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Plant phenomics and high-throughput phenotyping: accelerating rice functional genomics using multidisciplinary technologies

Wanneng Yang[1,2,3,5], Lingfeng Duan[2,5], Guoxing Chen[4], Lizhong Xiong[1] and Qian Liu[2]

The functional analysis of the rice genome has entered into a high-throughput stage, and a project named RICE2020 has been proposed to determine the function of every gene in the rice genome by the year 2020. However, as compared with the robustness of genetic techniques, the evaluation of rice phenotypic traits is still performed manually, and the process is subjective, inefficient, destructive and error-prone. To overcome these limitations and help rice phenomics more closely parallel rice genomics, reliable, automatic, multifunctional, and high-throughput phenotyping platforms should be developed. In this article, we discuss the key plant phenotyping technologies, particularly photonics-based technologies, and then introduce their current applications in rice (wheat or barley) phenomics. We also note the major challenges in rice phenomics and are confident that these reliable high-throughput phenotyping tools will give plant scientists new perspectives on the information encoded in the rice genome.

Addresses
[1] National Key Laboratory of Crop Genetic Improvement and National Center of Plant Gene Research, Huazhong Agricultural University, Wuhan 430070, PR China
[2] Britton Chance Center for Biomedical Photonics, Wuhan National Laboratory for Optoelectronics-Huazhong University of Science and Technology, 1037 Luoyu Road, Wuhan 430074, PR China
[3] College of Engineering, Huazhong Agricultural University, Wuhan 430070, PR China
[4] College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, PR China

Corresponding authors: Xiong, Lizhong (lizhongx@mail.hzau.edu.cn) and Liu, Qian (qianliu@mail.hust.edu.cn)
[5] Contributed equally to this review.

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Introduction

Rice is a major crop and a staple food throughout the world. In the face of the challenges presented by global environmental change and the rapidly growing human population, methods to exploit the high-production and high-quality potential of rice with reduced input are urgently needed [1]. Therefore, appropriate strategies for developing green super rice that would possess insect and disease resistance, high nutrient use efficiency, and drought resistance, has been proposed [2•]. Additionally, because of its importance as a model crop for monocotyledon plant science research, there have been many efforts to improve rice functional genomic research using high-throughput genomics tools [3]. Because of the rapid development of functional genomic and gene technologies over the past decade, particularly sequencing technology, the complete rice genome is now available, and the functional analysis of the rice genome has entered the high-throughput stage [4]. Although dozens of key genes, such as *S5* [5], *GS5* [6], *SNAC1* [7], *RID1* [8], *GS3* [9], and *pms3* [10], have been cloned or characterized for their functions in controlling important agronomic traits, the available genetic information has not been adequately exploited due to the outdated phenotyping tools. Indeed, it is apparent that high-throughput physiology and phenotyping have become a new bottleneck in plant biology and crop breeding [11].

To relieve this bottleneck and receive the full benefit of the available genomic information, plant phenomics, which integrates technologies such as photonics [12•], biology [13•], computers, and robotics, will permit the functional characterization of rice genes [14••]. Thus, reliable, automatic, multifunctional, and high-throughput phenotyping platforms should be developed to allow rice physiology and phenomics to better parallel rice genomics. Through such novel technologies, plant phenomics could offer plant scientists new insight into all the aspects of living plants [15••].

The most frequently investigated phenotypic traits include root morphology [16•], leaf characteristics [17], biomass [18,19••], yield-related traits [20••], photosynthetic efficiency [21], and abiotic stress response [22]. Here, we first introduce the key plant phenotyping technologies and highlight the photonics-based technologies. Next, we focus on the current applications in rice (wheat or barley) phenomics, as these three crops have similar morphological traits, such as multiple tillers and leaf characteristics. To conclude, we envision the application of a high-throughput phenotyping platform and note the major challenges in rice phenomics.

Key technologies in plant phenomics

Visible light imaging

Since the first digital camera was invented by Eastman Kodak in 1975 (www.letsgodigital.org/en/16859/ce-hall-of-fame), visible light imaging technology has been widely adopted in plant science due to its low cost and ease of maintenance. With a similar wavelength (400–700 nm) perception as the human eye, two-dimensional (2D) photography can be used to analyze shoot biomass [18,19••], yield-related traits [20••], leaf morphology [23], panicle traits [24], and the system architecture traits of washed roots or roots grown in transparent media [25].

Several approaches have been developed to overcome the loss of spatial and volumetric information upon compression to 2D images such as three-dimensional (3D) mesh algorithms, which could provide more accurate estimations of the morphological features of roots or shoots. A novel 3D imaging and software platform (RootReader3D) for the rice root system was developed to measure 27 root traits by acquiring 40 2D images, and this phenotyping platform can monitor a number of root growth and root system architecture (RSA) traits with an efficiency of over 100 roots per day [26]. Another novel 3D mesh-based technique was developed for the high-throughput analysis of stem height, leaf width and leaf length in cotton plants by capturing 64 side-view images when each plant is rotated 360°. This methodology exhibited a mean absolute error of less than 10% and an average execution time (including 3D mesh segmentation and data extraction) of approximately 4.9 min [27]. Additionally, a 3D digitizer and L-system formalism was used to reconstruct '3D virtual rice', with which it was possible to determine the growth function and estimate the tillering process and leaf accumulation [28]. Thus, when integrated with 2D or 3D image analysis, low-cost visible light-imaging techniques are always a first choice and are popular components of the integrated phenotyping platform. However, there is always a trade-off between the precision and efficiency of image analysis; with equivalent trait extraction accuracies, 2D digital image analysis is more efficient and suitable for high-throughput phenotyping than in 3D technology.

Infrared and hyperspectral imaging

Because of internal molecular movements, all objects emit characteristic infrared radiation [29]. Two popular infrared imaging devices can be used to screen radiation images: a near-infrared (NIR, wavelength of approximately 0.9–1.7 μm) imaging device and a far-infrared (Far-IR, wavelength of approximately 7.5–13.5 μm) imaging device. Healthy green plants reflect a large proportion of NIR light from 800 to 1400 nm, whereas the soil reflects little NIR light; moreover, soil and unhealthy plants reflect considerably more red wavelength light as compared with healthy plants. For these reasons, many studies have combined NIR imaging and visible imaging to detect vegetative indices. A Crop Phenology Recording System (CPRS) has been developed for monitoring rice growth. CPRS uses visible light imaging to derive the visible atmospherically resistant index and uses near-infrared imaging (830 nm) to derive the night-time relative brightness index and then establishes the relationship between the camera-derived indices and the agronomic traits [30]. Another approach integrates the visible red image (630–670 nm) and near-IR image (820–900 nm) to assess the rice leaf area index during the before-heading period [31] and then uses only near-infrared imaging to predict the leaf area index (LAI) [32]. Far-IR (also called IR thermal) imaging is the most general technique with which to visualize temperature differences, and it is used in plant drought resistance or insect inspection, as it will be discussed in the 'current applications' section below.

Beyond visible and IR imaging technology, hyperspectral imaging techniques can divide images into bands, thus providing a vast portion of the electromagnetic spectrum of the photograph. The reflected spectra of crop plants may carry information about plant architecture and health conditions and, therefore, can be used to evaluate plant growth characteristics. The high spectral resolution of hyperspectral technology makes it a promising method for detecting the severity of damage caused by insects [33], for assessing rice leaf growth [34], and for determining the condition of rice panicles [35]. Moreover, hyperspectral imaging of grain kernels would potentially provide information concerning internal infestation [36] and grain cleanness [37], which can be used to discriminate healthy kernels, insect-damaged kernels, and other materials. However, it is worth mentioning that, due to the low speed of scanning, hyperspectral imaging is more suitable for the preliminary investigation of waveband signatures to guide high-throughput inspection by reducing the redundant spectra.

3D structural tomography and functional imaging

In recent years, several modern optical imaging techniques, for instance, 3D structural tomography and functional imaging, have been developed and expanded to improve living plant visualization. The rice plants serve as 'patients' in a novel use of X-ray computed tomography (CT) scanners to estimate the tiller number [38••]. Equipped with an acceleration algorithm using the adaptive minimum enclosing rectangle (AMER) and graphics processing unit (GPU), the entire tiller inspection time of one plant is less than 200 ms [39]. Moreover, the incorporation of various optical sensors, such as visible and infrared digital cameras, provides this system with the potential to achieve the advanced screening of multiple traits for pot-grown rice plants within one chamber [38••].

The *in vivo* 3D imaging of plant structures (e.g., an *Arabidopsis* leaf) at a microscopic level has been permitted by optical coherence microscopy (OCM) [40]. OCM, sometimes called optical coherence tomography (OCT), is a new photonics-based technology with an approximately 1 μm spatial resolution. However, the image quality decreases rapidly when the inspection sections are deeper than 60–80 μm. With advantages such as a greater penetration and capability of detecting non-fluorescent signals, optical projection tomography (OPT) can be applied to visualize plant development and gene expression. One shortcoming of this technique is that large specimens must be cleaned before imaging, thus it is difficult to extend this technique to high-throughput phenotyping [41]. In contrast, X-ray CT is regarded as an appropriate tomography for the inner structural phenotyping of multitiller plants (such as rice plants) due to its relatively low cost and high spatial resolution.

In contrast to structural imaging, functional imaging technologies (such as fluorescence imaging and positron emission tomography, PET) focus on revealing changes in plant physiology. Chlorophyll fluorescence imaging is widely used to determine photosynthetic performance and stress in plants [42]. By tracing the positron-emitting radionuclides in plants (e.g., ^{11}C, ^{13}N, and ^{15}O), PET is able to visualize the distribution and transportation of radionuclide-labeled tracers involved in metabolism-related activities [43]. However, the disadvantage of functional imaging techniques is the low spatial resolution, thus the combination of the structural tomography (with a high spatial resolution) and functional imaging techniques can more accurately screen physiology activity. The combined application of PET and CT technologies provides simultaneous spatial and temporal root architecture data and links the observed morphology with the recently assimilated ^{11}C [44]. Magnetic resonance imaging (MRI) is another novel technique that is able to image the protons of water, thus providing structural information about the internal physiological processes occurring *in vivo* [45]. Moreover, the combination of MRI and PET is a novel structure and functional imaging technique for screening the dynamic changes in plant structures and functions [43]. In addition, several potential photonic technologies could be applied in plant phenotyping. Terahertz radiation (frequency range between 0.1 and 3 THz) is very sensitive to water and thus, could be adopted to determine the leaf water status and monitor drought stress [46]. Photoacoustic tomography (PAT) can provide anatomical and functional information from organelles to organs of up to 7 cm, and it is not constrained by the optical diffusion limit [47]. To date, compared with other expensive functional imaging techniques, fluorescence imaging, particularly chlorophyll fluorescence, is widespread in plant phenomics. The applications of the optical techniques currently used in rice, wheat and barley are listed in Table 1.

In addition, there are some other essential techniques or factors in plant phenomic research such as a flexible image analysis pipeline [48•], an accurate and integrated model [49], an effective data management system (e.g., 'PHENOME' with personal digital assistant [50]), an optimal transportation planning of robotics [51], a meticulous experimental setup [13•], and even the choice of the proper pot (or growth container) [52].

Current applications of phenomics in rice or other cereal crops

Abiotic stress resistance

Because of nondistinct and complex mechanisms involved in the responses of plants to abiotic stresses, including salinity, drought, extreme temperatures, and nutrient deficiencies [22], the phenotyping of abiotic stress resistance is often a big challenge. With the ability

Table 1

Applications of the current photonics-based techniques in rice, wheat or barley

Optical technique	Cost	Trait	Species	Reference
Visible light imaging	Low	Shoot biomass	Barley	[19••]
		Yield traits	Rice	[20••]
		Panicle traits	Rice	[24]
		Root architecture	Rice	[25,26]
Near-infrared imaging	Medium	Leaf area index	Rice	[30–32]
Far-infrared imaging	Medium	Shoot or leaf temperature	Barley, wheat	[55]
		Insect infestation of grain	Wheat	[75]
Hyperspectral imaging	Medium	Leaf health status	Rice	[33,59]
		Leaf health status	Wheat	[61]
		Leaf growth	Rice	[34]
		Panicle health status	Rice	[35]
		Grain quality	Wheat	[36]
Fluorescence imaging	Medium	Photosynthetic performance	Multivarieties	[42]
		Leaf health status	Wheat	[61]
X-ray digital radiography	Medium	Grain quality	Wheat	[72–74]
X-ray computed tomography	Medium	Tillers	Rice	[38••]

to reliably screen multiple traits under stress conditions, high-throughput phenotyping techniques are essential for improving this understanding [53]. Nondestructive measurements of plant growth have been developed using visible light imaging techniques with a Scanalyzer 3D to characterize overall plant salinity tolerance mechanisms, Na^{+} exclusion, osmotic tolerance, and tissue tolerance [54]. Using the plant age and plant area calculated by the Scanalyzer 3D, a modified model enables the more accurate high-throughput estimation of biomass for cereal plants under saline conditions [19••]. IR thermal imaging is also commonly used to quantify the osmotic stress response to salinity or drought in cereal crops [55]. By precisely controlling the environments of each pot-grown cereal plant in the greenhouse, these accurate, high-throughput phenotyping tools can overcome the limitations of current salinity-resistance or drought-resistance research.

Compared with chlorophyll meters or leaf color charts, visible and NIR digital imaging techniques [56] are more suitable for the high-throughput screening of nitrogen status. Furthermore, by incorporating accurate phenotyping, multi-environment statistical analyses and high-resolution genetic dissection, a reliable gene-to-phenotype model can bridge the gap between functional polymorphisms and tolerance to abiotic stresses in cereals [57•].

Insect and disease resistance

Both grain production and grain quality decrease as plants are damaged by insects and disease; it is, therefore, important to detect and classify the plant infestations at an early stage [58]. To identify rice blast disease at the seedling stage, a near-infrared hyperspectral imaging system was developed to scan clipped leaves, with an overall accuracy of classification (infected and healthy leaves) of approximately 92% [59]. In addition to these destructive measurement techniques, there are several approaches to achieve real-time and dynamic screening *in vivo* for pot-grown rice or field-grown cereals. With a color-based corner detection algorithm, visible image-based methods can detect plant-hopper infestations on the stems of pot-grown rice [60]. Integrating hyper-spectral and fluorescence imaging enables the detection of yellow rust in a winter wheat field, and the overall discrimination performance can reach 99% with a self-organizing map neural network [61]. Moreover, with a high-resolution web camera and a remote management system, an automatic field service system has been proposed to reduce the need for manual counting and to continuously monitor the presence of rice bugs. In addition, the system has the potential to achieve advanced field monitoring in conjunction with special sensors, such as infrared cameras [62]. Thus, optical screening methods could provide a high-throughput, high-accuracy, and time-lapsed inspection incorporated with advanced image analyses, surpassing human experts in monitoring plant diseases [63].

Yield and quality improvement

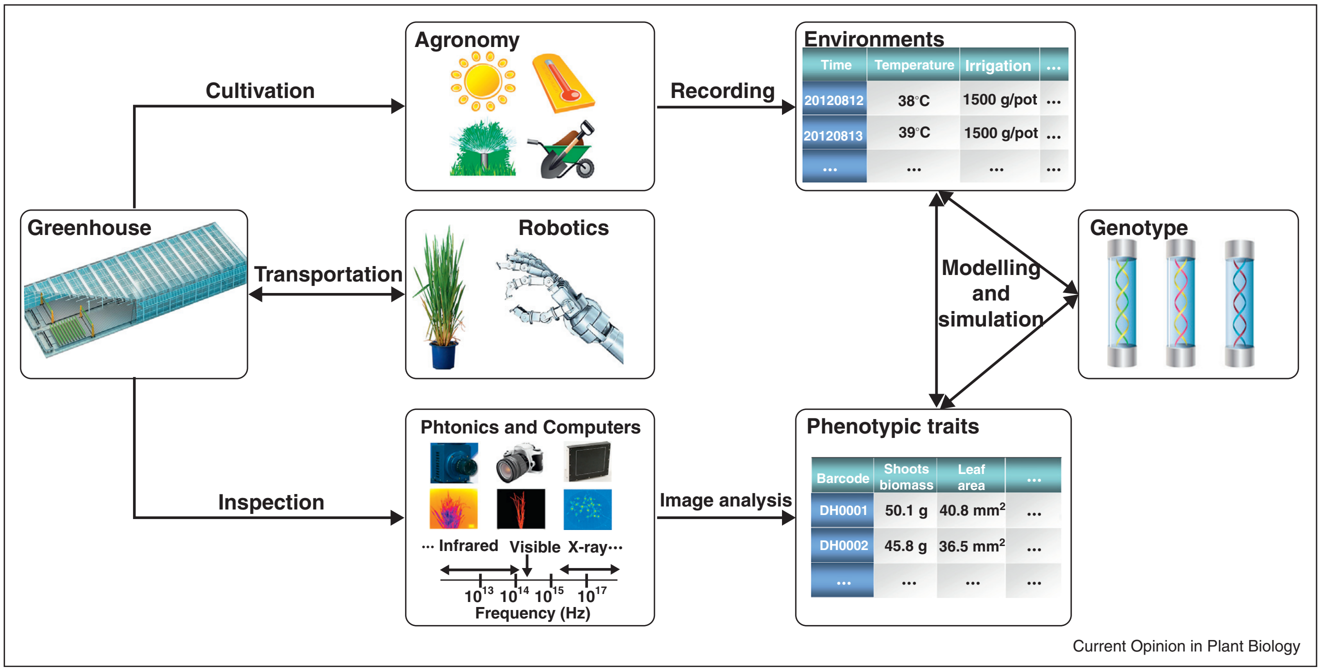
Yield is a complex agronomic trait that is determined by the grain number per plant and grain weight (influenced by grain size). Utilizing a less-expensive flatbed scanner for image acquisition, a user-coded ImageJ software plugin was developed to determine the major orthogonal dimensions of the grains (grain length, grain width) [64] and to analyze the sieveless particle size distribution [65]. To accelerate the measurements of spikelet number, a bimodal scanner using visible light imaging and X-ray digital radiography (DR) was employed for the rapid and simultaneous measurement of filled/unfilled rice spikelets [66]. Furthermore, to achieve fully automated yield trait scoring, an integrated facility has been developed to thresh rice panicles, evaluate rice yield traits, and pack filled spikelets. This novel machine vision-based facility is highly accurate (mean absolute percentage error is less than 5%) and highly efficient (1440 plants per continuous 24 hours workday) [20••].

Grain quality, encompassing grain appearance, health, and nutrition, are one of the most commonly quantified traits in rice breeding [67]. Using an inexpensive flatbed scanner and noncomplex image analysis, several rice quality traits, including chalkiness [68], protein content [69], and breakage [70], can be quantified with high accuracy. Equipped with a digital microscope and appropriate image analysis software, trans-illuminated imaging also can be used to assess wheat quality [71]. X-ray DR is used to image single kernels and detect internal wheat seed infestations [72], sprouted wheat kernels [73] and vitreousness in durum wheat by revealing changes in internal density [74]. Thermal imaging also permits the detection of insects inside wheat kernels [75]. In these studies, the samples must be manually placed on the sample platform before image capture; thus, high-throughput inspection cannot be achieved. An automatic rice-quality inspection system incorporating a near-infrared instrument and a visible light segregator has been developed to determine the protein content, moisture content, and sound whole-kernel ratio [76•]. Thus, with the appropriate optical imaging, image analysis and robotics tools, these techniques have the potential to achieve the high-throughput scoring of yield and quality, which will be popular in rice (or other cereals) science research.

Conclusions and future directions

Because of the robust genetic technologies, the functional analysis of the rice genome has entered into a high-throughput stage, and the RICE2020 project has been proposed to determine the function of every gene in the rice genome by the year 2020 [77]. To achieve levels of quality and speed comparable to those of genomics,

Figure 1



The main techniques (agronomy, robotics, photonics and computer analyses) needed in plant phenotyping platforms. Using robotics, the rice plants to be screened are transported to the inspection unit. The inspection chamber, which is the core of the phenotyping platform, carries out the noninvasive, high-throughput screening of plant phenotypic traits using photonics and computers. After image analysis, the quantified traits, environmental data (e.g., illumination, temperature, irrigation, fertilizer) and genotype are all managed in a database, which produces a 'phenotype–genotype model' and allows the simulation or predication of responses for special genotypes in different environmental scenarios.

reliable, automatic, multifunctional, and high-throughput phenotyping platforms should be developed using various novel technologies (Figure 1). There is advanced progress in plant phenomics, particularly in Europe (e.g., at the IPK, Leibniz Institute of Plant Genetics and Crop Plant Research) and Australia (at the APPF, Australian Plant Phenomics Facility). However, because rice is a staple food in many developing countries, more efforts to develop low cost and high performance rice phenomic technologies are needed.

Besides, with multifunctional phenotyping tools obtaining a large quantity of images and data, how to run the data-storage, handling and analysis will be another challenge in plant phenomics. The data volume mainly depends on the resolution of the imaging detectors and the numbers of acquired image from each inspection. And the data analysis methods, such as principal components analysis (PCA) [78], support vector machine (SVM) [79], and artificial neural network (ANN) [80], are often used for data dimension reduction and efficient parameters extraction. In future, to further promote the application of plant phenotyping, less expensive and sophisticated data analysis infrastructures (e.g., HTPheno [48•] and IAP [81] incorporating the open-source software ImageJ) need to be developed and popularized.

Thus, in our opinion, convincing rice scientists to accept or even rely on digital phenotyping platforms, reducing the platform costs, and developing efficient data storage and analysis infrastructures are the main challenges for the future. However, we are confident that these reliable, high-throughput phenotyping tools will give plant scientists new insights into the information encoded in the rice genome.

Conflicts of interest

The authors declare that there are no conflicts of interest related to this publication.

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