Future Scenarios for Plant Phenotyping

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Abstract

With increasing demand to support and accelerate progress in breeding for novel traits, the plant research community faces the need to accurately measure increasingly large numbers of plants and plant parameters. The goal is to provide quantitative analyses of plant structure and function relevant for traits that help plants better adapt to low-input agriculture and resource-limited environments. We provide an overview of the inherently multidisciplinary research in plant phenotyping, focusing on traits that will assist in selecting genotypes with increased resource use efficiency. We highlight opportunities and challenges for integrating noninvasive or minimally invasive technologies into screening protocols to characterize plant responses to environmental challenges for both controlled and field experimentation. Although technology evolves rapidly, parallel efforts are still required because large-scale phenotyping demands accurate reporting of at least a minimum set of information concerning experimental protocols, data management schemas, and integration with modeling. The journey toward systematic plant phenotyping has only just begun.

Contents

THE RESEARCH LANDSCAPE OF PLANT PHENOTYPING

One Plant, How Many Phenotypes?

In preparation for this review, we asked colleagues a few questions about terminology. A major outcome was that it became clear that the terms phenotype and phenotyping are interpreted in diverse ways. This finding is perhaps not surprising, given that even pioneers of this field have used different definitions in different contexts, not only among one another but also within their own work (88). Original definitions also tend to be forgotten over time (24), and scientific discovery provides unanticipated details. In certain publications—for example, large-scale surveys in model species—some colleagues also prefer to adopt a broad concept, interpreting molecular and biochemical signatures as phenotypes or phenotypic traits (85). However, there is no doubt that molecular mechanisms and patterns of activity represent intermediate layers that result in physiological regulation and in phenotypic manifestations at the macroscopic level. Strictly speaking, there is no good argument for dismissing such interpretations while adopting definitions of phene and phenotype that contain concepts such as the "observable" or "measurable" properties of an organism (88). Phenomics is no exception, because it can be viewed from a genome-wide perspective or a physiological one (9, 48).

For the purposes of this review, we refer to phenotyping as the set of methodologies and protocols used to measure plant growth, architecture, and composition with a certain accuracy and precision at different scales of organization, from organs to canopies. In particular, we use this term in reference to noninvasive technologies because the increasing importance of phenotyping in recent years has been clearly linked to the increasing availability of these methods. New techniques often allow researchers to analyze dynamic and spatially distinct parameters that were previously inaccessible. Beyond terminology, what matters most is to briefly recall the biological framework, which defines the phenotypic space. The genotype, the environment, and their interaction ($G \times E$) influence quantitative traits in a complex and dynamic manner. Although many lab studies are limited to a few sometimes-extreme environmental treatments or treatment-level combinations, plant phenotypic responses are generally characterized by response curves or norms of reactions to the environment, which for complex traits are inherently continuous and mostly nonlinear (118, 129). Additionally, different environmental factors influencing shoot and root growth covary and are characterized by different levels of heterogeneity in space and time (57, 63, 121). Based on results and interpretations developed for plants and other organisms, this framework can be further extended because (*a*) one environmental stress can trigger pervasive syndromes at later developmental stages (126), (*b*) phenotypes respond

to the environment but can, in turn, shape their environment (65), (*c*) life history can influence the expression and intensity of a specific phenotype at certain developmental stages (128), (*d*) the phylogenetic history of both a given gene and its associated phenotype should be used to infer causation relationships (73), and (*e*) intracellular stochastic processes exist that have wide-reaching effects on macroscopic phenotypes (117).

This complexity clearly must be reduced for plant phenotyping experiments to be designed for specific experimental purposes. This consideration applies, for example, to research on mechanisms of drought avoidance and tolerance, for which ideotypes with improved performance based on growth and physiological parameters need to be considered in relation to specific target environments and crop phenology (133).

Addressing the Plant Phenotyping Bottleneck

During the past 20 years, molecular profiling and classical sequencing technologies enabled significant advances toward the large-scale characterization of plant genomes (146). Next-generation sequencing technologies promise to provide another step change in plant genomics (119), and molecular technologies have provided valuable tools for plant breeding such as marker-assisted selection and, more recently, genomic selection to introduce desirable alleles at many loci that have small genetic effects when used individually (134 and references therein). However, the plant phenotypic landscape needs to be populated at a faster pace to accelerate research in model organisms (65), biotechnology industrial pipelines (27, 112), and plant breeding programs for yield and resource efficiency gain in major crops (1, 12, 113, 137). In a nutshell, there is a phenotyping bottleneck hampering progress in both knowledge- and application-oriented research (41, 42, 47, 48, 65).

Many of the ongoing developments in plant phenotyping are driven by increasingly available technologies, especially in the field of imaging sensors (e.g., high-resolution imaging spectrometers) and advanced software for image analysis and feature extraction for 2D and 3D analyses of shoot and root growth and architecture (11, 42, 98, 104). Although noninvasive technologies provide an important piece of the puzzle, they need to be integrated in a much broader context. Large-scale and relevant phenotyping calls for a broad and multifaceted approach. Considering a generic process scheme applicable to controlled experimentation in indoor growth facilities (**Figure 1**) helps us to focus on the main components required to analyze, rank, and select valuable germplasm. A similar conceptual framework and the associated infrastructure requirements could be applied with modifications to field phenotyping at different scales in plant breeding environments.

We argue that only by clearly specifying each necessary phenotyping component will it be possible to efficiently address each challenge. Cost reductions and time gains are desirable targets when designing phenotyping at a large scale (9). First, researchers need sound and robust knowledge about the phenes that are indicative of the intended performance. Here, mechanistic understanding and deep phenotyping play a key role in identifying useful parameters and proxies to measure. It is clearly valuable to identify a set of parameters before wasting resources by measuring a large number of data points, which could be highly autocorrelated or not indicative of the target performance. Second, successful deployment of robust noninvasive methodologies for large-scale plant analyses requires integrating automated cultivation systems, precise environmental monitoring, and information technology for data management. The development of structural and functional models of shoot and root growth and architecture (132) is highly complementary as it enables researchers to interpret large-scale results, perform in silico experiments, and recognize which types of experiments should be given priority, and for which environmental scenarios. Third, we highlight

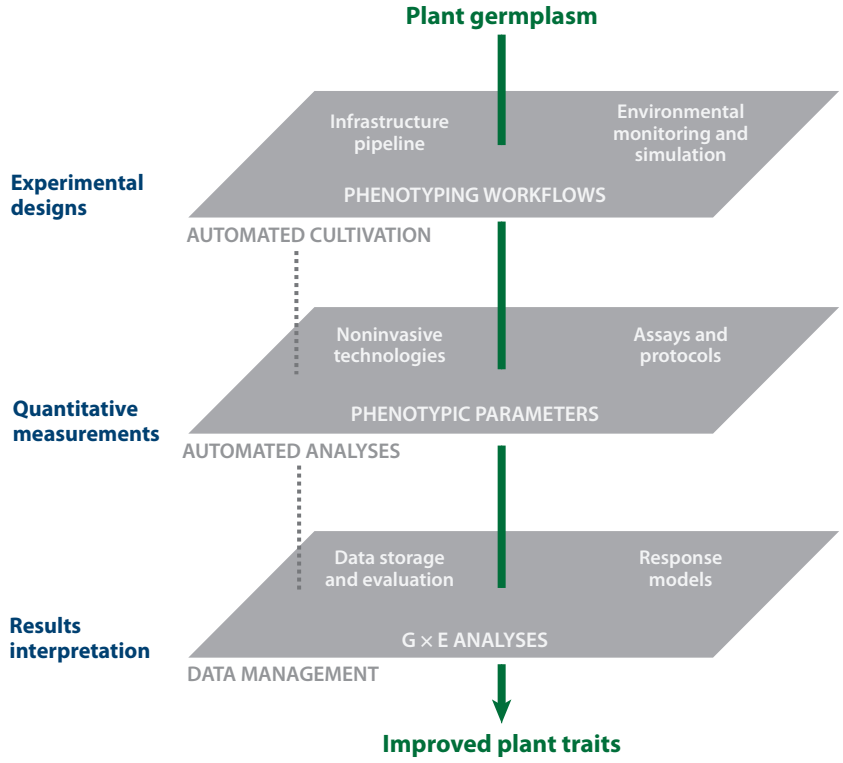

Figure 1

Conceptual scheme for plant phenotyping, applicable in particular to controlled-environment facilities. Building capacities to screen germplasm for enhanced agricultural traits requires a multidisciplinary approach. Globally, this scheme offers a quick overview of key layers and elements for a successful implementation of large-scale plant phenotyping. Experimental design includes sufficient capacity to support large-scale phenotyping, including adequate plant growth infrastructure, environmental monitoring and simulation, substrate handling, and, if needed, large-scale biosafety installations. Quantitative analyses strongly benefit from novel noninvasive technologies but require standardized experimental protocols, including sensor calibration and precise definition of raw data processing routines, as part of best practices in phenotyping. The data management layer includes hardware for data storage and software for numerical and statistical analyses. Results interpretation requires the integration of experimental metadata within data schemas for the measured phenotypic traits. This architecture implies a direct link between the measured plant parameters and the environmental conditions, enabling the analysis of gene-environment (G × E) interactions and modeling of phenotypic responses.

that the terms throughput and capacity of phenotyping facilities are not equivalent. For instance, the theoretical capacity of a phenotyping assay or an entire facility, which is often reported in the literature, may not directly translate into the actual operational throughput. Simply put, although one facility may have the (spatial) capacity to analyze twice as many plants per day as another facility, the two may have the same throughput on a yearly basis depending on the efficiency of each facility as a whole, which itself depends on the occupancy, duration, and timing of subsequent experiments as well as on resilience to technical failures. We argue that from a technical standpoint, thoughtful consideration of how to address the plant phenotyping bottleneck will require evaluating throughput on adequately long timescales—for example, number of plants or assays per year.

In summary, achieving high throughput (e.g., thousands of plants phenotyped per year) in facility settings will require comprehensive approaches and robust methods to quantify physiological traits (**Figure 1**). This also includes the challenge that academic research facilities often need to be flexible with respect to setups for different experiments (for example, by accommodating multiple species), which can reduce their overall throughput, whereas industrial facilities can achieve maximal throughput by dedicating facilities to one or several similar experiments. It should also be emphasized that irrespective of whether a facility is designed for high flexibility or maximal throughput, diverse areas of competence are required (**Figure 1**). This point must be taken into account when designing road maps and building research teams for large-scale, sustainable plant phenotyping (9, 48, 113), which calls for a medium-term perspective owing to the significant initial investments required. In the following section we evaluate some of the main advantages and disadvantages of different noninvasive methodologies that contribute richer and quantitative data matrices to populate the plant phenotypic landscape at an accelerated pace.

PRECISION AGRICULTURE: EFFICIENT FARMING FOR EFFICIENT PLANTS

Crop performance strongly depends on agricultural management. The release of crop varieties characterized by improved water and nutrient use efficiency is a primary target of the plant breeding and biotechnology industry. However, to sustain projected demand, the use of scarce and costly resources is also poised to globally increase. This calls for comprehensive approaches in crop management and monitoring of resource use across the whole food chain (50).

In the past 15 years, precision agriculture has emerged as a scientific discipline using a suite of in-field technologies for integral management of resource use. There is a significant potential for synergy with plant phenotyping both for exploiting knowledge in sensor technology and for designing varieties using the opportunities of precision agriculture. Considerable efforts are currently being made to develop applications for monitoring within-field spatial and temporal variability in crop quantity and quality, soil water and nutrient distribution, disease epidemiology, and weed infestation. New agricultural machinery or airborne platforms equipped with onboard sensors are deployed in combination with information systems. These approaches are designed to deliver a suite of supporting decision-making software tools to farmers, not only mitigating uncertainties inherent to farming but also leading to significant financial savings.

NONINVASIVE METHODS FOR PLANT PHENOTYPING AT THE LEVEL OF WHOLE PLANTS AND CANOPIES

Automated Phenotyping Platforms

Automation and robotics, new sensors, and imaging technologies (hardware and software) (see **Tables 1** and **2**) provide a range of applications for lab research and screening systems, horticultural production systems, and especially the food industry supply chain (6, 56, 79, 151). Applied to the scale of agro-ecosystems, the field of precision agriculture is growing rapidly (see sidebar, Precision Agriculture: Efficient Farming for Efficient Plants). In the past 10 years, automated phenotyping systems sold commercially or developed in the public domain have been deployed in growth chambers or greenhouses. These systems are specifically designed for research and large-scale phenotyping for a limited range of species, encompassing small rosette plants like *Arabidopsis* (4, 54, 58, 69, 124) and the main cereal crops (27, 52, 59, 112). Generic platforms and solutions enabling the simultaneous phenotypic evaluation of multiple species have not been implemented to date. This highlights that imaging methods, protocols, and workflows for large-scale phenotypic evaluation often need to be adapted and tailored to individual or small groups of species with similar growth habits and growth requirements, which may require significant time for protocol optimization.

Depending on the overall design, phenotyping systems can generally be classified as sensor-to-plant (4, 54, 58, 69, 124) or

Table 1 Commonly used and developing approaches for noninvasive analyses of plant morphology, growth dynamics, and physiological status

Parameters	Sensors	Raw data	Resolution	Applications	Advantages	Disadvantages
2D imaging[a]						
Shoot biomass (projected area),[b] growth dynamics, color, shape descriptors, root architecture, seed morphology and surface features, leaf disease severity assessments, seedling vigor	Broad range of cameras[c] sensitive in the visible spectral range	Gray or color value images (RGB channels)	Whole organs or organ parts, time series (minutes to days)	Automated screening systems (4, 13, 31, 34, 52, 54, 59, 71, 72, 101, 124, 130)	Rapid measurements, affordable solutions	Limited physiological information provided
Photosynthetic status (variable fluorescence),[d] quantum yield, nonphotochemical quenching, leaf disease severity assessments	Fluorescence cameras and setups (including active illumination)	Pixel-based map of emitted fluorescence in the red and far-red region	Whole shoot or leaf tissue, time series (minutes to days)	Automated or semiautomated screening systems, open field (19, 20, 53, 58, 69, 99, 145)[e]	Probe of photosystem II photochemistry in vivo	Complicated whole-shoot analyses for nonrosette species, preacclimation conditions required
Surface temperature	Thermal imaging (passive and active)	Pixel-based map of surface temperature in the infrared region	Whole shoot or leaf tissue, time series (minutes to days)	Automated or semiautomated screening systems, open field (1, 43, 70, 80, 99, 114, 137)[f]	Rapid measurements, potential information about leaf and canopy transpiration and heat dissipation	Measurements influenced by numerous factors, sound physics-based results interpretation needed
Water content, composition parameters for seeds	Near-infrared cameras, multispectral line scanning cameras, active thermography	Continuous or discrete spectra for each pixel in the near-infrared region	Time series or single-time-point analyses of shoots and canopies, single-point assessment of seeds	Automated screening systems, lab (8, 14, 23, 80, 99, 116, 122)	Estimates of biomass composition by chemometric methods	Extensive calibration required
Leaf and canopy water status, disease severity, pigment composition	Near-infrared instruments, spectrometers, hyperspectral cameras, thermal cameras	Continuous or discrete spectra	Crop vegetation cycles, indoor time series experiments	Lab, remote sensing of vegetation (13, 90, 138)	Large amount of information provided	Cost, large image data sets for hyperspectral cameras, complex data interpretation

3D imaging						
Shoot structure, leaf angle distributions, canopy structure	Stereo camera systems	Depth maps	Whole-shoot time series at various resolutions	Lab, some examples in the field (11)	High 3D accuracy, shoot and canopy models enabled	Complex data reconstruction
Shoot biomass and structure, leaf angle distributions, canopy structure	Laser scanning instruments with widely different ranges, time-of-flight cameras	Depth maps, 3D point clouds	Whole-shoot time series at various resolutions	Lab to field (28, 75, 103, 139)	High 3D accuracy, shoot and canopy models enabled	Specific illumination required for some laser scanning instruments
High-resolution volumetric imaging[g]						
Morphometric parameters in 3D	X-ray tomographs	Voxels and tissue slices	100 μm and lower, hours	Lab (44, 55)	3D root analyses enabled	X-ray source effects for imaging time series to be evaluated
Morphometric parameters in 3D, water content[h]	Magnetic resonance imagers	Water (^{1}H) mapping	200–500 μm, 1–600 s	Lab (61, 107, 111)	3D root analyses enabled	Removal of ferromagnetic elements in soil required
Transport partitioning, sectorality, flow velocity	Positron emission detectors [including single-photon emission computed tomography (SPECT)] for short-lived isotopes (e.g., $^{11}CO_2$)	Radiotracer mapping and coregistration with positron emission signals	1–2 mm, 10 s–20 min	Lab (68)	Short-term measurements of in vivo carbon flow	Access to short-lived radiotracers required

[a]For the purposes of this level of classification, we consider only the spatial dimension. An increasing number of applications also describe prototypes using sensor combinations for 3D reconstructions (139) and simultaneous measurements with different imaging modes (7).
[b]Current setups use multiple angles or camera views, typically achieved by a combination of plant rotations and multiple cameras (52).
[c]Root analyses are also performed using flatbed scanners (3) and cameras within mini-rhizotrons (26, 125).
[d]Emerging nonimaging techniques include sun-induced fluorescence (94) and laser-induced fluorescence transient (LIFT) methods (78).
[e]Nonimaging benchtop instruments are also available. Spot measurements of individual leaves can be done using portable instruments (1, 12). Similar considerations apply to near-infrared portable spectrometers.
[f]Infrared thermometers can also be used (1).
[g]A nonimaging prototype was described for integral measurements of shoot biomass at the lab scale using microwave fields (92).
[h]The possible MRI applications are numerous (15).

Table 2 Nonexhaustive list of commonly used software packages that allow advanced analyses of root morphometric and growth dynamics parameters based on 2D imaging of roots grown in a variety of growth media

Parameters	Software	Analysis	Remarks
Morphology, geometry, topology	SmartRoot (**http://www.uclouvain.be/en-smartroot**) (84)	Semiautomated: sampling-based strategy of manually picked nodes	Not used for global parameters
Morphology, geometry, topology, global parameters	EZ-Rhizo (**http://www.psrg.org.uk/plant-biometrics.html**) (2)	Semiautomated: skeleton reconstruction and identification of objects from contiguous white pixels	Detects 0.1-mm lateral roots on main root at 200 dpi
Length, topology	WinRhizo (3)[a]	Automated: overlap corrections	Analyzes washed roots; in situ analyses packages are available
Morphology, geometry, topology, global parameters	GROWSCREEN ROOT (98, 100)	Semiautomated: extraction of a tree model of plant roots	Has also been applied to image time series in rhizotrons (101)
Morphology, geometry, topology, global parameters	Root Reader 3D (40)	Automated: skeleton extraction method	Performs image acquisition with a 3D laser scanner or camera and rotation of the target (22)
Global morpho-geometric parameters	GiaRoots (49)	Automated: sequential threshold methods	Performs image acquisition with a visible camera and rotation of the target (67)
Morphology, geometry, topology, global parameters	DART (81)[b]	Manual: each root described by a series of ordered links	Uses human vision tracing to avoid analytical biases

[a]Commercial software platform.
[b]Includes extensive information about other root analysis software.

plant-to-sensor (27, 52, 59, 112) based on whether the plants occupy a fixed position during a measurement routine and an imaging setup moves to each of those positions or the plants are transported to an imaging station, respectively. Most of these platforms are suitable in particular for controlled water-limitation experiments that use gravimetric methods to measure daily evapotranspiration and automatically irrigate each pot to a predefined target weight (8, 54, 124). The most commonly used method for evaluating growth and rosette geometry time courses is 2D RGB (red, green, blue) imaging followed by image preprocessing and segmentation for the extraction of projected shoot area and geometric parameters (59, 82, 95, 131, 140). Initial validation experiments have been published with the aim of establishing standard protocols for dynamic responses of rosette growth to drought stress using *Arabidopsis* ecotypes and mutants (54). Platforms designed to evaluate larger plants and more complex shoot geometries, such as those of cereals, are currently used to screen for, e.g., growth responses to salinity (52, 110) and reduced water availability (8, 27, 59). To our knowledge, no published large-scale studies have used automated imaging platforms to simulate various nutrient regimes and analyze growth responses for the selection of genotypes based on morphological and physiological traits related to nutrient use efficiency.

Collectively, these studies demonstrate the applicability and validity of coupling automated plant cultivation with imaging routines for large-scale evaluation in time-course experiments. However, interpreting large-scale experiments requires the continued development of a comprehensive concept for automating the data stream as well, from the image-processing

RGB: red, green, blue

pipelines (59, 140) to experimental reports for data evaluation, automated filtering and annotation of outliers, and quality control (4). A central bottleneck remains the construction and maintenance of databases containing dynamic and quantitative data coupled with environmental data, an example in the public domain being the Phenopsis platform database, which contains data from several experiments with *Arabidopsis* ecotypes and mutants subjected in particular to water limitation (39). Importantly, simultaneous measurement of relevant environmental variables such as temperature, irradiance, and evaporative demand is a prerequisite for meaningful data interpretation (54, 108, 115).

A final consideration is that automated approaches are still generally expensive, especially because of the hardware required (robotics, conveyor belts, cameras, computing infrastructure). Local and cheaper solutions, particularly for labs with comparatively limited resources, will continue to be a necessity until more affordable plant phenotyping systems become common and are distributed to the community (**http://www.plataformabiotecsur.com.ar**) (150). The response of the community to this situation has been to enable access to existing phenotyping installations by forming networks of facilities such as the European Plant Phenotyping Network (**http://www.plant-phenotyping-network.eu**), the Australian Plant Phenomics Facility (**http://www.plantphenomics.org.au**), and the International Plant Phenomics Network (**http://www.plantphenomics.com**).

Noninvasive Methodologies for Shoot Phenotyping from Lab to Field

High-resolution imaging microscopy has made spectacular advances, enabling researchers to capture and visualize cell structure and molecular dynamic changes in unprecedented detail (32, 35). Organ- or tissue-level approaches based on time-lapse analyses enable investigation of the integrated responses of leaf and root dynamics in response to environmental challenges at high spatial and temporal resolutions (141). Here, we focus on noninvasive methods to measure shoot (or entire canopy) and root macroscopic plant phenotypes related to growth and performance in both indoor facilities and the field (**Table 1**).

RGB imaging. The simplest method consists of the construction of growth profiles of the shoot by acquiring time series using cameras sensitive to the visible range (400–700 nm) of the electromagnetic spectrum (4, 59, 82, 131, 140). The projected shoot area is extracted following image preprocessing and segmentation either in the RGB space or in the HSV (hue, saturation, value) space, which allows much finer color distinction without brightness losses (140).

Imaging setups vary widely depending on the cultivation format. For cereals, multiple view angles created by rotating the plants are generally used to reduce image occlusions (52). In addition, geometric parameters such as a convex hull (the smallest possible mathematically solved perimeter that envelops the imaged plant) can be extracted automatically for the classification of shoot morphology (69). Similar semiautomated approaches have been developed for individual shape and margin characterization of detached leaves, and have also been applied in association mapping studies and quantitative analyses of leaf shape variation (16, 144). The calibration of projected shoot area (the projected area of a 3D object onto a plane) based on total leaf area and fresh and dry shoot mass measured destructively throughout the growth trajectory was first performed in *Arabidopsis* (82), barley (52), and tobacco (140), leading to highly significant linear or polynomial correlations. Prior work on 27 grass species using generalized linear models to analyze the vertical distribution of leaf area and plant biomass led to similar conclusions (131).

Problems with RGB imaging are most commonly caused by the overlapping of leaves in developmentally older plants and by background soil noise. Also, the effects of specific treatments may change the slope of this

QTLs: quantitative trait loci

relationship, as was shown for salinity in barley (52). Consequently, new assessments should be performed on a case-by-case basis during phenology for the developmental stage of interest, especially for different species and camera setups. If biomass needs to be measured with higher precision, measurements of leaf thickness by using microscopic sections, minimally invasive methods, or noninvasive methods (123) would additionally be required. In any case, digitally reconstructed leaf area and leaf area growth rates can be used with relative confidence. In addition to analyzing shoot growth and geometry, RGB imaging can provide a quick way to quantify greenness parameters. In the field—as applied, e.g., to wheat breeding programs—the use of consumer RGB digital cameras might be an alternative to more expensive sensors to estimate green biomass via specifically developed indices (18).

Chlorophyll fluorescence. As with RGB imaging, chlorophyll fluorescence is commonly used from lab to field scales. It offers a rapid way to probe photosystem II status in vivo (5, 25, 91, 120). Active fluorescence protocols exploiting pulse amplitude modulation (120) of commercial instruments can measure the potential and effective quantum efficiency of photosystem II, the electron transport rate, and the extent of nonphotochemical quenching. Notably, the quantum efficiency of photosystem II can be measured much more easily than the other parameters (91).

Several possible uses of chlorophyll fluorescence have been recently proposed for diagnosing early stress responses to abiotic and biotic factors before a decline in growth can be measured (20, 69, 99). Likewise, there are numerous applications in the horticultural sectors (reviewed in 53). Semiautomated systems using fluorescence cameras have been described along with validation experiments (19, 69, 145). The F_v/F_m parameter (the ratio of variable and maximum fluorescence measured after saturating light pulses) appears to be relatively insensitive to severe water limitation but could be used to differentiate between responses during cold treatments (69). Also, in *Arabidopsis*, manual chlorophyll fluorescence measurements have been used to map quantitative trait loci (QTLs) for growth-related traits; the quantum yield of photosynthesis had a moderate heritability in these studies (36). In addition, portable fluorometers and fluorescence cameras have been used to screen large mutant collections (86) and to characterize mutants with different photosynthetic pigment compositions (102).

Applying chlorophyll fluorescence to large-scale evaluation workflows in order to derive dark-adapted basal rates (F_0) and F_v/F_m as an index of impaired photosystem II functionality under stress is somewhat challenging because preadaptation to dark may imply routines of a few minutes for each plant, potentially diminishing throughput (unless measured predawn). Measuring multiple plants at once could also be beneficial but is not possible with commonly available systems. Importantly, the application of active fluorescence protocols using 2D fluorescence cameras is still limited to rosette plants, such as *Arabidopsis* (58, 69). Significant developments are needed to analyze larger plants with different shoot geometries, implying the use of advanced 3D reconstructions (7, 42, 48). In the field, portable instruments are commonly used (1). Promising developments for measuring photosynthesis at the canopy level include methods utilizing laser-induced fluorescence transients (78) and sun-induced fluorescence (94), but technical challenges concerning robustness, reproducibility, and data analysis need to be addressed before these methods can be used in large-scale phenotyping.

Thermal imaging. Measurements of leaf and canopy temperature by thermal imaging (3–14-μm spectral range) have been introduced in the lab and in the field (70, 99) to evaluate leaf water status. Canopy temperature depression (the temperature difference between the canopy and the surrounding air) is currently used in cereal breeding programs as a selection trait for drought resistance in dry environments (1, 43, 137). Direct selection for canopy temperature

depression has contributed to yield gains (114). Thermography has also been used in the lab for mutant screens in *Arabidopsis* (93).

Several papers have discussed the limitations of thermography in the field, which include the need for soil background corrections and the impact of wind and transient cloudiness effects (70, 99, 137). Although taking pictures with thermal cameras is fast and attractive for large-scale evaluations, there are issues that should be critically evaluated because the physics of heat fluxes is complicated and highly variable in scale. In our experience, this applies equally to screening protocols in highly controlled growth chambers. Based on experiments conducted in a controlled cuvette system, an active thermography approach (e.g., short irradiation with infrared energy) was proposed as an option to reliably obtain plant water status (80). Taking this into account, we consider improved protocols based on a sound understanding of physical principles and different measurement methods (such as the protocols briefly sketched above) to be necessary for thermography measurements in plant phenotyping.

Imaging spectroscopy. Imaging spectroscopy applied to plant phenotyping originated from research in remote sensing of vegetation (77). Spectral measurements for a larger portion of the electromagnetic spectra have become possible through the use of multispectral and hyperspectral cameras capable of scanning wavebands of interest at high resolutions, in particular around the peak of green reflectance at 550 nm and the water absorption bands in the near-infrared (NIR) to mid-infrared region (138). This region includes strong water-absorbing bands at 970 nm, 1,200 nm, 1,450 nm, 1,930 nm, and 2,500 nm (76, 99). The best applied example of the use of spectral measurements is the derivation of a number of reflectance vegetation indices, from simple differences between two wavelength reflectance values to normalized reflectance values. Several indices have been introduced in both field research and breeding programs for large-scale phenotyping and dynamic estimation of biomass, greenness, nitrogen content, pigment composition, photosynthetic status, and water content (extensively reviewed in 42, 83, 149).

NIR: near infrared

Of particular interest for plant phenotyping is the possibility of using specific bands in the NIR to mid-infrared region to estimate tissue water content noninvasively and design screening protocols for genotypic differential responses to drought (99). In typical greenhouse experiments, demonstrating an advantage compared with classic physiological analyses requires detailed experimentation in crop species to quantitatively assess the sensitivity of NIR reflectance or transmittance to a range of water content (leaf water thickness) during leaf dehydration (99, 122). In the field, spectrometric measurements using various visible-NIR indices for water status estimation are performed. Detailed studies to establish correlations with leaf water potential show that variability due to developmental stage and date of measurement may prevent the establishment of widely applicable correlations (37).

Further extending the number of measured wavelengths, the assessment of plant composition using hyperspectral reflectance sensors is a burgeoning field of research for the remote estimation of plant composition, health, and disease status (13, 138). Interestingly, *Arabidopsis* mutants with weak and irregular reflectance phenotypes were recently identified using a hyperspectral camera setup and a supervised classification of the reflectance spectra, with wild-type leaf spectra as a reference (17, 90). For integration into phenotyping screening routines, cost, data reduction due to large image sizes, data analysis, and spectral classifiers (13) will need to be addressed, but researchers have begun to implement possible solutions, as shown by the example above. Because of the rich data matrices, imaging spectroscopy remains an attractive target for the future.

It is anticipated that research will eventually address the need for 3D reconstructions at different scales, from individual leaves to entire shoots and canopies (11, 104). It is difficult to precisely estimate the potential impact

of high-precision 3D reconstructions of shoot phenotyping for screening purposes, but we expect that these approaches will be invaluable for modeling. However, recent work has demonstrated the first applications of stereo camera systems and the simultaneous use of multiple sensors to enable 3D canopy reconstructions (7, 139). Remarkably, medium-scale greenhouse phenotyping applications were developed for QTL mapping in pepper plants using depth measurements and extraction of leaf size and leaf angles from color images, which resulted in heritabilities of 0.5–0.7 (139). In addition, light detection and ranging instrumentation (28, 103) and time-of-flight cameras (75) might be evaluated more comprehensively for 3D reconstruction in the future. Finally, a nonoptical method using a microwave resonator prototype at the lab scale has been recently described and applied to biomass measurements in tomato (92). Similar approaches may provide a noninvasive way to estimate plant biomass dynamically at various spatial and temporal resolutions. This short selection highlights the opportunities of integrating new sensors and discovering new traits in large-scale phenotypic analyses.

Alleviating the Root Phenotyping Bottleneck

Tissue-specific transcript profiling in *Arabidopsis* root tips has provided unprecedented detail in identifying regulatory genes and guiding reverse-genetics approaches (10). Time-lapse studies of root tip growth and development have revealed the interplay between hormonal and environmental cues, and imaging procedures based on optical flow measurements have been applied to leaves (141). Additionally, individual root tracking in transparent media has been successfully applied in a range of plant species, including both monocotyledonous and dicotyledonous models, to obtain high spatial and temporal resolution of local growth patterns in response to changing aerial and root environmental conditions during diel growth cycles. Root detection algorithms have been developed to analyze root tip dynamics (141, 147) and root curvature patterns in setups scalable to high throughput (45).

At a higher level of integration, there is now ample experimental evidence that root system architecture is intimately linked with water and nutrient use efficiency (64, 87). However, the need for phenotyping solutions is even greater in large-scale root biology than in shoot biology, considering that our ability to dynamically phenotype whole root systems and break down the associated anatomical and physiological traits for large numbers of genotypes is still limited. In the field, small- and larger-scale analyses still rely on destructive methods requiring the excavation of soil cores and partial or total uprooting of root systems from trees to cereals (26, 33, 136). Remarkably, experienced teams can afford medium- to large-scale experiments that either collect soil cores for tens of genotypes per day (143) or visually score root crown traits in maize recombinant inbred lines for hundreds of plants per day (136). Perhaps these approaches could be further mechanized and streamlined to enable association mapping studies.

The simplest minimally invasive method consists of using mini-rhizotrons equipped with a camera to partially visualize a rooting profile (26, 125). Noninvasive indirect methodologies are still being developed, including ground-penetrating radar, electrical resistance, and impedance tomography; these approaches have issues related to the resolution and variability depending on the soil moisture range for accurate measurements, which currently prevents their widespread adoption (143).

A variety of methods have been explored to study root systems dynamically in 2D in controlled experimentation and to enable large-scale research on root architecture traits in *Arabidopsis* and species with larger root systems. First, cultivation in transparent, agar-filled plates and vertical culture systems is easy. 2D imaging delivers rich data sets for major root architecture parameters, including branching angles and root density distributions, and often also does so for different root classes (45).

Second, different soilless media involve, for instance, the use of growth pouches and other transparent media such as gellan gums (21, 22, 40, 66, 67). Notably, there are examples of the use of gel-filled cylinders to derive 3D root architecture parameters by imaging roots from multiple view angles (**Table 2**). Novel classifying geometric indices based on these systems have been recently proposed (49, 67). Collectively, the systems above are amenable to scaling to high throughput. Third, soil-filled rhizoboxes have been used for quite a few years, with more recent developments including work in *Arabidopsis* and maize (30, 51). Scaling up this concept, we recently described the possibility of simultaneously acquiring dynamic parameters for roots, shoots, and shoot-to-root ratios using a first prototype of an automated platform (101). In these experiments, the proportion of roots visible at the transparent plate of the rhizotrons depends on the species and is loosely correlated with average root diameter. Linear estimates of total root length were established for six monocot and dicot species, and the experiment included soil compaction treatments, an important factor limiting growth in the field.

There is growing consensus that there is value in combining different methodologies in phenotyping chains (from agar plates to soil) to gain their respective advantages while remaining aware of the intrinsic limitations of each approach (29). Together with the development of advanced protocols mentioned above, in recent years there has been a proliferation of software tools for the manual, semiautomated, and automated analysis of root system architecture traits (**Table 2**). To our knowledge, a systematic comparison of the accuracy and robustness of the numerous available software packages and analytical tools has not been attempted. There are only limited examples of benchmarking approaches (62). A systematic comparison using selected image data sets of various qualities would be beneficial to help researchers improve current approaches and understand which under- or overestimation biases may be present for each parameter. This could lead to improved accuracy and provide generic software tools for screening platforms.

High-resolution and dynamic 3D imaging of soil-grown roots is at the frontier of phenotyping research. Two techniques, magnetic resonance imaging (MRI) and X-ray computed tomography (CT), have emerged in the past few years and may become relevant for precision phenotyping to dissect root traits and assist structural and functional models. MRI applications in plant biology have been recently and extensively reviewed and go well beyond unraveling the root dynamics of soil-grown plants, extending to imaging of seeds and dynamic imaging of water and metabolites (15). For example, MRI has been used to visualize the symptoms caused by cyst nematodes of sugar beet (61), bean root nodulation, the root distribution of maize grown with a heterospecific neighbor (111), and the potential effects of pot size in barley (107). For functional imaging of shoot-to-root carbon fluxes, coregistration of MRI with positron emission tomography—a technique capable of imaging the distribution of short-lived, positron-emitting radionuclides such as ^{11}C—demonstrates the ability to dissect sectors of a sugar beet taproot receiving photoassimilates in short-term experiments (minutes) (68).

X-ray CT is an imaging mode providing volumetric data of soil structural heterogeneity (106, 148) and plant structures (127). Acquisition times of approximately 80 min for small soil columns (25 mm diameter, 25 mm high) resulted in a 100-μm resolution in wheat seedlings (55). Further experiments in wheat have shown that root lengths measured by CT correlate with those measured independently, with a bias of 8% (44). However, CT could not differentiate effects due to layered phosphorus treatments, and a high variability was reported in root length estimation by CT, possibly owing to current limitations in image segmentation (44).

In all, both MRI and CT methodologies are making rapid progress. Besides improvements in image segmentation and reconstruction, which will accelerate processing and

MRI: magnetic resonance imaging

CT: computed tomography

increase accuracy, MRI requires the removal of ferromagnetic elements in soil, and potential negative effects of X-ray radiation on root growth have just begun to be addressed (44). We anticipate that in the near future these methodologies will be refined and the possible throughput at which they can be employed in root phenotyping will be thoroughly assessed. However, they already provide an unparalleled resource to investigate root dynamics in soil columns for mechanistic phenotyping.

The noninvasive methodologies reviewed here for their significance to plant phenotyping constitute significant advances that only a few years ago could not have been anticipated. Nonetheless, root phenotyping will remain challenging, and significant efforts will be required to increase the speed and accuracy of root architecture analyses for applicability to large-scale experimentation.

Seed and Seedling Phenotyping

Compared with shoot and root phenotyping, systematic seed phenotyping assisted by imaging and automated seed analyses has received less attention in research settings. Seed mass is a key trait both in ecology and for agricultural production. Plant biomass measured at any time during development depends on the seed mass, germination timing, and relative growth rate. Quantitative analyses of these components may reveal that small differences in seed mass can explain variation in relative growth rate that would normally be interpreted otherwise, as was shown, for example, in *Arabidopsis* and *Petunia* ethylene-insensitive genotypes (135). Likewise, small differences in seed weight are linked to QTLs that explain a moderate proportion of the overall variance in growth-related traits (36). Rapid and simple analyses of seed size and geometric features are possible using flatbed scanners and transmitted light (60) or by imaging. Depending on the seed type, they may provide good estimators of seed mass. Systematic evaluation of the seed yield of potted plants is usually not performed in public platforms. Interestingly, a fully automated evaluation pipeline for rice panicle yield-related traits based on imaging and seed weighing has recently been reported; the capacity exceeds 1,000 plants per day with relatively small process errors of 5% (34). Major changes in seed phenotyping could occur in the next few years, increasing the number of traits that can be measured systematically or at a medium scale.

Germination rates and seedling establishment are crucial for plant production and for designing experimental protocols in the lab. There are examples of automated and large-scale "early vigor" evaluation platforms based on RGB imaging, which allow the quantitative analysis of thousands of seeds (31). Similarly, automated platforms for seedling analyses enable large-scale assessment of the underlying genetic landscape (71, 72). Seed mass and germination timing are amenable to noninvasive, automated, high-throughput measurement, whereas measurements of relative growth rate on a mass basis still depend on destructive harvests (38). In addition, the systematic measurement of seed composition is of great importance for the agricultural industry and for plant breeders. NIR spectroscopy is already a well-established technique allowing the quantification of water, protein, oil, starch, and potentially other compounds (130). Although it requires extensive calibration, NIR spectroscopy enables large-scale studies, as was recently demonstrated for maize kernel composition in nested association mapping studies (14) and the characterization of soybean fast-neutron genetic resources (23). Furthermore, MRI and X-ray CT techniques can be employed to dissect macroscopic traits and link them to microscopic ones (15, 127).

In summary, automation and noninvasive methodologies are making rapid progress, and substantial benefits are becoming apparent for the phenotyping of shoot and root dynamic responses at multiple scales as well as for the measurement of seed biomass and composition traits. Phenotyping of root system architecture is still hampered by our limited ability to access roots noninvasively in soil. In a technology development road map, once methodologies and

a proof of concept for screening protocols are proposed, the robustness, precision, and accuracy in larger-scale experiments become a major focus. Globally, we consider plant phenotyping science to be in a transition between these two stages. We also anticipate that cheaper and more accessible solutions will receive increasing attention. Because technology changes rapidly, this should be viewed as a continuous discovery process. It is probably not possible to anticipate how exactly future phenotyping platforms for different plant species will be designed.

EXPERIMENTAL IMPLEMENTATION AND DATA INTERPRETATION

Indoor and Field Experimentation

Research in plant phenotyping includes indoor and field experimentation at widely different scales. One central question for both understanding phenotypic plasticity and linking results obtained in indoor experimentation to field performance when evaluating phenotypes for plant productivity traits is which plant-specific traits and measurements transfer from the lab to the field. Here, we briefly touch on some of the key points that need to be addressed to frame this topic in a tractable way.

Growth protocols in tightly controlled growth chambers are usually designed to minimize variability between individual plants and increase the power of statistical analyses (108). However, carefully designed efforts to use the same protocol, the same soil, the same seed source, and the same (target) environmental conditions may show the limits of trying to reproduce phenotypes or rankings (89). Also, confirmatory experiments conducted in different labs and with partly different protocols for quantifying the drought responses of characterized mutants may reveal a partial lack of robustness (124). Experiments that explicitly address the comparability of lab or greenhouse results and field results may lead to conclusions that phenotypes reproduce, do not reproduce, or partially reproduce, depending in part on the phenotypes surveyed (96, 97, 116). Differences can obviously be expected between traits characterized by relatively simple inheritance that can easily transfer to the field environment (e.g., strong architectural traits such as the number of seed rows in cereal ears) and quantitative traits (especially responses to abiotic stress) that depend on an entire suite of physiological adaptations.

As this short list of examples may be easily extended, and given that there is ample scope in linking lab and field research for both basic and applied research questions (1, 96), it is important to address this topic in a structured manner that focuses explicitly on the environment and its degree of spatial and temporal heterogeneity both in controlled conditions and in the field. First, far from being constant or standard, environmental conditions (e.g., light intensity) vary in both labs and greenhouses (108), even in highly controlled phenotyping platforms (54). Increasing the comparability of results requires accurate measurements of the environmental conditions in the lab and in the field at appropriate spatial and temporal scales (108). The more accurately information is captured, the easier it is to scale the principal environmental variables influencing the phenotype. Modeling approaches based on scaling phenotypic responses according to the environmental conditions have been undertaken, and meta-analytical studies have revealed the response of growth-related traits to a range of environmental factors (109) and crop responses to temperature (105).

Second, understanding the spatial and temporal heterogeneity of environmental conditions in the field helps in designing more representative experiments in indoor growth facilities. In this respect, soil temperature gradients that are commonly found in agricultural fields are a good example. We recently conducted indoor experiments in barley to study the effect of simulated soil temperature gradients on growth and shoot and root biomass allocation (46). These experiments revealed that, compared with uniform temperature conditions, soil temperature gradient treatments lead to increases in biomass and to

carbon-to-nitrogen ratios in both shoots and roots that are more similar to those found in the field.

Finally, there is ample room for improving current phenotyping cultivation protocols by optimizing factors that do not generally receive much attention, such as pot size and planting density. As this brief discussion highlights, there is also ample room for improving our ability to interpret phenotypic data for both basic and applied research questions. We envisage that closer links and communication between lab and field experimentalists will greatly benefit plant phenotyping science.

Toward Good Practice in Plant Phenotyping

The goal of modern plant phenotyping is to deliver quantitative data on the dynamic responses of plants to the environment. Much of the recent progress has been driven by increasingly available technologies to noninvasively measure plant growth and physiological status. Imaging phenotyping is becoming common. Achieving good accuracy with various types of sensors and tracking this accuracy over time depend on a correct understanding of the sensor physics and the sensitivity that can be obtained when image phenotyping is applied to quantitative screening protocols. Additionally, sensors (including camera systems) need regular calibration. Depending on the specific cameras, it can be important to perform both geometric and radiometric calibrations for each imaging setup (90). Routine use of cameras for large-scale analyses in phenotyping platforms requires special care because many factors can influence the performance of the cameras and of the automated algorithms for image feature extraction. In this respect, it is good practice to include internal reference objects that mimic plants and can be used to track the performance of imaging setups.

Experiment design depends on many considerations. Two important factors to consider are the experimental layout itself and the appropriate number of replications to address the desired questions (108). It is also important to stress that recording and capturing environmental conditions should not be optional, but instead should be seen as a necessary step enabling meaningful data interpretation. Similar considerations apply to good-practice definitions for field experiments (137). As global standardization is not feasible and may even be detrimental, this implies the need to capture environmental variables and protocol details in a systematic way as part of defining a minimum set of information for plant phenotyping experiments. This could be done on the basis of, for instance, the scheme of Poorter and colleagues (108). Quantitative measurements of plants and the environment during experiments are two sides of the same coin and should become easily available in plant phenotypic databases in the future.

CONCLUSIONS

Plant phenotyping for macroscopic and structural parameters has significant potential to increase our understanding of plant growth and development and of crop resource use for target environments and to systematically address the phenotyping bottleneck, complementing quantitative genetics and functional genomics. For large-scale phenotyping, 2D imaging in the visible range for plant biomass estimation is used routinely in controlled facilities, and well-established methods for chlorophyll fluorescence need further development for complex shoot geometries. Imaging spectroscopy, automated root analyses, and 3D reconstructions of plants and canopies will likely remain high priorities in the research agenda. High-resolution phenotyping technologies have started to open new horizons, especially for the analysis of root architecture. Fully realizing this potential will entail finding and applying robust solutions to measure with increasing accuracy and throughput specific traits for adaptation to water- and resource-limited environments in crops of high economic value for both indoor and field deployment.

There is also vast potential in applying noninvasive methodologies to determine the performance of specific crops, such as legumes (111), horticultural crops (139), and forage and turf species (142). Large-scale noninvasive phenotyping for macroscopic traits in model species like *Arabidopsis* will enable association studies in the medium term. Integral and volumetric measurements of biomass would also greatly benefit ecophysiological research in the future, because classical growth analyses depend on destructive measurements of plant biomass (38). Moreover, large-scale evaluation at the level of entire ecosystems is already a highly active field of research. A range of questions can be tackled in ecological, biodiversity, and climate change research using remote sensing at different spatial scales (74). Our understanding of the biology of plant phenotypic responses will benefit from stronger integration of field and lab work.

Data modeling is becoming crucial to reduce the complexity of the phenotypic landscape and generate new hypotheses. Scaling up experimentation increasingly requires appropriate data management schemas to make use of quantitative analyses. To alleviate the phenotyping bottleneck, we need rapid and robust methods for gathering quantitative data to enable gene-phene inferences about shoot and root plasticity traits and specific environments, as well as appropriate analytical frameworks for data interpretation.

Finally, successful plant phenotyping can be achieved only by integrating competences through the fostering of interdisciplinary teams comprising plant biologists, physicists, mathematicians, and engineers. This is currently leading to the development of multidisciplinary centers and networks of phenotyping platforms that will provide important routes of progress, especially if they can interact efficiently at the national and international levels and succeed in playing their roles in academic and industrial research. Linking indoor and field research in plant breeding will be a major target. Connecting the different scales and platforms will be the key to creating meaningful phenotypic concepts and obtaining data crucial to accelerating the discovery process as well as introducing into plant breeding novel selection protocols for complex quantitative traits such as drought tolerance and yield.

SUMMARY POINTS

1. Plant phenotyping is an expanding research field that combines plant biology, sensor technology, and automation engineering and is gaining increasing importance owing to the need to accelerate progress in plant breeding.
2. Noninvasive methodologies are maturing and theoretically ensure high throughput, but systematic approaches are still uncommon. Methods developed for model species will need to be adapted to a wider range of open field and horticultural crops.
3. An increasing number of publications are reporting about screening procedures in controlled environments and in the field for the selection of germplasm with enhanced resource use efficiency. As protocols between labs are necessarily different, it is crucial to report on a minimum set of experiment management conditions.
4. At the macroscopic level, shoot structure, growth, physiological status, and photosynthetic performance can be measured at medium to high throughput in a range of crop species using optical methods (2D imaging and 3D reconstructions in the visible and nonvisible regions of the electromagnetic spectrum) and nonoptical methods (e.g., using terahertz radiation or microwave).

5. Characterization of root architecture in soil-based assays in the lab and in the field remains challenging, and any useful methodology should also be exploited in specific chain combinations. Methodologies to study root growth and architecture in 2D and 3D are a frontier in plant phenotyping.
6. There are well-established noninvasive techniques to target seed traits, including composition, but these are surprisingly underutilized or not systematically exploited in plant research.
7. Plant phenotyping will remain a high priority and will benefit from a broad multidisciplinary approach that includes biostatistics and information technology for data integration with other "-omics" technologies and meta-analyses.

FUTURE ISSUES

1. Selection of germplasm with enhanced resource use efficiency requires stronger links between lab and field research. For growth chamber and greenhouse experiments, this will require research in designing assays that simulate the heterogeneous conditions found in the field.
2. Our understanding of how results transfer from controlled and semicontrolled environments to complex field situations needs improvement. What are the lab-to-field transfer functions for plant productivity phenotypes?
3. Robust and less time-consuming methodologies, including semiautomated or fully automated 2D and 3D reconstruction from images of roots grown in soil, will be required to measure root system architecture at medium scales.
4. Root phenotyping as a basis for resource use efficiency and breeding for low-input systems needs improvement. Which root system architecture traits are heritable?
5. Noninvasive technologies offer unique opportunities for high-throughput plant phenotyping, but the definition of standards is still lagging behind. What minimum set of information should be recorded in phenotyping experiments?
6. Bridging the genome-to-phenome gap will require defining parsimonious experimental approaches in plant phenotyping and thorough interfacing with modeling approaches.
7. Many approaches for indoor plant phenotyping are currently expensive. In addition to further development of dedicated phenotyping platforms, cheaper solutions with acceptable compromises in accuracy and sensitivity will need to be developed, especially for deployment in the field (from portable instruments to ground-based and flying platforms).
8. Extending the benefit of modern plant phenotyping to tackle primary questions in ecology would be desirable.

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RELATED RESOURCES

Australian Plant Phenomics Facility: **http://www.plantphenomics.org.au**
Camera Calibration Toolbox for Matlab: **http://www.vision.caltech.edu/bouguetj/calib_doc**
Carnegie Spectranomics: **http://spectranomics.stanford.edu**
Chloroplast 2010: **http://www.plastid.msu.edu**
European Plant Phenotyping Network: **http://www.plant-phenotyping-network.eu**
Jülich Plant Phenotyping Centre: **http://www.jppc.de**
Open Biological and Medical Ontologies: **http://www.obofoundry.org**
Phenopsis DB: **http://bioweb.supagro.inra.fr/phenopsis**
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