperspectral imaging can provide abundant spectral (visible and near-infrared) and spatial information simultaneously and can be assembled to assess disease severity, water status, and other physiological traits. However, the hyperspectral sensor is costly, and the processing of hyperspectral data (gigabyte per sample) can be complicated (Mahlein et al., 2018). (5) Compared with 2D imaging, 3D imaging techniques, which mainly include image-based (Fang et al., 2016) and laser scanning-based (Paulus et al., 2014) techniques, can generate 3D models and obtain more spatial and volumetric traits. According to the merits of the different imaging technologies, the current trend is to combine multiple imaging techniques. Some studies have compared the different imaging techniques in plant phenotyping (Fiorani and Schurr, 2013; Zhao et al., 2019).

More importantly, choosing the proper imaging-transfer mode (plant-to-sensor or sensor-to-plant) depends on the extracted traits, volume size of the measured species, greenhouse capacity, and other factors. CropDesign, Scanalyzer3D, and HRPf are all based on plant-to-sensor mode, which transfers the plants to imaging stations to extract biomass, shoot growth, and plant architectures. However, to measure certain physiological traits (such as canopy temperature) that are susceptible to environmental variation (air temperature and wind speed, etc.) (Ghanem et al., 2015), moving the camera and keeping the plants stationary would be better than transporting the plants to the inspection chamber via the long belt conveyor. Another example of sensor-to-plant mode is Phenodyn (Montpellier, France; Avramova et al., 2019), which has displacement transducers and a balance recorder for each plant, and measures leaf elongation rate and transpiration rate with the capacity of 500 plants in the greenhouse. In our opinion, combining both plant-to-sensor and sensor-to-plant modes would be interesting and have complementary advantages.

Going Underground: An Added Challenge in Plant Phenotyping

Unlike most animals, individual land plants live at the interface between two or more distinct media. While the shoots usually develop in the air (and are relatively easily accessible for inspection, measurement, and sampling), the roots normally function within the soil, limiting direct observation. Roots are crucial organs that determine water and nutrient uptake in most crops, directly influencing their resilience to drought as well as affecting yield and quality.

Root system architecture (RSA) phenotyping *in situ* is challenging, especially in comparison with shoot phenotyping (Atkinson et al., 2019). A wide range of solutions have been evaluated and can provide useful information. The use of transparent (hydroponic, aeroponic, and gel-based) growth media, such as PlaRoM, permits the use of RGB cameras to acquire and extract growth dynamics and hair development, with a maximum capacity of 50 seedlings (Yazdanbakhsh and Fisahn, 2009). Other systems include Rhizoslides (a paper-based root growth and observation system) (Le Marie et al., 2014),

Rhizoponics (a hydroponic rhizotron for the evaluation of the RSA of *Arabidopsis*) (Mathieu et al., 2015), RADIX (a rhizoslide platform used to screen the shoots and roots of 200 maize plants) (Le Marie et al., 2016), and RhizoTubes (an automatic “plant-to-sensor” platform comprising 1200 rhizotubes to obtain the RSA in approximately 6–8 weeks) (Jeudy et al., 2016). In addition, several open-source image analysis solutions evaluating the 2D RSA have been developed, including semiautomatic software (RootTrace, French et al., 2009; SmartRoot, Lobet et al., 2011; RootNav, Pound et al., 2013) and fully automatic software (EZ-Root-VIS, Shahzad et al., 2018).

Iyer-Pascuzzi et al. (2010) developed a 3D RSA imaging platform utilizing a gel-based growth cylinder to obtain 16 root traits. Other 3D image reconstruction and image analysis pipelines include GiARoots (Galkovskiy et al., 2012) and RootReader3D (Clark et al., 2011). To obtain the RSA similar to that observed in the field, Pineros et al. (2016) developed a plastic mesh vertical tower system to maintain the 3D root architecture and achieve 3D imaging in a hydroponic growth cylinder (Pineros et al., 2016).

However, it is not always apparent how results obtained from transparent media apply to normal growing conditions in the field. An intelligent mechanized root phenotyping platform, GROWSCREEN-Rhizo, was developed to image shoots and the roots simultaneously in transparent soil-filled rhizotrons. The throughput was 60 rhizotrons per hour out of a total capacity of 72 rhizotrons (Nagel et al., 2012). Moreover, additional sensors (i.e., hyperspectral) were integrated into this phenotyping system with the potential to dynamically reflect root chemical components, such as root water content and lignin change (Bodner et al., 2018).

X-ray CT, a 3D structural imaging application, can be used to visualize the inner 3D volume according to the differences in the X-ray attenuation of different materials, such as roots and soil. The first CT scanner (Cormack, 1963) was quickly followed by a clinically useful version (Hounsfield, 1976), for which the inventors received the 1979 Nobel Prize in Physiology and Medicine. The open-source software RooTrak was developed by the CPIB (Centre of Plant Integrative Biology, University of Nottingham) group (Mairhofer et al., 2012, 2013) to exploit the differential X-ray attenuation between soil and roots and reconstruct the RSA in 3D. Multiple interacting root systems could also be extracted and separately analyzed based on a modified RooTrak (Mairhofer et al., 2015).

In addition, some issues regarding the use of CT to scan roots must be noted: (1) to ensure the better contrast of X-ray attenuation between soil and roots, soil preparation to maintain homogeneity, including sieving and drip, is the first key step, and the RSA is also influenced by the soil type and soil compaction (Rogers et al., 2016); (2) CT scanning often involves trade-offs, for instance, the scanning volume and resolution both increase the scanning time so larger pots at high resolution will limit the number of samples or frequency of data acquisition (Mairhofer et al., 2012); (3) although several studies have shown that X-ray CT with a low dose of radiation (<30 Gy) had no impact on plant growth or soil microbial populations, the effect of X-ray radiation with high energy and high-throughput scan times should be further investigated (Zappala et al., 2013).

Other alternative approaches for recovering 3D RSA from soil included magnetic resonance imaging (MRI), positron emission tomography (PET), and a combination of both. Through the imaging of the absorption and re-emission of electromagnetic radiation from nuclei in a magnetic field, MRI can be used for structural imaging (such as root architecture) or functional imaging (water distribution in plant tissues) (Atkinson et al., 2019). The Jülich Plant Phenotyping Center (Jülich, Germany) utilized MRI to dynamically evaluate root morphological changes during *Cercospora beticola* infestation (Schmittgen et al., 2015), and used image analysis software (NMRRooting) to quantify root traits (such as root length, mass, angle, diameter, tip number, and spatial distribution) from 3D MRI data with a maximum measurement capacity of 18 pots per day (van Dusschoten et al., 2016). The group from Aarhus University used medical PET (functional imaging that detects positron-emitting radionuclides) to nondestructively scan plant roots down to 82 mm deep and dynamically screen carbon (photoassimilate) transportation over a prolonged time period (Garbout et al., 2011).

The techniques of CT, MRI, and PET all have their advantages and limitations: (1) CT tends to provide more detail from small pots (such as pots with a diameter of 34 mm) for which higher-resolution scanning is possible, but for larger pot diameters (>81 mm), MRI was found to detect more roots than CT (Metzner et al., 2015). (2) CT and MRI have higher spatial resolution ($\leq 30 \mu\text{m}^3$) than PET (approximately 1.4 mm), but PET, with its high γ -radiation, can provide high contrast between roots and soil (Jahnke et al., 2009). (3) Signal deterioration due to soil structure and water content is lower for PET than for MRI and CT, and a high soil water content affects CT more than MRI (Jahnke et al., 2009). In addition, soil with high sand and low clay (or silt) content yielded better image quality for both MRI and CT (Pflugfelder et al., 2017). (4) Finally, the time requirements for both PET scanning (≥ 60 min) and MRI scanning (approximately 40–60 min) are generally greater than those for CT, which means that it would be difficult to use MRI and PET for phenotyping populations on a large scale, as required for genetic studies (Jahnke et al., 2009; Metzner et al., 2015). Thus, because of their different merits, the use of a combination of these tomography techniques, such as MRI–PET (Jahnke et al., 2009), PET–CT (Garbout et al., 2011), and MRI–CT (Metzner et al., 2015), is popular not only in medical imaging but also in root phenotyping for addressing focused biological questions. Currently, many important functional genes influencing RSA likely remain undiscovered due to the bottleneck associated with root phenotyping (Wing et al., 2018), necessitating the development of breakthrough technologies for belowground imaging in the future.

FAST DEVELOPMENT OF PHENOTYPING TECHNIQUES IN THE FIELD

The outdoor environment, where the vast majority of crops are grown, is much more variable than the laboratory environment. Soil structure can vary within and among fields, and even small differences in topology and aspect can alter the wind speed, the effect of solar radiation, evaporation rates, and so on. Field crop breeding takes such variation into account through replicated multisite trials, but the application of appropriate pheno-

typing technologies remains a limitation for future genetic studies and breeding advances (Araus and Cairns, 2014). Field phenotyping can be broadly split into technological groups based on the sensor platform: ground-based, unmanned aerial vehicle (UAV), and handheld phenotyping (Table 1), which are discussed in the following sections.

Ground-Based Phenotyping: High Diversity of Phenotyping Solutions

Sensor platforms can be either fixed or mobile. Towers and other fixed platforms are easy to construct and maintain and have been used to record growth stages and monitor insect pests. For example, digital cameras attached to a fixed phenotyping tower (Crop Phenology Recording System [CPRS]) were used to monitor rice growth and the occurrence of the rice bug (Sakamoto et al., 2011; Fukatsu et al., 2012). Shibayama et al. (2015a) used two digital cameras at a height of 12 m above a rice field to evaluate the nitrogen content and leaf area index of paddy rice (Shibayama et al., 2015b). Zhou et al. (2017) developed a scalable and cost-effective field phenotyping platform (CropQuant), a web-based control system (CropMonitor), and a high-throughput trait analysis pipeline to measure dynamic crop growth, vegetative greenness, and plot orientation. The advantage of the use of the fixed phenotyping tower was that it was easy to install and maintain, whereas the disadvantage was that limited crop information in fixed areas was obtained, and costs for large-scale experiments increase in a near-linear fashion. A smart solution was an ambient sensor cloud system, which employed open-source sensors and a free cloud data service; this achieves long-time observations of individual plants (Hirafuji et al., 2011).

Rothamsted Research established a rail-based gantry system for field phenotyping, the Field Scanalyzer (Sadeghi-Tehran et al., 2017; Virlet et al., 2017), measuring 125 m (length) \times 15 m (width) \times 6 m (height) and equipped with an ~ 300 kg sensor array, including a visible camera, a 3D laser scanner, a thermal infrared camera, a visible to near-infrared (VNIR) hyperspectral camera, a four-channel amplified radiometer, a chlorophyll fluorescence sensor, and a CO₂ sensor. The acquisition of data from all sensors took approximately 7 min for one plot (1.5 m²). This platform can be used to produce a detailed description of crop canopy development through all life stages, for example, to determine wheat ear emergence and flowering times (Sadeghi-Tehran et al., 2017). Similarly, with an integrated LiDAR sensor, RGB camera, thermal camera, and hyperspectral imager in a mobile gantry system, Guo et al. (2018b) developed a high-throughput crop phenotyping platform (Crop3D) to measure 3D plant/leaf architectures and leaf temperature. However, there are two challenges that should be discussed: (1) variable ambient light leads to challenges, which are solved in part by nighttime imaging or the addition of an on-field reflectance standard (Virlet et al., 2017). (2) It is a challenge to effectively analyze terabytes of hyperspectral images and laser-scanned images (Vadez et al., 2015) as well as interpret temporal series of thermal, multi- or hyperspectral-based features in changing environments (Virlet et al., 2017). In addition, such large rail-based systems covering a limited area (e.g., 0.12 ha in the case of Rothamsted Research) are also expensive in terms of both construction and maintenance.

Sensors mounted on manually operated carts or self-propelled tractors overcome many of these issues. A cart carrying multiple sensors, including an ultrasonic sensor, a normalized difference vegetation index (NDVI) sensor, a thermal infrared radiometer, a portable spectrometer, an RGB camera, and a proximity sensor, has been used to obtain soybean and wheat canopy traits, including height, NDVI, temperature, reflectance spectra, and RGB imagery (Bai et al., 2016). To obtain 3D information and measure plant height, Wang et al. (2018b) built a ground-based high-throughput plant phenotyping system (HTPP) with various sensing technologies, which included an ultrasonic sensor, a LiDAR-Lite v2 sensor, a Kinect camera, and four digital single-lens reflex cameras. Stereo cameras, time-of-flight depth sensors, and infrared cameras have been used to evaluate individual plant architecture traits (Young et al., 2018), while a time of flight (TOF)-based 3D vision sensor provided the automated measurement of interplant distance (Nakarmi and Tang, 2012).

However, most imaging platforms in the open field are susceptible to variable environmental conditions, such as light intensity. The BreedVision system solves this problem simply by excluding ambient light and imaging within a mobile dark chamber (Busemeyer et al., 2013a). Because it is equipped with multiple sensors (3D time-of-flight camera, laser distance sensors, hyperspectral imaging, RGB sensors, and light curtain imaging) and specific trait calibrations, BreedVision can nondestructively measure plant traits, including plant height, tiller density, grain yield, moisture content, leaf color, lodging, and dry biomass, at an operating speed of 0.5 ms^{-1} . Remarkably, equipped with a pair of linear light barriers (one emitter and the other a receiver), light curtain imaging could be used to measure plant height, plant architecture, and biomass without the influence of indoor or field illumination conditions (Busemeyer et al., 2013a; Fanourakis et al., 2014). However, vehicle-based platforms have some limitations; they are restricted by soil and weather conditions and, in some cases, by topology. Specific crops, such as paddy rice, require specialized vehicles.

Remote Sensing with UAV: The Further You Look, the More You See

Drones (or UAVs) provide a flexible platform, quickly acquiring data over large areas and potentially providing high spatial resolution images ($\sim 1 \text{ mm}$ per pixel). Some advanced IT techniques, such as deep learning (DL), can handle of millions of remote sensing images with high accuracy and high speed (Bauer et al., 2019). Thus, remote sensing has been widely used to monitor drought stress response, assess nutrient status and growth, detect weeds and pathogens, predict yield (Maes and Steppe, 2019), and identify QTLs (Wang et al., 2019).

Canopy color and texture features obtained by UAV platforms (Yue et al., 2019) at high spatial and temporal resolution facilitate phenotyping tasks, since the improvement of image quality and quantity provides detailed information for feature mining and analysis. Accordingly, high-resolution UAV imagery has been adopted for various phenotyping purposes, such as leaf area index estimation (Yao et al., 2017), wheat ear identification (Madec et al., 2019), weed detection (Hung et al., 2014), and seeding performance evaluation in rapeseed (Zhao et al., 2018). In addition, researchers have paid great attention

to optimal resolution determination. For example, a recent study suggested that an optimal resolution of 0.3 mm allowed for high-throughput phenotyping in the field (Madec et al., 2019). Because of its combination of high spatial and temporal resolution (Burkart et al., 2018), UAV-based phenotyping opens new possibilities in field research.

For plant geometric morphometric analysis, such as that pertaining to plant height and aboveground biomass, 3D canopy modeling provides better estimates than vegetation indices (VIs) (Maimaitijiang et al., 2019). With the development of photogrammetric techniques, multiview stereopsis, and computer vision approaches, such as structure from motion (SFM), UAVs have emerged as a promising platform for obtaining 3D plant canopy structure information (Bendig et al., 2014; Hassan et al., 2019). SFM algorithms produce geometrically precise 3D point clouds by matching feature points from 2D images obtained by RGB sensors. SFM is effective for estimating plant height and biomass (Bendig et al., 2014; Malambo et al., 2018), but the penetration capability of photogrammetric techniques is limited compared with that of LiDAR. SFM can produce point clouds at the subpixel level with similar accuracy and quality to LiDAR (Hassan et al., 2019). Moreover, there is a strong correlation between digital surface model (DSM, obtained by SFM algorithms using UAV data) and LiDAR estimations of plant height (Madec et al., 2017; Jimenez-Berni et al., 2018), and the SFM-UAV-based method is less costly and more flexible (Jimenez-Berni et al., 2018).

Different imaging sensors mounted on UAVs can be used to capture spectral information regarding visible or NIR bands for crop nutritional diagnosis and monitoring various types of stress (Figure 1). A consumer-grade RGB camera can be used to generate a range of RGB VIs, which have been found to have good performance in the assessment of stress conditions, such as low nitrogen (Elazab et al., 2016) and biotic stress (Vergara-Diaz et al., 2015). In addition, the NIR or IR bands can provide more spectral information, which improves the measuring accuracy of crop growth monitoring. Multispectral cameras can respond to spectral information from red edge and NIR bands and have been utilized for chlorophyll-based diagnosis (Deng et al., 2018) and water stress monitoring (Zarco-Tejada et al., 2012). However, their spectral resolution is limited by the number of lenses on the sensors. Hyperspectral cameras cover a commonly used spectral region (400–1000 nm) for crop monitoring and can be used for assessing the leaf carotenoid content (Zarco-Tejada et al., 2013), nitrogen concentration (Zheng et al., 2016), heat and drought stress (Trachsel et al., 2019), and so on. This type of sensor can capture the most comprehensive spectral information, but it is expensive, and data processing may be relatively complicated. In addition to imaging sensors, a VNIR nonimaging spectrometer was even mounted on a UAV to collect accurate spectral reflectance signals (Garzonio et al., 2017). In this way, more detailed spectral information could be obtained rapidly. There are some reviews available on UAV spectral remote sensing technologies (Aasen et al., 2018; Maes and Steppe, 2019) and UAV-based hyperspectral data processing and applications in agriculture (Adão et al., 2017).

Remote sensing with UAVs has shown great potential for high-throughput phenotyping, which will enhance work in crop

functional genomics and crop breeding. However, some restrictions related to the use of UAVs should be addressed: (1) the fly time and the load capacity are limited; (2) local flight laws and regulations may be a constraint; (3) strict requirements for operating technicians should be implemented to ensure flight safety; (4) a low flight altitude will provide original images with higher quality but can also cause changes to the leaf architecture and physiology due to the strong wind produced by the UAV. Moreover, lower flights will result in longer flight time, which will make it difficult to screen large populations with low-altitude drone flights. Thus, reasonable flight altitudes and other camera settings associated with different phenotyping goals should be further investigated.

Pocket Phenotyping: The Flexible Future

Physiologists have long used specialized handheld portable tools to measure a range of functions (Kim et al., 2016). Many such instruments are necessarily complex, requiring training and good attention to detail to obtain useful data. The challenges and opportunities related to portable phenotyping include (1) decreasing the level of expertise required and integral data standardization (such as color calibration and smart user guides); (2) integrating multiple sensors into one portable device with corresponding software for data analysis; and (3) exploiting the 5th generation mobile network and artificial intelligence techniques, such as DL, could build robust model to face complex conditions in the field. Next-generation “pocket” or wearable phenotyping tools could provide the disruptive technology that will profoundly change and accelerate phenotyping.

The rapid development of smartphones with high-resolution RGB cameras and powerful computing has led to the creation of applications with ever-increasing utility. In addition, aspects of mobile phone technology have been incorporated into other bespoke portable instruments, increasing the range of optical and other sensors and enhancing the connectivity and portability of traditional phenotyping equipment. This is an emerging and rapidly evolving area, so we only provide a few examples to illustrate the possibilities. PocketPlant3D measures maize canopy structure (Confalonieri et al., 2017); the smartphone is moved parallel with the leaf lamina from base to tip, and all the leaves can be scanned to obtain the whole leaf architecture. A machine learning-based app can diagnose *Cercospora* leaf spot and other sugar beet leaf diseases better than experts (Hallau et al., 2018). A smartphone application for Android devices, vitisBerry, can quantify the number of grapevine berries (Aquino et al., 2018).

POST-HARVEST PHENOTYPING: APPLICATION IN RESEARCH

The harvested part of the crop is, generally, the most immediately economically relevant, and mechanized systems often already exist for handling and assessing its yield or quality, both in the harvesting process or post-harvest. Like the various sensors installed in the tractors discussed in the section on Ground-Based Phenotyping, during the process of harvesting, the sensors and global positioning system (GPS) could also be integrated in the (combine) harvester to monitor, for example, the yield of blueberries (Farooque et al., 2013), grain yield (Li et al.,

2016), and cotton yield (Pelletier et al., 2019). While several smart farming machines (e.g., advanced farming system, AFS, Case IH, US) have been commercialized in the harvest process, we focus on post-harvest phenotyping in this section.

Quality assessment in the seed and milling industry uses imaging technologies that readily lend themselves to research applications. The relatively simple integration of 2D image capture, feature extraction, and data export have streamlined seed phenotyping to allow a wide, cost-effective survey of wheat germplasm, revealing the genetic architecture underlying grain shape and size variation in bread wheat (Gegas et al., 2010). This approach can be applied to almost any harvested part of a plant and can be further refined. To relieve a bottleneck in phenotyping rice spikelets, an integrated and fully automatic machine was developed: the SEA unit can automatically thresh rice panicles, score yield traits, with a capability up to 1440 plants per day and a mean absolute percentage error of less than 5% (Duan et al., 2011). Similar approaches have been used for maize kernels (Miller et al., 2017), stalks (Mazaheri et al., 2019), for which candidate genes for vascular bundle and rind traits were identified, and tassels (Gage et al., 2017).

By utilizing a low-cost flatbed scanner or 2D digital camera, open-source and user-friendly image analysis software allows increased accessibility. SmartGrain, a free-use software package, extracts seed size and shape (Tanabata et al., 2012), while GrainScan provides information on both size- and color-related traits (Whan et al., 2014). With their user-friendly graphical user interfaces (GUIs), P-TRAP (AL-Tam et al., 2013) and PANorama (Crowell et al., 2014) are recommended as flexible tools to efficiently quantify both rice panicle traits and grain traits with good accuracy. Running on the Android operational system, a mobile application, SeedCounter, can measure grain number and grain size with high efficiency (20–60 s for 50 grains) (Komyshchev et al., 2016).

Although they are robust and rapid, 2D imaging/scanning approaches fail to capture much of the morphological and other complexity commonly found in biological material. The pheno-Seeder can extract 3D traits from individual seeds (Jahnke et al., 2016), which can then be traced through to early seedlings. Hyperspectral imaging can be used to infer grain cleanness (Wallays et al., 2009), insect-damaged wheat kernels (Singh et al., 2010), and grain protein content (Wang et al., 2004; Sun et al., 2019), potentially combining both 3D structural and physiological information in the future. An alternative approach to 3D morphometrics involves repurposing existing biomedical imaging techniques, such as X-ray microcomputed tomography (micro-CT) imaging (Hughes et al., 2017). The application of micro-CT to plant genetics will require the development of bespoke software as well as increased throughput (Hughes et al., 2019).

Ultimately, multidimensional seed traits must be combined with genetic analysis tools (such as GWAS or QTL) to dissect the genetic architecture of agronomic traits. Examples include sorghum panicle structure (Zhou et al., 2019) and the protein content of rice grains (Sun et al., 2019). Moreover, decreasing the cost of these novel photonics-based phenotyping tools and improving their reliability and extendibility could benefit crop grain research in the future.

HIGH-THROUGHPUT PHENOTYPING ENHANCES GENETIC STUDIES

In recent years, many phenotyping techniques, for example, root phenotyping (Atkinson et al., 2019), remote sensing (Maes and Steppe, 2019), DL for plant stress (Singh et al., 2018), and hyperspectral and other imaging technologies in phytopathology (Mahlein et al., 2018), have been widely discussed. However, the genetic studies and crop breeding applications that have already benefited from these technologies are rarely reviewed.

High-Throughput Phenotyping and Genetic Mapping

Here, we focus on genetic studies that have directly benefited from mechanized phenotyping platforms (Table 2), both within the laboratory and in the field. The phenotypic data listed in Table 2 can be categorized into organ-/tissue-related traits (Leiboff et al., 2015; Wu et al., 2019), plant morphological and leaf architecture traits (Bac-Molenaar et al., 2015; Yang et al., 2015; Condorelli et al., 2018), root anatomical traits (Courtois et al., 2013; Shi et al., 2013; Xie et al., 2017), biomass- or growth-related traits (Busemeyer et al., 2013b; Muraya et al., 2017; Zhang et al., 2017), drought- and salinity response-related traits (Honsdorf et al., 2014; Al-Tamimi et al., 2016; Guo et al., 2018a; Condorelli et al., 2018), and yield-related traits (Yang et al., 2014; Crowell et al., 2016; Zhou et al., 2019), which have been used in genetic mapping and are discussed in the following.

The size of shoot apical meristem (SAM) in the seedling stage correlates with early flowering phenotypes in maize that decrease the number of days to anthesis. Using a high-throughput image-processing pipeline, maize SAM morphological traits (shape and size) were extracted in an association panel and a backcross (BC) population. GWAS and QTL analyses demonstrated that the microscopic SAM morphology of seedlings is a predictor of adult phenotypes and that novel genes associated with SAM morphometric variation contribute to regulating SAM size (Leiboff et al., 2015, 2016). Combining a high-throughput micro-CT-RGB imaging system and GWASs, rice tiller traits and tiller growth traits as well as plant traits of nine growth stages could be obtained, and a total of 402 significantly associated loci were identified. In addition, two loci containing associations with both vigor-related traits and yield were identified for the further studies (Wu et al., 2019).

Leaves are primarily involved in photosynthesis and transpiration (Wang et al., 2011). The size, shape, degree of leaf greenness (chlorophyll content), and number of leaves determine a plant's photosynthetic and yield potential (Pérez-Pérez et al., 2010; Wang et al., 2015). Two genetic mapping studies of leaf traits in rice and maize were performed at the HRPF (Yang et al., 2015). They conducted a GWAS of 29 leaf traits (i.e., leaf size, shape, and color) in a panel of 533 rice accessions at three growth stages using a self-designed high-throughput leaf scoring (HLS) system and detected many loci, including nine loci containing known leaf-related genes associated with leaf traits. In maize, 22 leaf architecture traits of an RIL population at 16 time points were obtained and used for QTL mapping. Moreover, some leaf traits (i.e., leaf angle and leaf length distribution) are predictive indicators of final yield. Interestingly, a QTL hotspot for SDLC on

chromosome 10 overlapped with a QTL hotspot (Zhang et al., 2017) related to metabolic traits.

Root anatomical traits have important effects on the acquisition of nutrients and water from the soil and transportation to the aboveground parts of plants (Zhao et al., 2019). Uncovering the genetic basis of root traits will be helpful for improving the architecture of crop roots and promises to result in increases in water and nutrient use efficiency (Atkinson et al., 2019). Topp et al. (2013) dissected the genetic basis of 25 root-related traits obtained from semiautomatic *in vivo* 3D imaging and a digital phenotyping pipeline in a rice biparental mapping population. Courtois et al. (2013) performed GWASs of 15 rice root traits with a photography system, finding that most associations were identified for deep root mass and the number of deep roots, whereas no associations were detected for total root biomass or deep root proportions. In *Brassica napus*, the analysis of root architecture-related traits under high phosphate (Pi) and low Pi conditions resulted in the identification of 38 QTLs (Shi et al., 2013).

Drought and salinity are two important types of abiotic stress in many environments that can produce somewhat similar phenotypic effects (Munns and Tester, 2008; Munns et al., 2010). Drought stress responses interact with many other environmental parameters (e.g., temperature, relative air humidity, air flow, light, soil quality and drying, nutrient availability) (Granier et al., 2006) and are thus particularly challenging to replicate under field conditions. The quantitative measurement of drought resistance (DR) indicators (i.e., a leaf rolling indicator) combined with high-throughput phenotyping platforms provided an opportunity to measure both traditional and novel DR traits and mine for DR-related genes (Table 2). For example, based on LemnaTec's Scanalyzer3D, 44 and 21 DR QTLs were identified in a set of wild barley introgression lines (Honsdorf et al., 2014) and a wheat RIL population under water stress (Parent et al., 2015), respectively. In a rice study, 51 i-traits and traditional DR traits were identified in an association panel and an RIL population using a nondestructive phenotyping facility, and 93% of loci found in GWASs were colocalized with previously reported DR-related QTLs and some loci containing known DR-related genes. Sixty-nine i-trait-locus associations were identified by both GWAS and linkage analysis. The role of a DR gene, *OsPPT15*, was confirmed by genetic transformation experiments, demonstrating that the combination of high-throughput phenotyping and genetic mapping is a promising novel approach for the discovery of causal genes for DR (Guo et al., 2018a) and other traits.

Thanks to the various phenotyping platforms, sequencing technologies, GWASs (Huang et al., 2010), statistical methods for genetic mapping (van Eeuwijk et al., 2010), many loci controlling yield and its components in different crops have been identified (Table 2). However, most of the work listed in Table 2 is at the stage of QTL identification, and there are still several issues that need to be addressed. The first question is when we need high-throughput phenotyping, which depends on the research objectives: (1) characterizing dynamic QTLs for a complex trait at multiple developmental stages (Li and Sillanpää, 2015); (2) comparing QTLs for the same traits across different large-scale species (e.g. rice and maize); (3) the

| <i>Arabidopsis/ Crop</i> | Platform | Extracted traits or features | Population | Sample size ^a | No. of markers ^b | Methods | Reference |
|-----------------------------|---|---|------------|--------------------------|-----------------------------|-------------|---|
| <i>Arabidopsis thaliana</i> | Phenoscope (INRA, France) | Projected rosette area, relative expansion rate | RIL | 358 | – | LA | Tisné et al., 2013 |
| <i>Arabidopsis thaliana</i> | An imaging system (11 cameras) acquiring root images in Petri plates (University of Wisconsin, USA) | Root tip angle, root gravitropism | RIL/NIL | 162/92 | – | LA | Moore et al., 2013 |
| <i>Arabidopsis thaliana</i> | PHENOPSIS (Fondettes, France) | Projected leaf area, fresh weight, growth traits, and growth model parameters | IAP | 324 | ~215 000 | GWAS | Bac-Molenaar et al., 2015 |
| <i>Arabidopsis thaliana</i> | Near-infrared reflectance spectroscopic (NIRS, INRA, France) | Oil, protein, carbon, and nitrogen content | RIL | 164 | – | LA | Jasinski et al., 2016 |
| Rice | Rhizoscope phenotyping platform (Montpellier, France) | 15 traits: root system architecture, tiller number, and root/shoot biomass | IAP | 167 | 16 664 | GWAS | Courtois et al., 2013 |
| Rice | 3D root imaging, GiA Roots 2D and 3D image analysis platform | 2D and 3D root system architecture (RSA) traits | RIL | 171 | – | LA | Topp et al., 2013 |
| Rice | High-throughput rice phenotyping facility (HRPF, Huazhong Agricultural University, China) | Plant morphological traits, biomass, and yield-related traits | IAP | 529 | 4 358 600 | GWAS | Yang et al., 2014 |
| Rice | LemnaTec3D Scanalyzer system (University of Nebraska, USA) | 32 salinity-responsive fluorescence color classes | IAP | 373 | 26 258 | GWAS | Campbell et al., 2015 |
| Rice | High-throughput leaf scoring (HLS) (Huazhong Agricultural University, China) | 29 leaf traits: leaf size, shape, and color traits | IAP | 529 | 4 358 600 | GWAS | Yang et al., 2015 |
| Rice | PANorama (Cornell University, USA) | 49 panicle phenotypes | IAP/RIL | 242/168 | 700 000 and 30 984 | GWAS and LA | Crowell et al., 2016 |
| Rice | Australian Plant Phenomics Facility, The Plant Accelerator, Australia | Relative plant growth rate, transpiration rate and transpiration use efficiency, and select salinity tolerance traits | IAP | 553 | 700 000 | GWAS | Al-Tamimi et al., 2016 |
| Rice | High-throughput hyperspectral imaging system (HHIS, Huazhong Agricultural University, China) | 1540 hyperspectral indices, chlorophyll content | IAP | 529 | 4 358 600 | GWAS | Feng et al., 2017 |

Table 2. Trait and Genotype Variation Discovery Platforms.

(Continued on next page)

| Arabidopsis/ Crop | Platform | Extracted traits or features | Population | Sample size^a | No. of markers^b | Methods | Reference |
|------------------------------|---|---|-------------------|------------------------------------|-----------------------------------|----------------|---|
| Rice | Tractor-based high-throughput phenotyping (HTP, Colorado State University, USA) | Canopy height, canopy temperature depression, and three reflectance ratios | RIL | 986 | 4046 | LA | Tanger et al., 2017 |
| Rice | HRPF and HLS | 51 image-based traits (i-traits), 10 leaf color-related traits, 11 yield traits to reflect drought response | IAP/RIL | 507 | 4 358 600 and 2499 | GWAS and LA | Guo et al., 2018a |
| Rice | High-throughput micro-CT- RGB imaging system (HCR, Huazhong Agricultural University, China) | 74 traits: tiller traits, tiller growth traits, biomass, shoot morphological and shoot growth traits | IAP | 234 | 2 863 169 | GWAS | Wu et al., 2019 |
| Triticale | Breedvision (University of Applied Sciences Osnabrück, Germany) | Biomass accumulation at three developmental stages | DH | 647 | 1710 | GWAS | Busemeyer et al., 2013b |
| Wild barley | Australian Plant Phenomics Facility, The Plant Accelerator, Australia | 14 traits to detect drought tolerance: shoot area, height, growth, color, biomass, water-use efficiency, etc. | ILs | 47 | 1536 | LA | Honsdorf et al., 2014 |
| Wheat | Australian Plant Phenomics Facility, The Plant Accelerator, Australia | Biomass, leaf area, relative growth rate, transpiration, and water-use efficiency | RIL | 250 | – | LA | Parent et al., 2015 |
| Hexaploid wheat | A root imaging system and RootNav software (University of Nottingham, UK) | 25 root seedling traits: root angle, root length, root number, etc., mature plant height, grain yield, and nitrogen (N) uptake | DH | 94 | – | LA | Atkinson et al., 2015 |
| Bread wheat | A germination paper-based “pouch and wick” phenotyping system, coupled with digital image analysis (University of Nottingham, UK) | Seedling root traits, yield, yield components, and phenology in field trials | RIL | 226 | 230 | LA | Xie et al., 2017 |
| Wheat | Unmanned Aerial Vehicle (UAV) and a ground-based platform (University of Bologna, Italy) | Normalized Difference Vegetation Index, leaf chlorophyll content, phenology score, leaf rolling, and dry biomass, and select drought adaptive indicator | IAP | 248 | 17 721 | GWAS | Condorelli et al., 2018 |

Table 2. Continued

(Continued on next page)

| Arabidopsis/ Crop | Platform | Extracted traits or features | Population | Sample size ^a | No. of markers ^b | Methods | Reference |
|----------------------|---|---|------------------|--------------------------|---|---------|--|
| Wheat | High-throughput plant phenotyping system in National Plant Phenomics Centre (Aberystwyth University, UK) | Plant area, height, water use, and senescence and fitted parameters | MAGIC population | ~1000 | 80 000 | GWAS | Camargo et al., 2018 |
| Bread wheat | A tractor-based semiautomatic phenotyping system (Beijing Forestry University, China) | Sensor-based traits, yield-related canopy architecture | IAP | 221 | 68 958 | GWAS | Jiang et al., 2019 |
| Sorghum | Time-of-flight depth (Microsoft Kinect) sensor and open-source 3D image analysis pipeline (Texas A&M University, USA) | Shoot and leaf morphological traits | RIL | 98 | 10 787 for single QTL mapping and 1209 for multiple-QTL mapping | LA | McCormick et al., 2016 |
| Sorghum | Phenobot 1.0, an auto-steered and self-propelled field-based high-throughput phenotyping platform equipped with stereo RGB cameras (Iowa State University, USA) | Plant height and stem diameter | IAP | 307 | 127 992 | GWAS | Salas Fernandez et al., 2017 |
| Sorghum | RGB imaging box and semi-automated imaging analysis pipeline: Toolkit for Inflorescence (Iowa State University, USA) | Panicle shape and panicle size | IAP | 272 | 146 865 | GWAS | Zhou et al., 2019 |
| Maize | Axio Imager.Z10 (Carl Zeiss Microscopy, LLC, Thornwood, NY, USA) and image analysis using ImageJ (Cornell University, USA) | Shoot apical meristem (SAM) morphology | IAP | 369 | 1 281 000 | GWAS | Leiboff et al., 2015 |
| Maize | High-throughput plant phenotyping system (Leibniz Institute of Plant Genetics and Crop Plant Research, IPK, Germany) | Biomass, growth traits | IAP | 252 | 35 682 | GWAS | Muraya et al., 2017 |
| Maize | HRPF | 106 traits: plant morphological traits, leaf architecture traits, color traits, biomass-related traits, and growth traits | RIL | 167 | 2496 | LA | Zhang et al., 2017 |

Table 2. Continued

(Continued on next page)

| <i>Arabidopsis</i> / Crop | Platform | Extracted traits or features | Population | Sample size ^a | No. of markers ^b | Methods | Reference |
|------------------------------|--|---|------------|--------------------------|-----------------------------|---------|------------------------------------|
| Maize | PhenoArch phenotyping platform (INRA, France) | Plant leaf area, fresh biomass, transpiration rate | IAP | 254 | 758 863 | GWAS | Prado et al., 2018 |
| Maize | Unmanned aerial vehicle (Beijing Academy of Agriculture & Forestry Sciences, China) | Plant height and growth rate at four growth stages | IAP | 252 | 1 227 441 | GWAS | Wang et al., 2019 |
| Large pepper plants | SPICY: a sensor-to-plant phenotyping device equipped with RGB cameras and time-of-flight depth sensor (Wageningen University, The Netherlands) | Leaf size, leaf area, leaf angle, plant height | RIL | 148 | 493 | LA | Gerie et al., 2012 |
| <i>Brassica napus</i> | Acquire root images using a flatbed scanner and image analysis using ImageJ (Huazhong Agricultural University, China) | Primary root length, lateral root length, lateral root number, lateral root density, and biomass traits under low Pi and high Pi conditions | DH | 190 | 798 | LA | Shi et al., 2013 |
| <i>Brassica napus</i> | High-throughput plant phenotyping system (Leibniz Institute of Plant Genetics and Crop Plant Research, IPK, Germany) | Estimated biovolume, projected leaf area, early plant height, color uniformity | IAP | 477 | 16 311 | GWAS | Knoch et al., 2019 |

Table 2. Continued

IAP, inbred association panel (consists of a set of inbred lines); RIL, recombinant inbred lines; DH, double haploid population; BC, backcross population; NIL, near isogenic lines; IIs, introgression lines; MAGIC, multiparent advanced generation inter-cross. LA, linkage analysis; GWAS, genome-wide association study; –, not mentioned in the reference.

^aThe values separated by slash (/) indicate sample size corresponding to multiple populations.

^bThe values separated by slash (/) indicate marker numbers corresponding to multiple populations.

measurements of some traits are subjective and error prone (e.g., visual scoring of leaf rolling), or some traits cannot be manually measured nondestructively (e.g., digital biomass, root system architectures). The second question is how to utilize extensive lists of phenotypic traits in genetic mapping. One good example is performing principal component (PC) analysis to extract PCs of a specific phenotypic trait category and combining the PC scores and GWAS to identify a gene controlling rice architecture (Yano et al., 2019). The third question is how to rapidly clone candidate genes from the large number of QTLs. One solution is to combine genetic transformation technology, CRISPR/Cas9 technology, other omics data and statistical methods for speeding up QTL cloning (e.g., QTG-seq; Zhang et al., 2019a). This information could be used in omics-based strategies as well as in systemic and synthetic biology in molecular design breeding or molecular module programs and will greatly facilitate future agronomic improvements (Figure 2A) (Li et al., 2018; Wing et al., 2018; Zhang et al., 2019b).

High-Throughput Phenotyping and Genome Selection

Genome selection (GS) uses genome-wide markers and statistical modeling to select complex traits controlled by many alleles with small effects. It was first applied to cattle breeding (Meuwissen et al., 2001), but with the ever-decreasing cost of sequencing in recent years, GS is emerging as a powerful tool for estimating breeding values in crop breeding (Taylor, 2014). Its advantage is in predicting how crops will perform before a field test. To develop an accurate and robust predictive model, huge amounts of genotype and phenotype data from individuals or lines are necessary (Meuwissen et al., 2001). With the development of next-generation sequencing technology, markers can be easily and accurately obtained. However, phenotyping represents a serious bottleneck.

High-throughput phenotyping platforms have been demonstrated to enhance GS in grain crops. For example, an UAV carrying a remote-sensing unit with either an RGB or near-infrared, green and blue (NIR-GB) camera has been used for the high-throughput phenotyping of sorghum plant height and different genomic prediction models (Watanabe et al., 2017). UAV remote sensing will be an important and indispensable tool for high-throughput genomics-assisted crop breeding due to its relatively low cost and easy operation (Watanabe et al., 2017). Data on secondary traits (canopy temperature and green and red NDVI) obtained with remote sensing could be used in wheat GS to improve the prediction accuracy for grain yield (Rutkoski et al., 2016). Moreover, the International Maize and Wheat Improvement Center (CIMMYT) found that GS strategies are a tractable way for crop breeders to increase the rate of genetic gain and select superior higher-yielding crop varieties more efficiently after investigating several GS methods combining dynamic high-throughput phenotyping data and 2254 genotyping-by-sequencing (GBS) markers of 1170 advanced wheat lines (Crain et al., 2018).

Sensor-Based Phenotyping-Assistant Breeding

Image-based phenotyping technologies can rapidly and accurately quantify biotic/abiotic resistance traits and grain yield and quality and have a high potential to accelerate crop breeding. A comparison of a traditional long-term breeding experiment with

a more recent technology-supported example illustrates this. In Illinois in 1896, Hopkins began long-term directional recurrent selection for oil concentration in maize, increasing the oil content from 4.69% to 20.37% over approximately 100 generations (Dudley and Lambert, 2004), while Song et al. (1999) developed synthetic populations in China in which the oil content increased from 4.71% to 15.55% over just 18 cycles of selection (Song and Chen, 2004). This relative acceleration can be largely attributed to MRI and NIR spectroscopy.

Another example involves double haploid (DH) technology, which is already widely used in commercial crop breeding programs (Ishii et al., 2016). There is an ongoing need for better discrimination of haploid and hybrid kernels. An automatic screening system for maize haploid kernel identification based on the xenia effect value of the oil content and nuclear magnetic resonance (NMR) detection, developed by China Agricultural University, identified *in vivo*-induced haploid seeds with a mean accuracy of over 90% (Liu et al., 2012), which would favorably accelerate the breeding process. Furthermore, digital imaging (Yang et al., 2014; Liang et al., 2016; Makanza et al., 2018) can rapidly assess yield- and quality-related grain traits. For example, a high-throughput maize kernel trait scorer had an efficiency that was seven times greater than that of manual operators (Liang et al., 2016). Furthermore, maize vascular bundles, as key transportation paths for delivering water, mineral nutrients, and organic substances, can be measured by X-ray micro-CT (Zhang et al., 2018).

Phenotyping is a key informant for establishing the accuracy of statistical models in conventional breeding, marker-assisted selection (MAS), or GS; however, high-throughput field phenotyping is still a bottleneck in these fields (Araus et al., 2018). Indeed, developing new varieties and focusing on cost-effective returns are key objectives of breeding companies and breeders. To improve the management of breeding data and optimize breeding programs, many commercial plant breeding software programs have been developed, such as AGROBASE Generation II (<https://www.agronomix.com/>) and Plant Research Information Sharing Manager (PRISM, <http://www.sytseed.com/>). However, to date, most commercial breeding software has limited functionality. Thus, the development of multifunctional breeding software that integrates genomes, phenotypes, environments, data management, and multiomics data analysis and can perform genetic mapping is urgently needed for crop breeding.

CHALLENGES AND FUTURE PERSPECTIVES

With the aim of enabling cooperation by fostering communication among stakeholders in academia, industry, government, and the general public, 17 institutions established the IPPN in November 2015, increasing to 44 in 2019 (<https://www.plant-phenotyping.org/>). The IPPN carried out regular surveys of trends in crop phenotyping, and key findings were that (1) static phenotyping infrastructures were concentrated in Europe and Australia (Figure 3A) but with increasing investments in the USA and China; (2) the infrastructures have developed rapidly over the last 5 years (Figure 3B); and (3) over 82 mechanized indoor phenotyping platforms have been established across the globe, with the

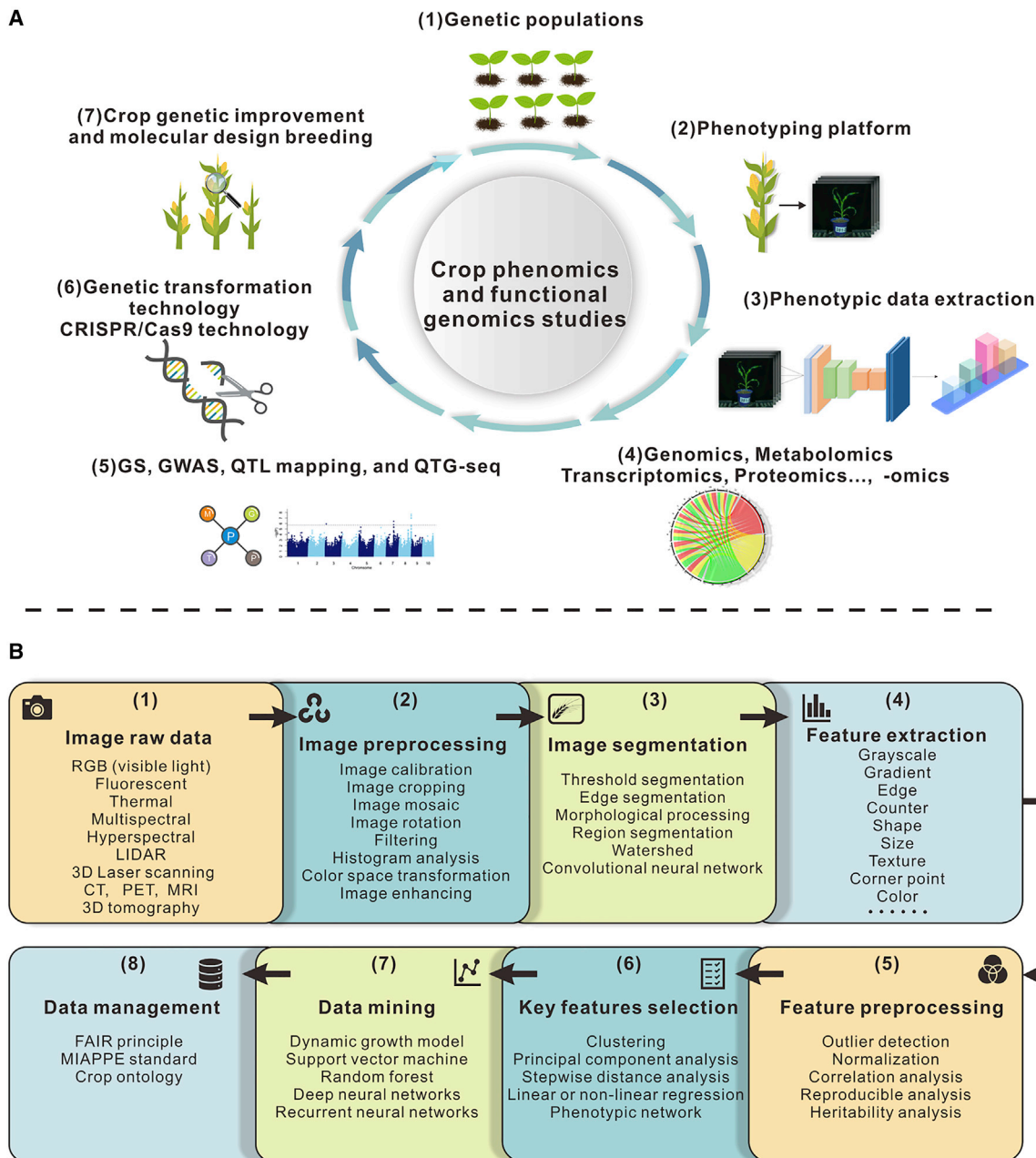


Figure 2. The Loop of Crop Phenotype-to-Genotype and Image Data Analysis.

(A) Linking crop phenotyping to functional genomics studies and crop improvement with multiomics: The high-throughput and multiscale phenotyping platform (2) can obtain the dynamic phenotypic traits of large crop genetic populations (1). The combination of phenotypic data (3) and other omics data (i.e., genomics, transcriptomics, proteomics, metabolomics) (4) can be used in mining QTL/genes via QTL mapping and GWASs (5), and combined with genetic transformation techniques to benefit crop genetic improvement (7).

(B) The workflow of image data analysis: (1) image raw data obtained; (2) image preprocessing; (3) image segmentation; (4) feature extraction; (5) feature preprocessing; (6) key feature selection; (7) data mining; (8) data management.

largest proportion of these infrastructures within controlled environments (59%) and field platforms accounting for only 18% (Figure 3C). Referring to the three surveys in 2014–2018, in our opinion, the key topics were root phenotyping, abiotic stress, field phenotyping, data management, and costs (Figure 3D), which are challenges that are discussed in the following sections. In addition, we searched for the topics of crop phenomics or high-throughput crop phenotyping in Web of Science, and

observed the similar trends in the number of published papers (Figure 3B) and the most mentioned species (Figure 3E).

Future Root Phenotyping in the Field: A Need for Innovation Below the Ground

An effective methods for assessing the RSA is still simply to dig up the root system and shake or wash off the soil; often termed

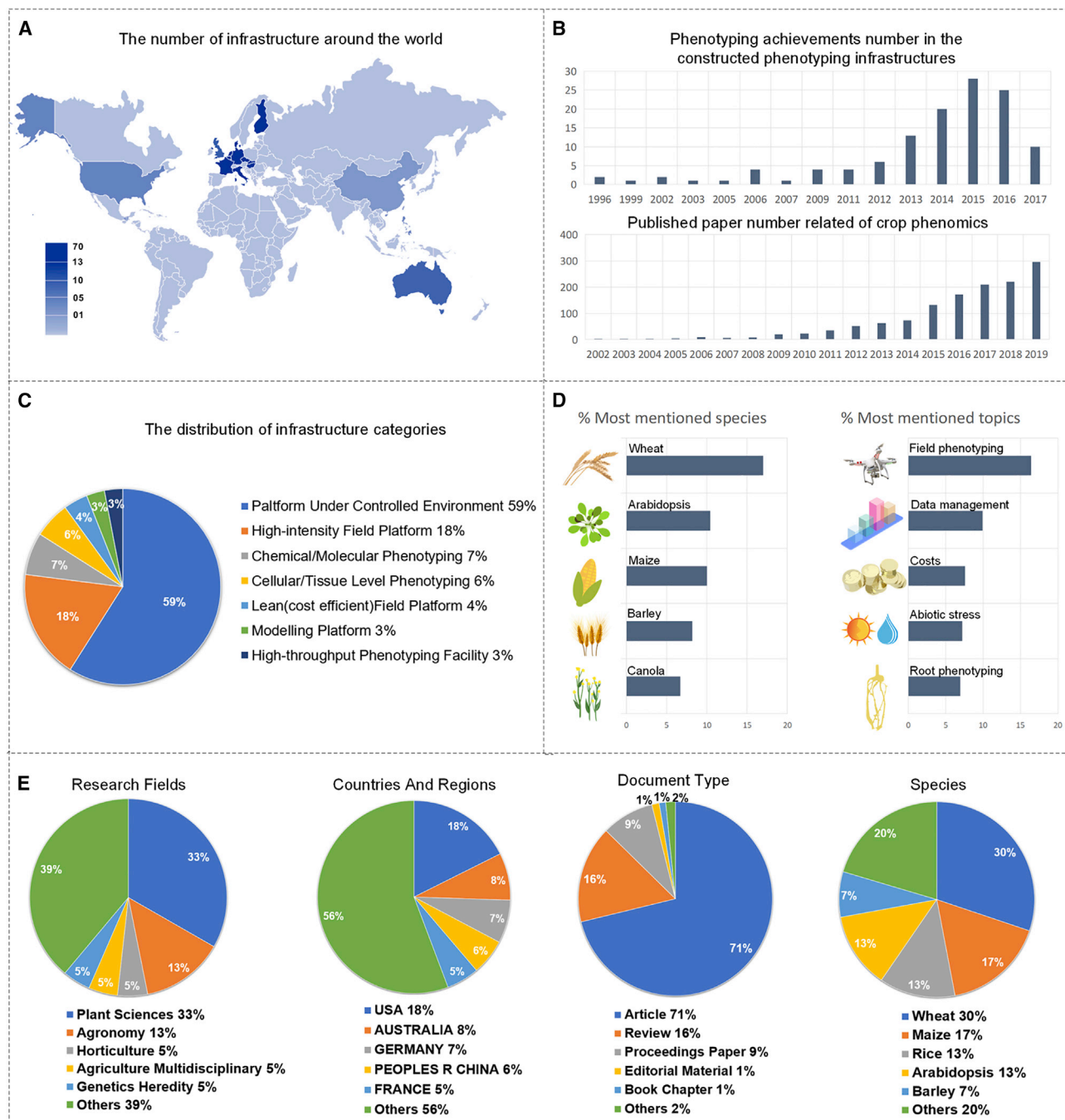


Figure 3. The Current Status of Global Phenotyping.

(A) The distributional map of plant phenotyping infrastructures around the world.

(B) Number of phenotyping achievements and number of published crop phenomics-related papers in the past 20 years.

(C) Classification of infrastructures.

(D) Summary of species and topics from IPPN surveys (2014 and 2016). Reference: database of the International Plant Phenotyping Network (IPPN, https://www.plant-phenotyping.org/ippn-survey_2016).

(E) The distribution map of crop phenomics or high-throughput crop phenotyping-related papers by research field, region, document type, and species (searched in Web of Science).

“shovelomics” when scaled to the field level (Trachsel et al., 2010). Although this method is destructive and somewhat unpleasantly messy, image acquisition can be relatively rapid (up to 5000 images/h), and those images can be rich in trait

information (39 root traits). Other root phenotyping methods include, for example, the core-break method, which was proposed to obtain the root depth (Wasson et al., 2017) and minirhizotrons with imaging sensors to observe root growth

(Svane et al., 2019). But both the core-break and minirhizotrons methods can only detect limited roots, and the root measurements were subject to the sample positions. Some nondestructive detection methods, such as ground-penetrating radar (Delgado et al., 2017) and electrical impedance tomography (Corona-Lopez et al., 2019), could be used to phenotype root biomass and root development. However, the spatial resolution of both methods is much lower (~cm/pixel), and it is hard to detect individual fine roots.

To date, nondestructive root phenotyping in the field remains a challenge due to a lack of suitable technology. In an attempt to solve this problem, the US Department of Energy funded the ROOTS project in 2017 (<https://arpa-e.energy.gov/?q=programs/roots>), which aims to evaluate and develop novel approaches, such as thermoacoustics tomography (TAT), associated particle imaging (API) using neutrons, tomographic electrical rhizosphere imaging (TERI), low-cost X-ray CT, and backscatter X-ray platforms, to dynamically image RSA. The long-term justification for the investment is associated with the production of crops that increase carbon uptake and decrease N₂O emissions under field conditions. As the advancement of UAV and sensor techniques currently benefits canopy phenotyping in the field, we believe that breakthrough technologies could provide similar benefits below ground.

State-of-the-Art Phenotyping Techniques for Abiotic Stress

The genetic architecture of abiotic tolerance, such as DR, is complex and influenced by many alleles with small effects (Fukao and Xiong, 2013). Thus, the search for generic drought tolerance using single major-effect genes has almost always been disappointing (Passioura, 2012). Several issues still need to be addressed with regard to high-throughput phenotyping platforms and high-throughput image analysis pipelines that can be used to extract an extensive list of 2D and 3D phenotypic traits related to stress and have the potential for understanding tolerance over time:

- (1) Can the complexities of tolerance be decomposed into simple and more heritable traits? Using nondestructive phenotyping and image-based traits, this may be possible; complex DR, for instance, can be dissected into highly heritable and simple image-based traits (i-traits), including digital stay-green and digital leaf-rolling traits (Guo et al., 2018a). If the loci (genes or QTLs) identified by these simple traits can also affect yield under different stress scenarios, then the effects of the identified loci could benefit crop breeding (Tardieu and Tuberosa, 2010).
- (2) How do root architectures contribute to yield and abiotic stress tolerance? The gene *DRO1*, for example, which controls the root growth angle and depth, has been used to increase rice yield under drought conditions by backcrossing with a shallow-rooting rice cultivar (Uga et al., 2013). However, variation in root architecture and function has not often been directly exploited in breeding, largely due to the huge challenge in assessing root traits.
- (3) Can DL resolve the data analysis bottleneck? DL is a versatile tool that can be used to make sense of large datasets for crop stress ICQP (identification, classification,

quantification, and prediction) (Singh et al., 2018). However, due to the outstanding identification of diseases, most DL models have been applied in biotic stress and disease detection. In the future, with different sensor technologies (hyperspectral, thermal, CT, terahertz, MRI, radar, etc.) and large multiscale (lab-to-field) phenotypic datasets that include weather and other environmental data, DL could also be used to quantify abiotic stress and predict the loci that control stress tolerance through integration with genetic information and other omics information. This approach will likely work best with large and well-annotated datasets, making the case for open data.

Field Phenotyping Bottleneck and Future Perspectives

In the previous section, we discussed the applications of ground-based, UAV, and handheld field phenotyping techniques, which have advantages and limitations. With the rapid development of UAV and peripheral equipment, many novel platforms for low-altitude remote sensing are rapidly emerging, such as mooring UAV platforms, cameras with 100 000 000 pixels, multisensor integrated platforms, miniaturization, and simplification UAV platforms. The UAV platform will partially replace the ground-based phenotyping platform and will likely be widely deployed based on new technologies in the future. In addition, integrated special UAV image processing and standardized analytical software will likely become available for a broader range of users, allowing nonexperts to process and analyze hyperspectral, thermal, or LiDAR data, for example.

For ground-based phenotyping, one of the trends is the development of field-intelligent robots, such as BreedVision (Busemeyer et al., 2013a). Equipped with multiple sensors integrated within an imaging chamber to ensure good image quality and automatic navigation, these types of robots are flexible to move to phenotype different fields and in the future could collaboratively work in a field. In terms of handheld phenotyping tools, one trend is to integrate multiple sensors into one portable device and develop on-chip data analysis software to obtain and manage more traits together. Another trend is to decrease human intervention and add data standardization processes to improve measurement reliability and efficiency. With the advancements in artificial intelligent analysis techniques, fifth-generation mobile networks, and cloud-based technologies in recent years, more smart “pocket” phenotyping tools will likely be developed and change manual field phenotyping, which has been around for thousands of years. In addition, to achieve a real sense of “cost-effective phenotyping”, the trade-off between investment of phenotyping techniques and manpower costs should be considered, which mainly depend on the different objectives (Reynolds et al., 2019b).

Data collection in the field using high-throughput technology has undergone rapid advances in recent years. However, the limiting factor now is how to manage and mine the vast amounts of data collected from fields through high-throughput technology. First, the biological objective of data collection should be clearly stated. Next, robust and user-friendly postprocessing and analysis tools for processing and interpreting raw data are urgently needed and should be improved (Araus and Cairns, 2014). For

example, combining DL and multiple optical images obtained under different field conditions will create robust models for disease phenotyping and even the early detection of plant stress (Singh et al., 2018).

Each year, the thousands of phenotyping experiments in environmentally controlled growth facilities or in the field will produce large amounts of phenotypic data. However, the replication of results by the same researcher or the reproducibility of results by different laboratories in independent experiments are often not satisfactory because of the unexplained variation of environments (Poorter et al., 2012). Thus, environmental factors are vital and should receive at least the same amount of attention as the traits that are measured, which leads to the next question: how to measure all the environmental impacts? Envirotyping, defined as a full set of next-generation high-throughput accurate envirotyping technologies, could help to address this issue (Xu, 2016). Moreover, integrating multityping information, the genotyping \times environment \times management (G \times E \times M) interaction could also be investigated, and predictive phenomics would be possible (Xu, 2016; Araus et al., 2018). In recent years, crop yield growth (genetic gain) has been slowing down, affected by several factors, for example, population, genotype, heritability, GS model, and breeding scheme (Xu et al., 2020). Integrated with optimized experimental design, high quality of field trials, robust crop model, envirotyping, and other strategies, high-throughput and accurate phenotyping will improve the heritability and potential for genetic gain (Araus et al., 2018).

Image Data Analysis and Big Data Organization

The imaging data formats vary widely depending on the different imaging sensors (e.g., RGB, thermal, hyperspectral, CT; Figure 2B), thus it is challenging to conclude a common process of image analysis. Although phenotypic data analysis is not the focus of this review, we summarize a workflow of image data analysis (Figure 2B): (1) image preprocessing to clip or merge raw images, enhance image contrast, remove the noise, and transform the space to get the image easy to handle in the next step; (2) image segmentation (e.g., threshold, morphological processing) to obtain the objects of interest from the background; (3) feature extraction to get the raw features according to the experimental targets, which mainly include grayscale, gradient, edge, counter, shape, size, texture, corner point, color features, and so on; (4) after extraction of huge amounts of raw features, feature preprocessing and traits selection should be adopted to filter and explore the key features; (5) data mining, for example, building dynamic growth using mechanistic models (Chen et al., 2014) or using DL (e.g., deep neural networks [DNNs] or recurrent neural networks [RNNs]); (Singh et al., 2018) to explore more spatial and temporal information. One challenge of using DL is an insufficient number of training datasets, which could be smartly resolved by citizen science (Giuffrida et al., 2018). There are some reviews on the software and algorithms of image analysis (Fiorani and Schurr, 2013; Perez-Sanz et al., 2017; Atkinson et al., 2019). Interestingly, an online database referencing more than 90 plant image analysis software solutions would benefit users to quickly find the proper solutions and has been recommended (Lobet et al., 2013). After huge amounts of data

have been obtained, the next question is how to manage the Big Data?

To manage and integrate the extensive amounts of data from multi-optical and other sensors, Wilkinson et al. (2016) proposed the FAIR (findable, available, identifiable, and reusable) principle to allow the finding and reuse of data across different individuals or groups, which means all the necessary metadata, such as resource and data acquisition information, measurement protocols, data description, and environmental conditions, should be clearly addressed and capable of being accessed. According to the FAIR principle, several efforts have also been made for data management and analysis, for example, PHENOPSIS DB (Fabre et al., 2011) and CropSight (Reynolds et al., 2019a).

However, phenotypic data are rarely reused, in contrast to genomic and other omics data. Much of the phenotypic data—despite complying with the FAIR criteria—are not openly accessible, and we encourage the development of OPEN data infrastructures or the publication of primary data with DOIs. This would facilitate data reuse, and equally importantly, the development, testing, and comparison of technologies. To pave the way for data exchange and reuse, some common management standards and data formats should be clearly defined. One good case for plant biology is iPlant cyberinfrastructure (CI), an open-source project supported by the United States National Science Foundation (NSF), which provides high-performance computing, easy-to-use bioinformatics software, and large data access, and have been facilitating collaborations across the diversity of multi-disciplines, such as plant biology, bioinformatics, and computer science, etc. (Goff et al., 2011). A document of Minimum Information About a Plant Phenotyping Experiment (MIAPPE) was proposed and recommended as a necessary metadata set with ISA-Tab formatting, which mainly included phenotypic data, environments, experimental design, resource, and so on (Ćwiek-Kupczyńska et al., 2016). To support data annotation and avoid ambiguous description, ontology is the key to facilitate dataset construction. The Crop Ontology of the Generation Challenge Program was proposed by the Consultative Group on International Agricultural Research (CGIAR), aiming to develop an online integrated G-P (genotypic and phenotypic) data management tool across several species and several international agricultural research institutes (Shrestha et al., 2012). Oellrich et al. (2015) used shared ontologies, annotation standards, and shared formats to annotate 2741 genotypes with 2023 unique entity-quality statements and achieve cross-taxon phenotypic data analysis (e.g., determine the same pathway both in *Arabidopsis* root tip gravitropism and inner ear defect in humans). More open-source databases or software, such as Phenobook (open software for data collection in plant breeding; Crescente et al., 2017), Planteome database (an integrated ontology resource; Cooper et al., 2018), BrAPI (an application programming interface for plant breeding; Selby et al., 2019), were developed to benefit both basic plant biology research and plant breeding. Some reviews describe data standardizing using MIAPPE (Bolger et al., 2019) and data integration (Coppens et al., 2017).

Crop functional genomics has entered the large-scale and multi-omic stages. There is growing interest in combining phenomics

data with genomic data and other omics data, such as metabolomics, proteomics, and transcriptomics. Data storage should be standardized to facilitate the joint analysis and reanalysis of data from independent experiments or interconnections with other available biological databases and resources (Billiau et al., 2012). Comprehensive metadata description and agreed-upon ontologies are therefore needed to construct a database of multiomics information. However, the vast amounts of collected data are scattered as small pieces in many labs and vary in format and quality (Li et al., 2018). Data sharing and standardization across different communities remain a big challenge. Another challenge is the lack of funding and data infrastructure to handle these data (Bolger et al., 2019). To face the challenges, synergism efforts should be made and integrated resources and bioinformatics technologies will facilitate crop functional genomics and crop breeding efforts.

Key to Success in the Future Challenge: Talent and Joint Cooperation

In the coming decade, to address the challenges discussed earlier, emphasis should be placed on innovative techniques below the ground, state-of-the-art methods to dissect abiotic stress or other complex traits, intelligent and easy-to-promote field phenotyping, data standardization, and multiomics data dissection. Besides, the creation of phenomics talent should also be considered, even before construction of a phenotyping facility. Unfortunately, phenotypic experts are scarce, and the loss of researchers (e.g., image data analysis specialists) is common nowadays. To relieve the bottleneck, in our opinion, requires the following: (1) technical innovation: the value of traditional agriculture should be improved to attract more talent from industry; (2) more multidisciplinary training in phenomics, particularly in agricultural university or college; Huazhong Agricultural University (Wuhan, China) plans to initiate a new major of Intelligent Agriculture in 2020, which we believe will promote multidisciplinary talent in the future; (3) data sharing: sharing the right data and the right questions to attract more computer specialists to help solve problems for free, even if the solutions are not perfect (Tsafaris and Scharr, 2018).

Due to the fast development of sensor techniques, machine vision, automation technology, fifth-generation mobile networks, cloud-based technologies, and artificial intelligence (DL), phenotyping has shown great power to promote fundamental crop research and crop breeding. In addition to the technical advances and talent cultivation, some international cooperation would also relieve the challenges. The European phenotyping community projects, including EPPN2020 (<https://eppn2020.plant-phenotyping.eu/>) and COST Action (http://www.cost.eu/COST_Actions/), have shown the power of connecting phenotyping and various omics scientists to promote crop breeding. To promote the next green revolution in crop breeding, the development of an International Crop Phenome Project (ICPP) should also be encouraged. To achieve this goal, we call for the coordinated efforts of multiomics international networks and diverse disciplines and coordinated financial support within many countries.

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