# Chapter 10 Advances in Integrated High-Throughput and Phenomics Application in Plants and Agriculture



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## 10.1 Introduction

Since the new millennium, emerging next-generation sequencing (NGS) technology has assisted researchers in measuring intractable and complex traits in biological data acquisition (Furbank et al. 2019). Among these technologies, crop functional genomics and whole-genome sequencing (Li et al. 2018) allowing in the acquisition of genome-wide association studies (GWAS) and quantitative trait locus (QTL) mapping

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(Xiao et al. 2017) on the large-scale phenotypic and genetic architecture of complex traits (Shi et al. 2019; Wang et al. 2019) have stepped into the high-throughput and big-data era. For instance, plant genome annotation revealed 26500 loci for *Arabidopsis* to 41000 genes in rice. Recently, the poplar genome was revealed to encode 45000 genes, and more than 40000 genes regulate multidimensional physiological and biological processes in Medicago and Lotus (Sterck et al. 2007). However, a genome size of 950 MB encoding about 35,000 genes in the tomato revolution our understanding of tomato biology (Barone et al. 2008). In the last two decades, thousands of genes in tomato (Barone et al. 2008), rice (Yao et al. 2018), and *Arabidopsis* (Bouché and Bouchez 2001) were functionally characterized through various traditional phenotypic techniques. The challenge is to attach the functions of these enormous numbers of genes restricting functional genomics studies and crop breeding (Deery et al. 2016).

The term "phenome" was first characterized by Davis (1949): approach to systematically explore the comprehensive set of extragenic, non-autoreproductive portions of the cell and represented the set of phenotypes, either cytoplasmic or nuclear. Later on, phenomics was described as a complex interaction of an organism's genotype and phenotype (Houle et al. 2010). Conventional crop phenotyping is laborious, tedious, intensive, and potentially injurious to plants (Chen et al. 2014). Recently, plant phenomics has been growing and advancing rapidly in the last decades includes the set of approaches used to precisely assess individual cells, tissue, leaf, or plant to the large scale, i.e., ecosystem (Fiorani and Schurr 2013). In addition, crop phenomics is the inter/intradisciplinary study of high-throughput phenotyping platforms for accurate acquisition and an organism-wide scale analysis of phenotypes in crop development (Fig. 10.1) (Zhao et al. 2019). In recent years, next-generation genotyping and phenotyping have been advantageous over traditional breeding approaches due to the accuracy of these methods and their robust capability to accelerate crop breeding (Pasala and Pandey 2020).

Advanced sophisticated sensors, vision-guided robotics, automation technology, and machine learning, with applications in harvesting, quality assessment, sorting, screening, and packaging, have been extensively implemented in the agri-production industry to promote efficiency (Ruiz-Garcia et al. 2009) and for breeders to have a breakthrough in making rapid genetic progress (Furbank et al. 2019). The integration of genomics and phenomics can accelerate genetics gain in breeding programs and identify new traits in diverse plant germplasms that help breed populations through the crossing and artificial genomics (Bortesi and Fischer 2015). However, these approaches should apply at the early stages of plant development. To search for how and why to measure the whole genome and whole-plant phenotypes has been extensively explored. Over the last few decades, the answer to the former has been examined in detail. Recent achievements in high-throughput technologies allow us to conclude how and why to measure organism-level phenotype in the coming decades (Houle et al. 2010). For instance, RICE 2020 has been initiated to systematically and functionally characterize all protein transcripts and gene transcripts in rice by 2020 (Zhang et al. 2008). This chapter tries to cover the significant advances in applying integrated high-throughput and phenomics approaches in genetics studies. Finally, we discuss the challenges in agri-phenomics and specify our standpoint on phenomics-related studies.



Fig. 10.1 Timeline of significant achievements in the deployment of phenomics approaches, including genomics, phenotyping, machine learning, and artificial intelligence

# 10.2 Phenomics

The plant being sessile organisms interact with multiple environmental stress across the life cycle and developed multiple avoidance strategies (Lymperopoulos et al. 2018). Conventional phenomics approaches focus on crop productivity and yield, while advanced sensing approaches enable plant scientists to record the environmental history of plants, together with their dynamic responses (Pratap et al. 2019). Robust phenomics is vital to plant breeding due to its fundamental basis for developing new varieties. However, advancement in phenomics including robotics, image processing, and deep learning enables non-destructive monitoring of plants development and function to extract valuable information (Hickey et al. 2019). The ongoing challenges with new generation phenomics are data handling and continuous contribution by computational technologies critical to maintaining rapid advancements in accelerated breeding programs (Tester and Langridge 2010).

## 10.2.1 Phenomics for Crop Microphenotypic Traits

Phenotyping at organ, tissue, and cellular levels requires complex procedures, and several automated high-throughput and large-scale phenotyping platforms have been developed for screening and assessing Arabidopsis, rice, and maize under controlled environmental conditions or in the field (Table 10.1) (Kaul, Koo et al. 2000). Phonoscope, a large-scale high-throughput phenotyping platform, monitors the plant growth rates of more than 700+ plants (Tisné et al. 2013). Renovator, accurate quantification for genotypic variation in natural genetic population using growth and photosynthesis as a phenotypic indicator of plant performance (Flood et al. 2016), utilizes a monochrome camera on the moving rail system platform with 1440 plants carrying capacity. The renovator can collect leaf area and light-use efficiency. Instead, PlantScreen with automatic weighing and watering moving conveyor belts transported plants from growing chamber to RGB imaging cabinets, chlorophyll fluorescence, and dark acclimation chamber (Awlia, Nigro et al. 2016). Plant root traits play a critical role in nutrient acquisition and the transport of water from the soil to the aerial parts of plants. Microimaging and sensing have improved our understanding of root anatomy and functions. Wu et al. (2011) developed a low-cost computer-aided 3-D visualization and quantitative analysis technique based on classical paraffin embedding serial sections and microtome techniques. In 2012, a highthroughput, high-resolution phenotyping platform, RootSlice, aided with laser and 3-D visualization was introduced to analyze (Burton, Williams et al. 2012) and quantify root anatomy with semi-auto RootScan (Chimungu, Loades et al. 2015). RootAnalyzer, automatic versatile root tissues, and root cells image phenotypic analysis tools. RootAnalyzer has more than 90% accuracy and improves image segmentation efficiency in quantifying the properties of tissues (Chopin, Laga et al. 2015). Walter et al. (2007) introduced an automated imaging pipeline GROWSCREEN to acquire the dynamics of seedling growth acclimation such as relative growth rate, total leaf area, and root area. Moreover, GROWSCREEN FLUORO allowed the simultaneous phenotyping of chlorophyll fluorescence in rosette plants (Jansen, Gilmer et al. 2009). The mapping power of GROWSCREEN for GWAS or QTL analyses reduced due to relatively limited carrying capacity in of platform in combination with micro-environmental heterogeneity.

TraitMill by CropDesign (Belgium) yield-related morphometric traits measure exclusive bioinformatics tools and a high-throughput phenotyping platform (Reuzeau et al. 2010). Computer-controlled Scanalyzer3D platform accelerating plant phenomics aided with automated watering and weighing, a conveyor with a capacity of 2400 plants, and RGB imaging stations to estimate plant biomass using RGB images (Virlet et al. 2016). In addition, the salinity tolerance of chickpea can be assessed by Scanalyzer3D (Hairmansis, Berger et al. 2014). In addition, rice (Hairmansis, Berger et al. 2014), and nutrient starvation in crops, in field diseases monitoring, and more physiological features including growth date. In recent years, an array of algorithms and tools has been developed (Table 10.1). However, there is

Plant	Platform	Parameters	References
Canopy	LabVIEW	Growth parameters Bai et al. (2016) and Zhang et al (2017)	
Leaf	PHENOPSIS		Granier et al. (2006)
	WIWAM	_	Clauw et al. (2015)
	GROWSCREEN	Leaf discs	Nagel et al. (2012)
	LemnaTec	Growth and yield	Neumann et al. (2015)
	Phenodyn/Phenoarch	Leaf elongation	Sadok et al. (2007)
	Integrated Analysis Platform	Leaf orientation	Klukas et al. (2014)
	LAMINA	Leaf parameters	Bylesjö et al. (2008)
	Leaf Analyser	Leaf architecture	Weight et al. (2008)
	Phenovator	Photosynthesis	Flood et al. (2016)
	LeasyScan	Canopy traits	Vadez et al. (2015)
Root	Shovelomics	Root growth parameters	Burridge et al. (2016)
	Self-construction		Bucksch et al. (2014)
	LemnaGrid		Guo et al. (2017)
Shoot	PlantScreen <sup>TM</sup>	Imaging/nonimaging chlorophyll fluorescence and plant growth parameters	Humplík et al. (2015)
	Rosette Tracker	Leaf area, perimeter diameter	De Vylder et al. (2012)
	PHENOSCOPE	Vegetative growth and homogeneity	Tisné et al. (2013)
Whole plant	TraitMill	Growth and yield parameters	Reuzeau et al. (2010)
	PlantScan	_	Sirault et al. (2013)
	HRPF		Yang et al. (2012)
	GlyPh	Growth and soil water content estimation	Pereyra-Irujo et al. (2012)
	BreedVision	Growth and genetic parameters	Busemeyer et al. (2013)
	OloPhen	Leaf area, growth, and survival rate	De Diego et al. (2017)

 Table 10.1
 Phenomics tools deployed for microphenotyping

still a need to simplify complex phenotypic procedures at cellular and tissue levels. The introduction of advanced imaging techniques will accelerate microscopic phenotyping and assist in advanced phenotyping studies, particularly on specific cell phenotypes and crop organ characteristics.

# 10.2.2 High-Throughput Plant Phenotyping Platforms in a Controllable Environment

In crops, the breeding and selection of desirable traits is vital for sustainable agriculture, global food security, and the growing global demand for fiber, feed, and fuel (Dungey et al. 2018). High-throughput plant phenotyping with non-destructively image approaches facilitate efficient screening of plants based on their morphological and physiological traits, may assist in increasing productivity, shorten the crop cycle, improve plant efficiency in the environment, and help in linking phenomics to genomics (Li et al. 2014). However, the application of high-throughput plant phenotyping in agriculture is still in its infancy. It needs to have the accuracy and efficiency to assess the growth and morphological traits of plants, such as growth patterns, development rate, plant aerial architecture, root architecture system, and plant biomass. These features are fundamental to understand function-structure of plant in assesing biotic and bitoic responses for sustainable management of crops.

Global warming has posed a risk to global food demand as it has impacted agricultural productivity in the past few decades. Despite the negative influences of climate change, however, the selection and breeding of environmental resistance crops are needed today, but they should be done without compromising the quality and quantity of crops (Langstroff et al. 2021). Controlled environment phenotyping (CEP) is a nondestructive approach for exploring plant behaviors, which enable breeders to search for genotypes capable of coping with future environments (Xue et al. 2019). The primary problems in upcoming plant breeding programs are the lack of infrastructure and the diversity of users (Carpentier et al. 2019). Currently, a practical approach that is being used for searching scientific opportunities is bibliometric science mapping, which is done by analyzing scientific publications (Van Raan 2004). This method has been applied for analyzing phenomics. Under such a scenario, there is a need to develop dedicated tools, infrastructures, and resources for phenotyping genomics resources. The production of such high-throughput plant phenotyping resources could only be possible through a public-private partnership.

Luckily few initiatives arose to integrate fully controlled environment facilities climate-specific locations between laboratory-based work and "real-world" scenarios (Carpentier et al. 2019; Costa et al. 2019). For instance, the projects EPPN (http://www.plant-phenotyping-network.eu/), the COST Action FA1306 (http:// www.cost.eu/COST\_Actions/fa/FA1306), and EPPN2020 (https://eppn2020.plantphenotyping.eu/). Similarly, European Infrastructure for Pan-Phenomics and simulation for global food security, the ESFRI-project EMPHASIS, jointly launched synergistic pan-European excellence in phylogenomics for developing relevant approaches and shared infrastructures. German Plant Phenotyping Network (DPPN) provides a robust phenotypic portfolio and shares productive and efficient infrastructure. On a global scale, China, USA, and Canada national phenotyping efforts include Asia-Pacific Plant Phenotyping Network (NAPPN, https://www.plantphenotyping.org/), and the International Plant Phenotyping Network (IPPN; https://www. plant-phenotyping.org/) develops integrated approaches beyond the national and regional perspectives.

High-throughput plant phenotyping can be integrated into greenhouses which allow crop phenotyping at the whole-population level and observe natural-variation in GWAS citrus (Minamikawa et al. 2017), rice (Crowell et al. 2016; Rebolledo et al. 2016; Yang et al. 2014), maize (Gage et al. 2018; Wang et al. 2019), wheat (Beyer et al. 2019; Rasheed et al. 2014), barley (Bergsträsser et al. 2015; Neumann et al. 2017), soybean (Bergsträsser et al. 2015). Besides acting as a shield from light, rain, and extreme temperatures, greenhouses provide a straightforward environment conducive for plant nutrient, salinity, and drought studies (Neumann et al. 2015). During drought, identification, and selection of precisely multitude drought heritable traits beneficial to characterize a phenotype (Chen et al. 2014). Global warming impacts agriculture productivity at global scale proxies drought indices to account and predict drought severity (Mukherjee et al. 2018) owing to few crop species spatiotemporal adaptations with varying productivity. Temperatures have drastic effects on crop yield and productivity (Zhao et al. 2017); for example, an elevated temperature decreases crop yields in maize up to 90% (Hatfield and Prueger 2015). Control environment integrated with high-throughput phenotyping enables identification of QTL and GWAS necessary for repeated phenotyping to ultimate phenotype (Muraya et al. 2017). The ecosphere is highly sensitive to temperature changes and differentially affects plant growth at different altitudes (Rosenzweig et al. 2014), influencing plant adaptation and productivity. Flowering in plant is controlled by daily temperature fluctuation, day length, light intensity, and seasonal cues to help understand the dynamic genetic components to plant adaptation (Li et al. 2010). In general, intercepted light can increase total plant biomass (60%) (Poorter et al. 2016). Dynamic environmental components are integrated with high throughput approaches to explore genetic variations in field or controlled environments.

## **10.3** Application of Machine Learning in Phenomics

Integration of artificial intelligence in interdisciplinary fields has been grown exponentially in the last decade. Artificial intelligence applications such as deep learning, sensors, and machine learning successfully enable high-throughput phenotyping of plant traits into non-invasive imaging approaches (Nabwire et al. 2021). The accuracy and efficiency of data collection and analysis Improve through deep learning and machine learning for vigorous image analysis and influential study of phenotypes. Conventional breeding approaches of phenotyping are destructive with sufficient resolution and require crop harvesting at specific plant growth stages (Furbank and Tester 2011). The plant breeding programs are significantly lagging behind genomics, slow, time consuming, and require repeated experiments to validate certain traits pivotal for crop improvement (Fahlgren et al. 2015). The noninvasive high-throughput imaging approaches enable phenotype visualization at a cellular scale. The imaging techniques such as chlorophyll fluorescence (Zarco-Tejada et al. 2009), thermography (Oerke et al. 2006), spectroscopic imaging (Montes et al. 2006), and digital imaging (Jensen et al. 2007) carry a large amount of extractable data to support biological interpretations of plant growth (Walter et al. 2010). Hight-throughput artificial intelligence architecture applied in phenotyping are listed in Table 10.2.

Currently, high-throughput approaches like growth chambers (Bai et al. 2016), imaging sensors (Chaerle and Van Der Straeten 2000), data acquisition, and statistical software are employed for data collection, management, and interpretation at laboratory and field levels. Integration of these techniques into artificial intelligence in the form of machine learning (Kruse et al. 2014) and computer vision (Casanova et al. 2014) attribute to the non-invasion aspect of phenomics (Montes et al. 2007). Artificial intelligence applications are expanding with a public-private partnership in developing and disseminating these phenomic approaches that address the challenges of costly infrastructure and proprietary data formats. Thus far, computer vision, deep learning, and machine learning have been applied in phenomics. Since 1970, various machine learning models, such as Bayesian networks, support vector machines, and perceptron, have been developed, but none have proved to be the best

Model	Sensor	Crop	Trait	Reference
Machine learning	e RGB/NIR	Macrotyloma uniflorum	Plant height, shoot length, flower percentage and pods, pod length, seeds per pod	Amal et al. (2020)
		Brassica napus, Camelina sativa, Fabaceae, Cicer arietinum	Flowering detection UAV	Obidiegwu et al. (2015)
		Zea mays	Identifying growth rate	Dutta et al. (2016)
	Scanner	Vicia faba	Root system architecture	Mula et al. (2016)
	RGB, IR, HS	Beta vulgaris	Water, nutrient stress	Mula et al. (2016)
		Glycine max	Canopy wilting	Howarth et al. (2011)
	3D laser scanning	Cicer arietinum	Evapotranspiration	Leport et al. (2011)
Deep learning	RGB/ multispectral	Glycine max	Plant yield estimation	Waring and Cleary (1967)
		Zea mays	Water stress	De Bei et al. (2011)
		Triticum	Root system architecture	Zakaluk and Ranjan (2008)
	HS	Zea mays	Relative water content	Patanè et al. (2016)

Table 10.2 Integration of artificial intelligence tools in plant phenomics

as they all have certain limitations (Roscher et al. 2020). Later on, neural networks integrated with data collection and information processing infrastructure enable machine learning to determine best-fitting models (Roscher et al. 2020). One of the advantages of phytogenomic machine learning is their ability to simultaneously processing massive amounts of data in combination with other related features (Roscher et al. 2020) assist in the identification and classification of plant traits, including disease or pest detection (Wetterich et al.), floral transition (Wetterich et al.), and seeds classification (Sabanci et al. 2017).

Deep learning is a subset of machine learning instead of several complex high throughput sensors with a wide range of phenotype applications (LeCun et al. 2015). However, the data collected through this versatile tool contained high variability making its application more complicated but providing more reliable prediction (Singh et al. 2018). Deep learning in computer vision-based phenomics ensures the more reliable processing of phenotypic images involving multilayered approaches network, each performing its operation in succession, improving prediction and discrimination ability (Pound et al. 2017) by a process called transfer learning. Table 10.2 lists some deep learning phenomics approaches that have been used for plant morphology and stress identification. However, applying machine learning and deep learning subsets of artificial intelligence enables plant scientists robust identification, classification, and detection of environmental variability influences on plant growth, development, and other related physiological parameters.

#### 10.4 High-Throughput Phenomics Enhances Phytogenetics

High-throughput phenomics has been widely used in remote sensing, root phenomics, deep learning for plant stress, and vision sensing technologies in disease and pest detection (Atkinson et al. 2019; Mahlein et al. 2018). However, applying phenotyping technologies and genome sequencing is still limited in genetics and crop breeding studies both in the field and within the lab. A plethora of studies have identified QTL in many plant species, but still sveral issues need to be resolved. Among them is how to charctarizes dymanic QTLs for complex traits at mutiple grwoth stage or at different species level or districtive tarit measurements including root architechture, biomass allocation, and nutrient assessment. Identification and efficient functional characterization of potential QTLs. Integrating various genomics approaches with systemic and synthetic molecular biology approaches will significantly facilitate future breeding programs.

The regulation of the size of maize shoot apical meristems (SAMs) is correlated with flowering. High throughput analysis enables integration of SAM morphological traits with GWAS and QTL, demonstrated their contribution to SAM development (Leiboff et al. 2015). High-throughput non-destructively micro-CT-RGB phenotyping and genomics enable large-scale assessment of rice tiller traits, tiller growth, and plant traits nine growth stages. Among these traits, 402 significantly influence grain yield, vigor-related traits and yield (Wu et al. 2019). However, such

integration of genomics and phenomics is beneficial in crop breeding programs required for high yields and compact planting. Genome selection is another robust genotype-phenotype approach that involves statistical modeling and genome-wide markers. It allows efficient and accurate markers to be identified, but associated phenotype prediction is still a bottleneck in crop breeding (Taylor 2014). The process of photosynthesis and transpiration in leaves (Wang et al. 2015) depends on the number of leaves inm plants as well as the leaf size, shape, and greenness (Wang et al. 2011). The genetics study of rice and maize leaves by high-throughput leaf scoring revealed nine loci associated with leaf traits in 533 rice accessions at thee growth stages. In maize, QTL mapping of 22 leaf traits of a RIL population at 16 growth stages predited leaf tarits (leaf angle and length) being an indicator of yield (Yang et al. 2015).

Integration of high throughput phenomics with large-scale GWAS or QTLs expanded our understanding of crop developmental dynamics and emerged as a tool for plant genomics, gene expression, and characterization. The root system architecture is a promising trait for nutrients and water acquisition from the soil. Dissecting the root genetic will be helpful in increased nutrient and water acquisition from the soil. Two genetic studies on root traits were performed on rice and *Brassica* (Courtois et al. 2013; Shi et al. 2013). They conducted a GWAS of 15 root traits using vision sensors and detected associations between deep root number and mass. In *Brassica napus*, 38 QTLs were predictive indicators under phosphorus variability. Shi et al. (2013) used an agar-based high-throughput root phenotyping system to identify QTLs associated with phosphate variability correlated with *Brassica napus* root architectural traits.

Environmental factors, including both abiotic and biotic factors, can produce a variety of phenotypic effects. The rapid development of non-destructive high-throughput plant phenotyping approaches has been popularized in a plethora of crop populations to reveal the genetics of complex quantitative traits to various environmental factors (Yang et al. 2020), such as phosphate deficiency tolerance of Brassica napus (Shi et al. 2013), drought response of wheat (Parent et al. 2015), salinity tolerance of rice (Al-Tamimi et al. 2016), and drought resistance of rice (Guo et al. 2018). Most of these studies have focused on external responses, such as the morphology, biomass, and greenness-related traits. The internal response of plants to drought is mainly unknown. Wu et al. (2021) develop a non-destructively image-based traits (i-traits) approach to plant responses to drought. The i-traits are highthroughput image analysis pipelines aided by RGB optical sensors, X-ray computed tomography and hyperspectral imaging. In maize, i-traits identified 4322 drought-responsive loci encoding 1529 QTLs, including 15 QTLs containing potential markers for drought tolerance breeding in maize. Combining crop genetics information with genotype-phenotype approaches revolutionizes researchers' understanding of complex traits and reinforces the new era of crop breeding.

#### **10.5** Conclusion and Future Perspective

Crop breeding evolved from conventional approaches to phenotypes-genotype aided breeding through advancement in next-generation sequencing of crops. Plant breeding enters the next era of phenomics which enables breeders accurate sampling to phenotype various traits. In the last few decades, phenomics has entered a new period of advancements as it integrated machine learning, deep learning, and artificial intelligence to predict the phenotypic characteristics of different crop populations. These approaches inherent data from various sources tend to accelerate crop breeding programs.

Considerable efforts have been placed in agriculture and global food security to maintain sustainable crop growth and productivity. Sustainable agriculture and crop production by integrating genetic tools lead to the advancement in phenomics, but demand for financial investments diminishes the development of agriculture. Phenotypic studies focus on aerial plant parts and underground plant parts; however, the integrated role of aerial-phenomics to underground phenomics influenced, but various biotic and abiotic stresses need to consider. For instance, the primary root of plant roots determines the plant's capacity to store water or other essential plant nutrients in the stem. Integrated phenotype, the ratio of root length to stem height, could be related to crop yield. For such integrated phenotypes, algorithms to compute plant imagery are required to be developed.

Furthermore, for early detection of environmental stresses, including water, drought, salinity, temperature, etc, effort should be placed to investigate the phenotypes for characterizing the propagating stress and classified them into different stages such as moderate, extreme, or exceptional. Similarly, controlling the root growth angel may contribute to the speed of recovery.

The advancement of phenomics in "big data" enables the plant science community to establish new theories in plant phenotypic approaches to integrate artificial and collaborative research at global levels. Crop phenotypic information should focus on developing tools that comprehensively integrate multi-tudinal scale emphasize on pheno-envir-genotype and physiological parameters to systematically and complete phenomic information. The critical problem of functional phenomics is its development and application in phenotyping. The introduction of new methodologies integrated with artificial intelligence and machine learning help minimize environmental challenges. These throughput approaches collect digital features efficiently. These features' precise and robust interpretation dig out critical quantitative and qualitative phenotypic traits for functional genomics. High throughput approaches also facilitate the integration of multi-tudinal phenotypic information for big-data development, management, shareability, and globality in crop genopheno-envirotype analysis and utilization. In short, for the future of phenomics, we urgently need synergism at the global level. Search for novel tools and methodologies offer powerful tools to dissect the processes in plant growth, development, and producing high-yielding and climate-resilient crops.

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