

Field high-throughput phenotyping: the new crop breeding frontier

José Luis Araus¹ and Jill E. Cairns²

¹ Department of Plant Biology, Unit of Plant Physiology, University of Barcelona, 08028 Barcelona, Spain

² CIMMYT Southern Africa Regional Office, Harare, Zimbabwe

Constraints in field phenotyping capability limit our ability to dissect the genetics of quantitative traits, particularly those related to yield and stress tolerance (e.g., yield potential as well as increased drought, heat tolerance, and nutrient efficiency, etc.). The development of effective field-based high-throughput phenotyping platforms (HTPPs) remains a bottleneck for future breeding advances. However, progress in sensors, aeronautics, and high-performance computing are paving the way. Here, we review recent advances in field HTPPs, which should combine at an affordable cost, high capacity for data recording, scoring and processing, and non-invasive remote sensing methods, together with automated environmental data collection. Laboratory analyses of key plant parts may complement direct phenotyping under field conditions. Improvements in user-friendly data management together with a more powerful interpretation of results should increase the use of field HTPPs, therefore increasing the efficiency of crop genetic improvement to meet the needs of future generations.

Why is phenotyping so important in the breeding pipeline?

Crop production must double by 2050 to meet the predicted production demands of the global population [1]. However, achieving this goal will be a significant challenge for plant breeders because crop yields would have to increase at a rate of 2.4% per year, yet the average rate of increase is only 1.3%, with yields stagnating in up to 40% of land under cereal production [2,3]. Extensive breeding and agronomic efforts over the past 50 years have been responsible for tripling cereal yields [4]. Continuing advances in the techniques available to breeders offer the potential to increase the rate of genetic improvement [5]. Attempts to exploit new molecular tools to their full potential (Figure 1), particularly the ability to dissect the genetics of quantitative traits such as yield and stress tolerance [6–10], are limited by our ability to phenotype. However,

plant breeders and farmers have been making selections based on phenotypes long before the discovery of DNA and molecular markers. The development of improved varieties relies on the ability to identify the best genetic variation for advancement. Breeding is essentially a numbers game: the more crosses and environments used for selection, the greater the probability of identifying superior variation. Plant breeders want to be able to phenotype large numbers of lines rapidly and accurately identify the best progeny. To meet future needs there is a need to increase breeding efficiency. Advances in high-throughput genotyping have provided fast and inexpensive genomic information. Low cost, high-throughput genotyping has paved the way for the development of large mapping populations and diversity panels of thousands of recombinant inbred lines for phenotyping [11]. Although molecular breeding strategies, such as marker-assisted recurrent selection (MARS) and genomic selection, place greater focus on selections based on genotypic information, they still require phenotypic data [12]. In genomic selection, phenotypes are not used for selection but are used to train a prediction model [13]; whereas in MARS, a single phenotyping cycle is used to identify markers for subsequent selection through generations. Similarly, phenotyping is necessary to identify promising events in transgenic studies [14,15]. Given that molecular breeding populations can include up to 5000 lines, the ability to accurately characterize all lines simultaneously is challenging [11]. Advances in phenotyping are likely to be essential to capitalize on developments in conventional, molecular, and transgenic breeding and ensure genetic improvement of crops for future food security.

High-throughput phenotyping

In recent years, there has been increased interest in high-throughput phenotyping platforms (HTPPs) [16,17]. Most HTPPs, both those run by the big transnational seed companies and the most advanced public plant research institutions around the world, such as the Australian Plant Phenomics Facility (<http://www.plantphenomics.org.au/>), the European Plant Phenotyping Network (<http://www.plant-phenotyping-network.eu/eppn/structure>), and the USDA (http://www.nifa.usda.gov/nea/plants/pdfs/whitepaper_finalUSDA.pdf), (http://www.wheatgenetics.org/downloads/Projects/HTP_ProjectNarrative_20130219.pdf) are fully automated facilities in greenhouses or growth chambers with robotics, precise environmental control, and remote sensing techniques to assess plant growth and performance. However, low-cost HTPP approaches are now

Corresponding author: Araus, J.L. (jaraus@ub.edu).

Keywords: crop breeding; phenotyping; remote sensing; spatial variability; stress tolerance.

1360-1385/\$ – see front matter

© 2013 Elsevier Ltd. All rights reserved. <http://dx.doi.org/10.1016/j.tplants.2013.09.008>



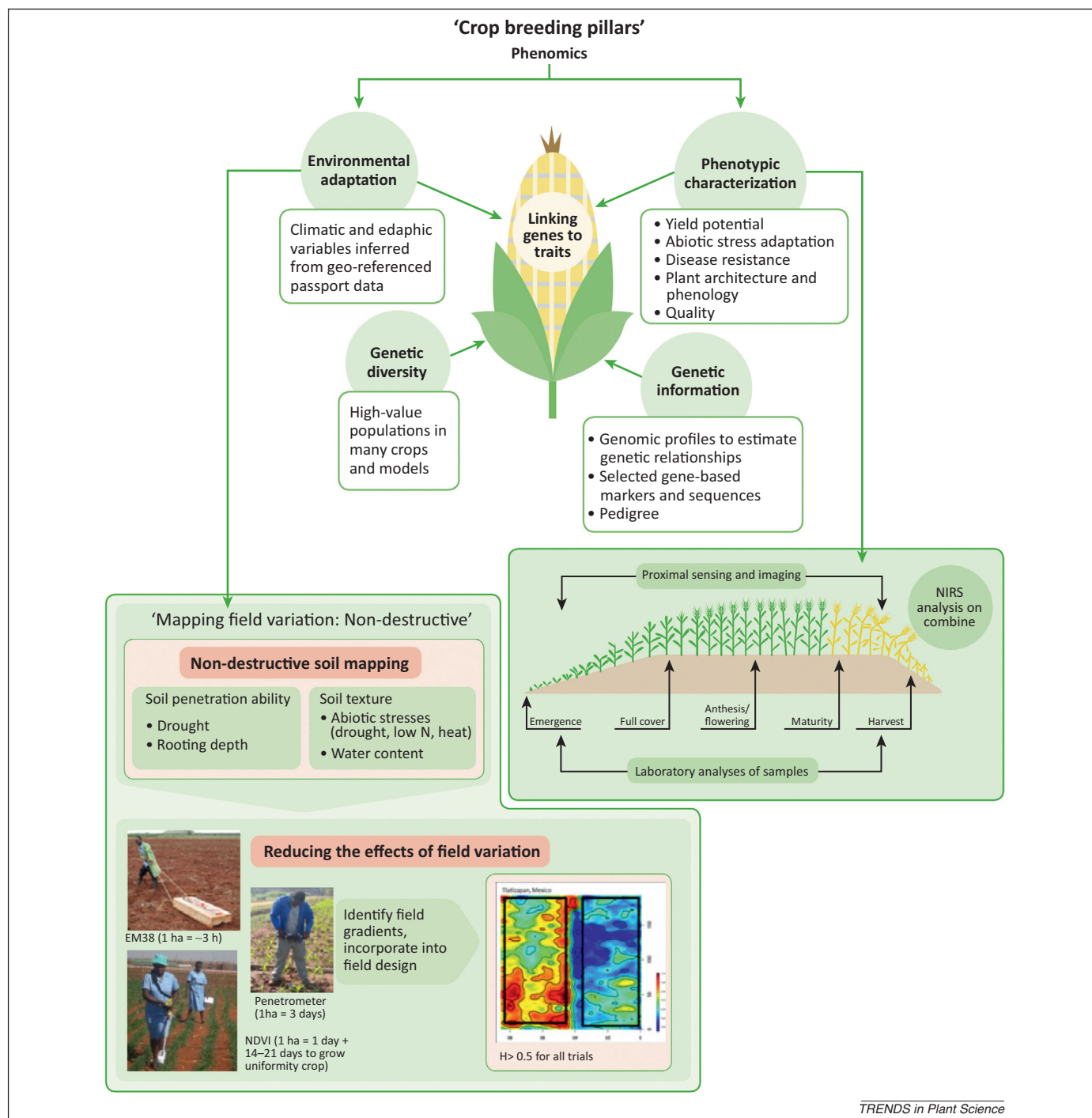


Figure 1. (Upper) The four pillars of the crop breeding pipeline (environmental adaptation, phenotypic characterization, genetic diversity, and genetic information) and the implications of phenotyping. The importance of phenotyping is highlighted by its involvement in two of these pillars. **(Lower left)** Mapping field variability in a non-destructive manner implies the use of different methodological alternatives and its further integration. **(Lower right)** Diagram of the main categories of phenotyping techniques deployed over the life cycle of an annual seed crop. Types of data acquisition include: proximal sensing and imaging at frequent intervals, laboratory analyses of samples taken at specific intervals, and near-infrared spectroscopy (NIRS) of seed for oil or protein content during combine harvesting. Redrawn from [20].

starting to be developed [18]. In this review, we examine the need for high-throughput field phenotyping, the current technical developments, and the factors that limit its implementation, together with the future avenues that will pave the way for its wide adoption in practical breeding.

Field phenotyping

Although HTPPs enable detailed, non-invasive information to be captured throughout the plant life cycle in a

carefully controlled environment, quantitative trait loci and candidate genes identified within controlled environments have generally not translated into gains in grain yield in the field [19–21]. Field conditions are notoriously heterogeneous and the inability to control environmental factors makes results difficult to interpret. However, results from controlled environments are far removed from the situation plants will experience in the field and, therefore, are difficult to extrapolate to the field. The problems

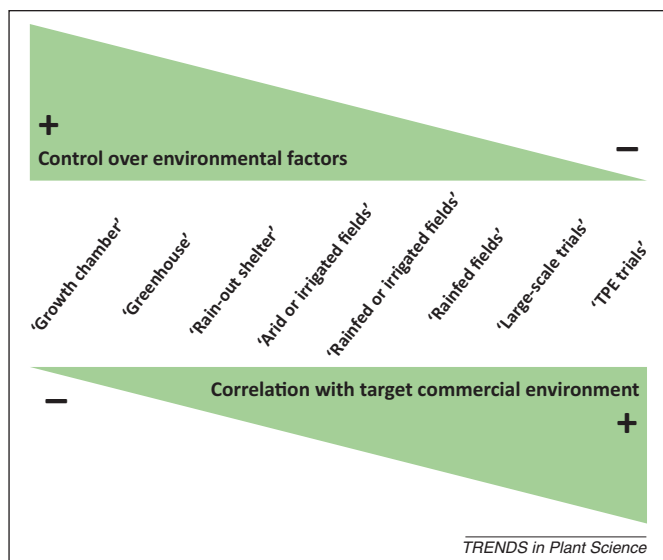


Figure 2. Continuum of environments for drought-resistance screening. The control over environmental factors decreases from the use of growth chambers to the target population environment (TPE), whereas the correlation of performance with the target commercial environments increases. Rainout shelters are designed to protect a selected area of land against receiving precipitation so that an experimentally controlled drought stress can be imposed on that area. Redrawn from [24].

associated with controlled environments are well established [6,22,23] (Figure 2). For example, the volume of soil available to roots within a pot is considerably smaller than in the field, thereby reducing the amount of water and nutrients available to plants [24–26]. The soil environment plays a crucial role in plant growth and development and is difficult to simulate under controlled conditions [27]. Drought stress phenotyping is particularly challenging because declining soil moisture content is associated with increased mechanical impedance in the field, which is an effect that is difficult to replicate within pots [28]. Moreover plants in the field do not grow in isolation but configure a canopy. Varietal improvement has heavily relied on multilocation screening within the target environment [29]. Here, plants experience a range of stresses throughout their life cycle. In many cases, the environmental characteristics are not monitored and, hence, are poorly understood. This further complicates the ability to mimic the field environment under controlled conditions. Phenotyping under field environmental conditions remains a bottleneck for future breeding advances [7,10,19,30,31]. Besides the above considerations, the choice of phenotyping under controlled conditions versus field environments largely depends on the purpose of phenotyping and the heritability of the trait, together with the logistical considerations of collecting the data [10]. For example, there are no feasible spatial or temporal options for testing high atmospheric CO₂ in the field [32].

Traits for phenotyping

The most successful traits for evaluation integrate in time (throughout the crop cycle) and space (at the canopy level) the performance of the crop in terms of capturing resources (e.g., radiation, water, and nutrients) and how efficiently these resources are used [19,33]. Different methodological

approaches have been proposed to evaluate these traits in the field (Figure 1). Using the criteria proposed in [20] they can be summarized into three categories: (i) proximal (remote) sensing and imaging, (ii) laboratory analyses of samples, and (iii) near-infrared reflectance spectroscopy (NIRS) analysis in the harvestable part of the crop. Besides the choice of the most appropriate traits, it is also crucial to determine the key time(s) for their evaluation. Measuring these traits at more or less frequent intervals during the crop cycle together with the measurements of the harvestable components would be unfeasible (or at least impractical) and may even be negative in terms of the impact of the trait for breeding.

Proximal (remote) sensing: different categories of sensors

Remote sensing phenotyping methods are non-destructive, non-invasive approaches [20,34,35] based mostly on the information provided by visible/near-infrared (VIS-NIR) radiation reflected (or transmitted) and far-infrared (thermal) radiation emitted by the crop [36–41]. These methods are termed proximal in the sense that information is gathered ‘near’ the crop. Remote sensing techniques may be deployed in *in situ* screening for a wide range of breeding objectives, including yield potential, adaptation to abiotic (water stress, extreme temperatures, salinity) and biotic (susceptibility to pests and diseases) limiting conditions, and even quality traits. Many categories of traits may be measured under different environmental conditions using remote sensing approaches, ranging, for example, from green biomass through to photosynthetic transpirative gas exchange and on to quality traits or even to predict grain yield [39]. For example, the same authors [39] working with a set of 300 maize testcrosses grown under different water and temperature regimes reported that partial least square regression (PLSR) models from hyperspectral reflectance of maize explained up to 40% of the variation in grain yield in each trial, with a relative efficiency of selection of 0.88 and 0.68 using leaf and canopy reflectance, respectively. Moreover, strong agreement has been reported between remote sensing and imaging values of traits measured by HTPPs at the land and aerial levels, as well as through manual phenotyping approaches, with determination values ranging between 0.35 and 0.82 in the case of cotton under different water regimes [42]. These results confirmed the ability of the phenotyping system to measure multiple traits rapidly and accurately. Concerning NIRS, it is already routinely used in breeding for a wide array of food and feed quality traits [43]. In fact, NIRS can be applied to drought or nutrient use efficiency screening, or other more general breeding/gene discovery objectives.

The implementation in imaging formats of proximal sensing with VIS-NIR and far-infrared radiation has enabled the process of taking measurements to be upscaled: for example, from measuring a single plot to dissecting an entire trial composed of different plots, providing that the image has enough resolution (pixels). However, imaging speed is limited by post-processing, which may include image alignment, geometric and radiometric calibrations, atmospheric correction, automatic mosaicking, and algorithms for automatic image segmentation [44,45].

Proximal (remote) sensing and imaging techniques include different approaches. The approaches that are most implemented (feasible) for field phenotyping can be grouped into three different categories (see [Box 1](#) for images): (i) VIS-NIR spectroradiometry (including multispectral and the even less developed hyperspectral imaging) [45], (ii) infrared thermometry and thermal imaging, and (iii) conventional digital photography (RGB color cameras). The first approach is undergoing rapid technical advances associated with the use of high-resolution full-range spectroradiometers that reach into the NIR region

wavelengths around 2500 nm. This, together with the use of special adaptors with their own light source, enables such spectroradiometers to be used as field-portable NIRS devices, generating large amounts of data with a wide range of potential applications. Examples include the development of empirical models to assess specific photosynthetic characteristics related to radiation use efficiency [46], or to predict a complex trait such as grain yield [39], and the use of single spectroradiometrical indices to predict yield [41]. At the imaging level, hyperspectral sensors are being developed along similar lines, although

Box 1. Cameras for crop monitoring

Different categories of imaging systems for remote sensing evaluation of vegetation are detailed below with examples of prototypes capable of being carried by UAPs of limited payload ([Figure I](#)).

RGB/CIR cameras

The combination of color infrared (CIR) and red, green, and blue light (called visible or RGB) imagery enables the estimation of green biomass (NDVI type of information). Miniaturization is advancing fast with cameras that weigh only 100 g and have spectral range bands in red, green, and NIR. [Figure 1A](#) shows an image taken with a multispectral mono lens camera (ADC Lite) of three bands.

Multispectral cameras

Multispectral imagers are widely used for crop monitoring via remote sensing. They can acquire a limited number of spectral bands at once in the VIS-NIR regions. Besides vegetation indices for evaluating green biomass, multispectral imagers can be formulated to other different spectral indices targeting senescence evaluation, nutrient status, pigment degradation, photosynthetic efficiency, or water content [84]. [Figure 1B](#) shows an image taken with a multispectral camera (miniMCA-6) of six lens.

Hyperspectral visible and near-infrared imager

The hyperspectral visible and near-infrared imager enables the acquisition of hundreds of images at once, covering the entire

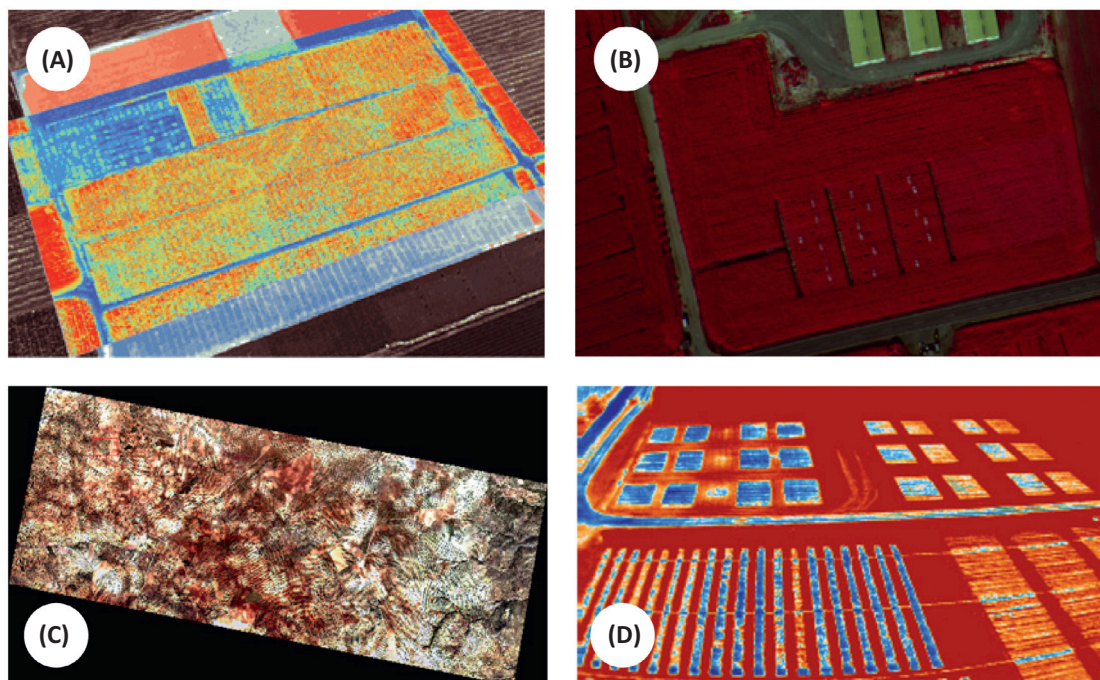
electromagnetic spectrum between the visible and the near-infrared regions in a continuous mode (wavelengths ranging from 400 to 900 nm). Other configurations cover the range from 1000 to 2500 nm. Therefore, it is possible to run empirical calibrations (like in a 'NIRS mode') against a wide and miscellaneous set of traits. [Figure 1C](#) shows an image taken with a hyperspectral (Microhyperspec VNIR) camera.

Long-wave infrared cameras or thermal imaging cameras

Long-wave infrared cameras and thermal imaging cameras render infrared radiation in the range of μm as visible light. Potential use of thermal imaging in phenotyping includes predicting water stress in crops, disease, and pathogen detection in plants, evaluating the maturing of fruits and bruise detection in fruits and vegetables [50,37,85,38]. The low resolution of the imaging (current cameras are in the range of 640×500 pixels) may limit the use of such cameras from aerial platforms. Thermal images obtained from a UAP using this type of camera have a resolution in the range of 20–40 cm [41]. [Figure 1D](#) shows an image taken with a thermal (MIDAS) camera. For more information about imaging sensors, visit <http://quantalab.ias.csis.es/pdf/>.

Conventional digital cameras (NDVI type work)

Conventional digital cameras are low-cost instruments that enable, for example, plant cover (green biomass) or senescence to be estimated. Moreover, the software needed is usually freely available.



TRENDS in Plant Science

Figure I. Examples of false-color images taken with different categories of cameras: (A) RGB/CIR, (B) multispectral, (C) hyperspectral, and (D) thermal imaging.

it is not possible for them to carry their own light source (specifically for large-scale images), thus this may represent a limitation [44]. Nevertheless, hyperspectral imagery enables assessment of complex traits, such as canopy photosynthesis and fluorescence under natural sunlight conditions [45,47]. There are several software programs available to process information to extract phenotype data from images, including free options [10].

A general limitation of spectroradiometrical approaches lies in the differences in plant architecture and developmental stage, together with the anisotropic characteristics of the leaf (mostly due to the leaf surface: roughness, trichomes, epicuticular waxes). These may negatively affect the estimation of traits (mostly of biochemical traits) by spectroradiometrical approaches [10]. The effect of leaf anisotropy is more evident in the remote sensing-based approaches that use reflected instead of transmitted radiation, which is the situation in the majority of systems. Moreover, care should be taken, for example, to standardize measures with plants at similar developmental stages and exhibiting a narrow range of variability in plant architecture, whereas sun elevation should also be taken into consideration because the majority of spectroradiometrical evaluations in the field use passive sensors. In the case of canopy temperature, variation in development and canopy architecture must be taken into account (e.g., plant height, soil covering, emerged spikes, leaf angle and size) when interpreting performance and selecting cooler breeding lines [48,49]. This is in addition to the environmental variability (e.g., in light intensity, temperature, relative humidity, wind speed) and timing of measurements, which affects the accuracy of thermal measurements [50].

Digital photography is also a promising approach given the low cost of the sensor (i.e., a conventional camera) and access to free software for data processing that enables estimation of green biomass, soil cover, plant color [51,52], or even agronomical components such as plant or inflorescence density.

Moreover, alongside these approaches, other techniques are starting to be adopted for field phenotyping, such as the use of laser imaging detection and ranging (Lidar). This is an active remote sensing technique that uses Lidar sensors to directly measure the 3D distribution of plant canopies as well as subcanopy topography, thus providing high-resolution topographic maps and highly accurate estimates of vegetation height, cover, and canopy structure [53–55]. Furthermore, laser scanning and fluorescence enables evaluation of photosynthetic performance and has potential in areas such as plant pathology [56].

Field-based high-throughput phenotyping platforms

By combining advances in remote sensing, aeronautics and high-performance, computing is paving the way for the development of field-based HTPPs. Recently, several platforms have been developed, ranging from ground-based to aerial systems (e.g., the Australian Plant Phenomics Facility, <http://www.plantphenomics.org/hrpcc/capabilities/technology>). Ground-based HTPPs include modified vehicles equipped with a global positioning system (GPS) navigation device and sensors often referred

to as ‘phenomobiles’. Different ‘phenomobiles’ have been developed within the past few years [20,42,57–60] (http://www.plant-phenotyping-network.eu/eppn/inra_diaphen). For example, in the case of cotton a system has been developed that carries four sets of sensors to measure canopy height, reflectance, and temperature simultaneously on four adjacent rows, enabling the collection of phenotypic data at a rate of 0.84 ha h⁻¹ [42]. The mounting of sensors (such as NIRS) on agricultural harvesters may also be included within the category of ‘phenomobiles’ [61,62]. Ground-based HTPPs enable data to be captured at the plot level and require little post-processing. However, this also limits the scale at which ground-based HTPPs can be used. Furthermore, simultaneous measurements of all plots within a trial are not possible with ground-based platforms. Aerial platforms are increasingly being considered as an alternative option to overcome limitations associated with ground-based HTPPs. Aerial HTPPs enable the rapid characterization of many plots within minutes. Initial aerial HTPPs used small airplanes (e.g., crop-dusting airplanes); however, this is costly and it is difficult to safely achieve the low speeds required for high-definition images at low altitude. The current generation of aerial HTPPs significantly varies in payload, initial costs, maintenance costs, and control. Recently developed alternatives include ‘phenotowers’ [63] and blimps [64]. However, this type of aerial HTPP has a maximum height of 50 m. Blimps are helium-filled balloons that can be held in a stationary position and have sensors mounted underneath. They can carry a heavy payload (several kilograms), enabling many sensors to be used concurrently; however, they require many people for control and considerable room for storage when inflated. Alternatives to blimps are unmanned aerial platforms (UAPs) such as polycopters (e.g., Ascending Technologies: <http://www.asctec.de>; Mikro-Kopter: <http://www.mikrokoetter.de>) and airplanes (see ‘Innovative agricultural technologies for sustainable intensification’ at the MAIZE Annual Report of the CGIAR Research Program: <http://repository.cimmyt.org/xmlui/bitstream/handle/10883/3209/98018.pdf?sequence=1>). Although the payload of UAPs is lower than blimps, they can generally carry up to 2 kg, enabling at least two sensors to be mounted for simultaneous image capture. UAPs enable greater flight control and autonomy and are becoming increasingly affordable. Unlike airplanes, polycopters can be maneuvered into a stationary position; however, advances in aeronautics and sensors now enable high-quality images to be obtained from unmanned airplanes. Furthermore, the autonomy and area covered by airplanes is larger and the risk of destruction by crashing lower than for polycopters. Most UAPs carry an RGB/CIR camera, together with a thermal-imaging sensor [and sometimes a conventional color (RGB) digital camera]. Replacing the RGB/CIR camera with a multispectral or hyperspectral imaging sensor increases the payload but opens up a huge range of new possibilities (Box 1). The software requirements for UAPs include programs to: (i) plan flight missions, (ii) gather the images, and (iii) extract the data for plots within the images. UAPs are controlled by an autopilot for autonomous flying; the ground control station and the UAP are radio linked, transmitting

position, altitude, and status. The imager is operated from the ground station.

Laboratory and NIRS analyses

In addition to proximal sensing approaches, the analysis of plant samples, for example, the analysis of stable isotopes [65], may complement direct phenotyping under field conditions. When breeding for yield potential and adaption to abiotic stresses such as drought, carbon isotope discrimination ($\Delta^{13}\text{C}$) in dry matter is a promising tool that frequently exhibits high heritability and genetic correlation with yield [66–68], and has already been applied to breeding programs [69]. The use of grain $\Delta^{13}\text{C}$ relies on a good understanding of tested populations and assumes no confounding of genotypic differences in phenology, anthesis biomass, pre-anthesis water use, and remobilization of stem carbohydrates [19,66,69]. Whereas $\Delta^{13}\text{C}$ is still an expensive tool for use in large-scale phenotyping, there are low cost surrogates (see next paragraph). In addition, if analyzed through the regular (mass spectrometry) technique, $\Delta^{13}\text{C}$ can still be used on just the tails to complement HTPP approaches. Other traits that may be deployed as indicators of transpiration and, hence, of water use are the total minerals accumulated in transpiring organs [70].

NIRS is regularly deployed not only at the laboratory level but also in harvesting machinery to analyze grain characteristics. When used in harvesting machinery, this technique secures a broad distribution of measurements within plots and covers substantially larger amounts of plot material than analyses at the laboratory level using conventional sample-based methods [61,62]. In any case, the near-infrared spectrum captures physical and chemical characteristics of the samples, either of vegetative plant tissues or harvested seeds. By using calibration models, several traits can be determined on the basis of a single spectrum. Thus, NIRS is regularly used to analyze, for example, the protein, nitrogen, starch and oil content, grain texture, and grain weight of (intact) seeds [20,61,62,71]. However, the same spectrum may be used to develop prediction models for analyzing traits of potential interest for phenotyping for stress adaptation, such as $\Delta^{13}\text{C}$, mineral content, or the composition of other stable isotopes [34,72,73]. Although the precision of these indirect estimations may be lower than those of direct analysis, the rapid, low-cost, and non-destructive nature of NIRS may justify its use, at least in the early generations of a breeding program, as a first screening approach when thousands of genotypes need to be evaluated.

Although considerable advances have been made for evaluating the aerial parts of plants, roots are still hidden in terms of phenotyping, despite their importance in capturing resources for the crop. A broad overview of the different approaches proposed for root phenotyping under field conditions is set out in Box 2.

More than traits and tools: spatial variability and environmental monitoring

Earlier HTPP initiatives have largely focused on phenotyping and little emphasis has been placed on environmental monitoring and reducing error variances. The phenotyping environment plays a vital role in the quality

Box 2. Phenotyping roots

Roots are notoriously difficult to phenotype in the field [26,86]. Besides technical considerations, screening under field conditions is limited by significant soil environment–genotype interactions [87]. Traditional studies have focused on excavation techniques, from which root depth and root length density can be determined. Trenching is labor intensive and slow. Recently, the Australian Commonwealth Scientific and Industrial Research Organization (CSIRO) implemented a high-throughput soil-sampling system to evaluate the maximum root depth and distribution [86]. This system uses a hydraulic push press: 2–5 min per core and 200 cores (200 cm long) per day. The system is being used to assess the impact of root architecture traits on water uptake. To that end, continuous logging of soil water content (gypsum blocks) and canopy temperature is combined with root architecture. Another approach that has been proposed for use in graminaceous crops and that is less intensive in terms of the resources deployed is ‘shovelomics’ [88]. Values for root architectural traits are derived from the visual scoring of roots, which includes in the case of maize (*Zea mays*), numbers, angles, and branching patterns of crown and brace roots.

Several non-invasive (i.e., indirect) techniques have been proposed. These include, for example, electrical capacitance [89]. At the level of root imaging, ground-penetrating radar has been explored, but resolution limitations mean that it is still likely to be restricted to trees and woody plants [86,90]. The use of electrical resistance tomography, even if still mostly at the container level [91], is perhaps more promising for mapping soil moisture and root biomass in herbaceous crops in the field [92]. Other techniques such as magnetic resonance imaging are still restricted to use at the container level [63].

Root biomass and performance can also be assessed (indirectly) through the evaluation of shoot proxy traits. This is the case for the natural abundances of stable oxygen and deuterium isotopes in shoot water [93]. The increasing availability of laser analyzers is making such determination faster and less expensive than conventional mass spectrometry. Canopy temperature has been used for remote sensing of wheat (*Triticum aestivum*) lines with deeper roots [94]. In the same way, $\Delta^{13}\text{C}$ in dry matter may also be a valuable indicator of differences in access to water by mature root systems [95,96]. Shoot biomass may also be considered as an indirect indicator of root biomass and performance [86,96]. However, problems of reliance on indirect measures of root growth may be encountered under field conditions [60,86].

of phenotypic data generated through experiments and, consequently, in the efficiency of breeding. Field variation increases error variances, thereby masking important genetic variation for key traits and reducing repeatability, regardless of the cost and precision of a phenotyping platform [74]. Spatial variation can be caused by several factors, including the soil, which is inherently heterogeneous even in relatively uniform experimental sites. HTPPs allow a larger number of genotypes to be phenotyped, thereby increasing the likelihood of soil variability masking genetic effects. In general, as the land requirement of an experiment increases the harder it becomes to find an area with minimum soil heterogeneity. Information on field variability can be incorporated into field designs by avoiding areas of high spatial variation [75]. The basic concept is to create homogeneous blocks in which the ‘noise’ factors are held constant, whereas the factor of interest (‘signal’) is allowed to vary. Earlier measurements of field variation relied on direct (i.e., destructive soil sampling) and subsequent laboratory analysis. However, advances in proximal and remote sensing technologies enable high-resolution mapping of spatial variability [76] (Figure 1). Proximal sensors include cone penetrometers, which can

be used to measure soil mechanical impedance and depth. However, measurements are time consuming and, therefore, soil penetrometers are of limited use for obtaining the necessary detail required to create field maps over large areas [28]. Apparent soil electrical conductivity is closely related to clay, water, and ionic content and electromagnetic surveys can be used to determine field gradients in soil texture [30,60]. Crop performance provides the best indicator of field variability. Imaging techniques in parallel with wireless sensor networks and geographical information systems will allow a more precise mapping and monitoring of, for example, spatial variability [77]. For example, aerial HTPPs that enable fast non-destructive GPS-linked measurements of biomass using the normalized difference vegetation index (NDVI) can be used to measure field variability either on a single variety planted in the off-season to develop subsequent planting maps or within experiments to build up performance maps to guide planting of the next season. Compared with proximal sensors of field variability (Figure 1), aerial HTPPs allow the rapid characterization of experimental blocks (less than 30 min for 1 ha plus data processing).

Environmental variability inconsistently affects phenotypic observation over both space and time and must be accounted for in any statistical models that are used to estimate parameters of interest [10]. Spatial modeling is a key requirement because of the size of the trials, the time taken to collate traits, and the use of high-throughput phenotyping approaches. In that context, multitrait mixed modeling looks promising for the analysis of traits collected by aerial remote sensing platforms where multiple traits derived from the same procedure may produce correlated errors [26]. Linear models have long been the mainstay of

quantitative genetic experiments and are the most commonly applied statistical approach to understanding phenotypic variation. However, they have inherent limitations when measurements are not adequately replicated and not normally distributed. In this regard, alternatives such as the Bayesian inference may overcome the limitations imposed by a maximum-likelihood approach [10].

In HTPPs, the implementation of environmental characterization is essential to facilitate data interpretation, meta-data analysis, and, in the case of drought phenotyping, understanding patterns of water availability [74]. Weather data are frequently sourced from nearby weather stations at the end of experiments; however, soil moisture is often not measured [75]. The need for environmental data is particularly pertinent in drought screening where knowledge of soil moisture availability is necessary to ensure that the field environment and the type of drought imposed are representative of the target environment [56]. Real-time information on soil moisture can be used to facilitate irrigation decisions, which is becoming increasingly important with increased climatic variability in the off-season when managed drought phenotyping is often conducted. The development of wireless sensor networks to characterize both climatic and soil moisture conditions should enable real-time monitoring of environmental conditions (Figure 3).

A better understanding of the stresses prevailing under the target conditions is still needed [78]. Managed Environment Facilities are becoming more important as they permit selection under controlled stress [60]. Controlling environmental conditions increases the accuracy of performance measurements and the attribution of phenotypic effects to individual traits and the underlying genetic makeup [79].

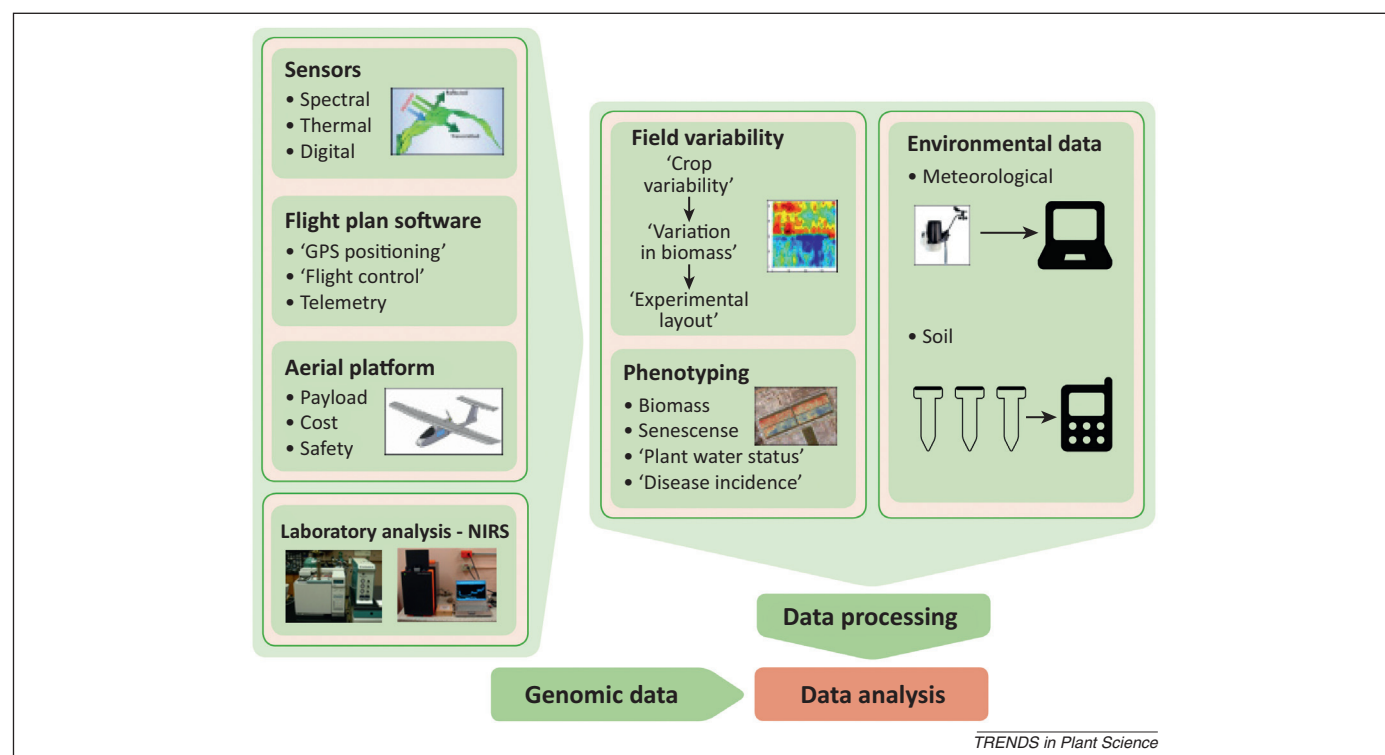


Figure 3. Summary of the different components of the breeding process where high-throughput phenotyping is involved. These include evaluation of key traits at the right moment, assessment of spatial variability, environmental characterization, and further integration of all the information.

Field phenotyping bottleneck

HTPPs have benefitted in recent years from the rapid progress that has been made in the development of a wide array of technologies including novel sensors, image analysis and modeling, robotics, and remote control and data mining. Previously, time was the largest limitation to phenotyping, but HTPPs now allow plot-level measurements within seconds. However, the bottleneck that may slow down the full implementation of these platforms is the management of the huge amount of information generated [10,50]. Two of the main limiting factors that need to be improved are: data management and use of bioinformatics to mine the volumes of phenotypic data, and the way in which modeling is used to incorporate genotypic, phenotypic, environmental, and management data [48,80]. Advanced analysis tools are required beyond even the usual statistical tools [20]. We do not even have a physical concept of what some of the numbers obtained by HTPPs mean in terms of plant or crop performance [10]. Much of the data are just mathematical transformations of numbers, but perhaps some linear combination of them will, for reasons we do not yet understand, have some correlation with important traits such as leaf angle and planting density [21]. However, in the case of information generated through hyperspectral sensors, the generation of a set of indices, even of a miscellaneous nature, does not enable full advantage to be taken of the hundreds of wavelengths measured. In this regard, the 'NIRS approach', although it represents construction of a purely empirical model, might use the most relevant information regardless of whether it has a clear physiological meaning or not [7]. Moreover, such an approach might share with genomic selection the possibility of training a model by adding new sets of data periodically.

Pyramiding all levels of information (different categories of traits measured at different times, spatial variability, environmental information) in a coherent manner requires the setting down of a strong modeling foundation based on a wide but deep understanding of the ecophysiological and genetic factors determining crop performance. Above all, more user-friendly post-processing of the raw data generated is needed. Improved software tools to optimize automation and speed up robust data analysis should support such a trend [81].

Concluding remarks and future perspectives

The capacity for undertaking precision phenotyping, particularly under repeatable and representative growing conditions in the field, is lagging far behind the capacity to generate genomic information. This is constraining breeding advances. Developments (e.g., sensors, platforms, and analytical capability) in HTPPs have enabled these measurements to be made *in situ*; however, the integration of data is lagging behind.

Field phenotyping of the appropriate traits, using low cost, easy-to-handle tools, should become an integral and key component in the breeding pipeline. Using technological advances with regard to phenotyping instrumentation should also go hand-in-hand with methods to characterize and control field site variation (for improving repeatability), adopting appropriate experimental designs, selection of the right traits, and, finally, proper integration of

heterogeneous datasets, analysis, and application, including prediction models [8,19,21,82,83]. Efficient integration of all the components of the system is needed to pave the way for the adoption of field HTPPs in the near future. This includes more user-friendly data management combined with data gathering and processing.

Acknowledgments

The preparation of this article was supported by grants from the MAIZE CGIAR Research Program (to J.L.A. and J.E.C.) and the Spanish and Catalan projects AGL2010-20180 and SGR2009-00327(J.L.A.). We thank Dr Pablo Zarco, Consejo Superior de Investigaciones Científicas (CSIC), Spain for providing some of the illustrations and Dr. L. Cabrera-Bosquet, Laboratoire d'Ecophysiologie des Plantes sous Stress Environnementaux (LEPSE), France for his suggestions on literature.

References

- 1 Tilman, D. *et al.* (2011) Global food demand and the sustainable intensification of agriculture. *Proc. Natl. Acad. Sci. U.S.A.* 108, 20260–20264
- 2 Ray, D.K. *et al.* (2012) Recent patterns of crop yield growth and stagnation. *Nat. Commun.* 3, 1293
- 3 Ray, D.K. *et al.* (2013) Yield trends are insufficient to double global crop production by 2050. *PLoS ONE* 8, e66428
- 4 Pingali, P.L. (2012) Green revolution: impacts, limits, and the path ahead. *Proc. Natl. Acad. Sci. U.S.A.* 109, 12302–12308
- 5 Phillips, R.L. (2010) Mobilizing science to break yield barriers. *Crop Sci.* 50, S99–S108
- 6 Blum, A. (2011) *Plant Breeding for Water-limited Environments*, Springer
- 7 Cabrera-Bosquet, L. *et al.* (2012) High-throughput phenotyping and genomic selection: the frontiers of crop breeding converge. *J. Integr. Plant Biol.* 54, 312–320
- 8 Araus, J.L. *et al.* (2012) Phenotyping maize for adaptation to drought. *Front. Physiol.* 3, 305
- 9 Tuberosa, R. (2012) Phenotyping for drought tolerance of crops in the genomics era. *Front. Physiol.* 3, 347
- 10 Cobb, J.N. *et al.* (2013) Next-generation phenotyping: requirements and strategies for enhancing our understanding of genotype–phenotype relationships and its relevance to crop improvement. *Theor. Appl. Genet.* 126, 867–887
- 11 McMullen, M.D. *et al.* (2009) Genetic properties of the maize nested association mapping population. *Science* 325, 737–740
- 12 Jannick, J.L. *et al.* (2010) Genomic selection in plant breeding: from theory to practice. *Brief. Funct. Genomics* 9, 166–177
- 13 Lorenz, A.J. *et al.* (2011) Genomic selection in plant breeding: knowledge and prospects. *Adv. Agron.* 110, 77–123
- 14 Gaudin, A.C.M. *et al.* (2013) Taking transgenic rice drought screening to the field. *J. Exp. Bot.* 64, 109–118
- 15 Saint-Pierre, C. *et al.* (2012) Phenotyping transgenic wheat for drought resistance. *J. Exp. Bot.* 63, 1799–1808
- 16 Furbank, R.T. and Tester, M. (2011) Phenomics – technologies to relieve the phenotyping bottleneck. *Trends Plant Sci.* 16, 635–644
- 17 Fiorani, F. and Schurr, U. (2013) Future scenarios for plant phenotyping. *Annu. Rev. Plant Biol.* 64, 17.1–17.25
- 18 Pereyra-Irujo, G.A. *et al.* (2012) GlyPh: a low-cost platform for phenotyping plant growth and water use. *Funct. Plant Biol.* 39, 905–913
- 19 Araus, J.L. *et al.* (2008) Breeding for yield potential and stress adaptation in cereals. *Crit. Rev. Plant Sci.* 27, 1–36
- 20 White, J.W. *et al.* (2012) Field-based phenomics for plant genetics research. *Field Crops Res.* 133, 101–112
- 21 Von Mogel, K.H. (2013) Taking the phenomics revolution into the field. *CSA News* March, 4–10
- 22 Passioura, J.B. and Angus, J.F. (2010) Improving productivity of crops in water-limited environments. *Adv. Agron.* 106, 37–75
- 23 Passioura, J.B. (2012) Phenotyping for drought tolerance in grain crops: when is it useful to breeders? *Funct. Plant Biol.* 39, 851–859
- 24 Passioura, J.B. (2006) The perils of pot experiments. *Funct. Plant Biol.* 33, 1075–1079
- 25 Poorter, H. *et al.* (2012) Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. *Funct. Plant Biol.* 39, 839–850

- 26 Reynolds, M.P. *et al.*, eds (2012) *Physiological Breeding I: Interdisciplinary Approaches to Improve Crop Adaptation*, CIMMYT
- 27 Whitmore, A.P. and Whalley, W.R. (2009) Physical effects of soil drying on roots and crop growth. *J. Exp. Bot.* 60, 2845–2857
- 28 Cairns, J.E. *et al.* (2011) Influence of the soil physical environment on drought stress and its implications for drought research. *Field Crop Res.* 121, 303–310
- 29 Cairns, J.E. *et al.* (2013) Adapting maize production to climate change in sub-Saharan Africa. *Food Sec.* 5, 345–360
- 30 Cairns, J.E. *et al.* (2012) Dissecting maize productivity: ideotypes associated with grain yield under drought stress and well-watered conditions. *J. Integr. Plant Biol.* 54, 1007–1020
- 31 Cairns, J.E. *et al.* (2012) Maize production in a changing climate: impacts, adaptation, and mitigation strategies. *Adv. Agron.* 114, 1–58
- 32 Gleadow, R. *et al.* (2013) Crops for a future climate. *Funct. Plant Biol.* 40, iii–vi
- 33 Araus, J.L. *et al.* (2002) Plant breeding and water stress in C3 cereals: what to breed for? *Ann. Bot.* 89, 925–940
- 34 Cabrera-Bosquet, L. *et al.* (2011) NIRS-assessment of $\delta^{18}\text{O}$, nitrogen and ash content for improved yield potential and drought adaptation in maize. *J. Agric. Food Chem.* 59, 467–474
- 35 Monneveux, P. *et al.* (2013) Drought tolerance in potato (*S. tuberosum* L.): Can we learn from drought tolerance research in cereals? *Plant Sci.* 205–206, 76–86
- 36 Berger, B. *et al.* (2010) High-throughput shoot imaging to study drought responses. *J. Exp. Bot.* 61, 3519–3528
- 37 Munns, R. *et al.* (2010) New phenotyping methods for screening wheat and barley for beneficial responses to water deficit. *J. Exp. Bot.* 61, 3499–3507
- 38 Vadivambal, R. and Jayas, D.S. (2011) Applications of thermal imaging in agriculture and food industry – a review. *Food Bioprocess Technol.* 4, 186–199
- 39 Weber, V.S. *et al.* (2012) Prediction of grain yield using reflectance spectra of canopy and leaves in maize plants grown under different water regimes. *Field Crops Res.* 128, 82–90
- 40 Zia, S. *et al.* (2013) Infrared thermal imaging as a rapid tool for identifying water stress tolerant maize genotypes of different phenology. *J. Agron. Crop Sci.* 199, 75–84
- 41 Lobos, G.A. *et al.* (2013) Wheat genotypic variability in grain yield and carbon isotope discrimination under Mediterranean conditions assessed by spectral reflectance. *J. Integr. Plant Biol.* (in press)
- 42 Andrade-Sanchez, P. *et al.* (2013) Development and evaluation of a field-based high-throughput phenotyping platform. *Funct. Plant Physiol.* <http://dx.doi.org/10.1071/FP13126>
- 43 Grieder, C. *et al.* (2012) Breeding maize as biogas substrate in Central Europe: II. Quantitative-genetic parameters for inbred lines and correlations with testcross performance. *Theor. Appl. Genet.* 124, 981–988
- 44 Bernie, J.A. *et al.* (2009) Thermal and narrow and multispectral remote sensing for vegetation monitoring from an unmanned aerial vehicle. *IEEE Trans. Geosci. Remote Sens.* 47, 722–738
- 45 Zarco-Tejada, P. *et al.* (2013) Relationships between net photosynthesis and steady-state chlorophyll fluorescence retrieved from airborne hyperspectral imagery. *Remote Sens. Environ.* 136, 247–258
- 46 Serbin, S.P. *et al.* (2012) Leaf optical properties reflect variation in photosynthetic metabolism and its sensitivity to temperature. *J. Exp. Bot.* 63, 489–502
- 47 Zarco-Tejada, P. *et al.* (2013) Spatial resolution effects on chlorophyll fluorescence retrieval in a heterogeneous canopy using hyperspectral imagery and radiative transfer simulation. *IEEE Geosci. Remote Sens. Lett.* 10, 937–941
- 48 Maes, W.H. and Steppe, K. (2012) Estimating evapotranspiration and drought stress with ground-based thermal remote sensing in agriculture: a review. *J. Exp. Bot.* 63, 4671–4712
- 49 Rebetzke, G.J. *et al.* (2012) Genomic regions for canopy temperature and their genetic association with stomatal conductance and grain yield in bread wheat (*Triticum aestivum* L.). *Funct. Plant Biol.* 40, 14–33
- 50 Costa, J.M. *et al.* (2013) Thermography to explore plant–environment interactions. *J. Exp. Bot.* 64, 3937–3949
- 51 Casadesús, J. *et al.* (2007) Vegetation indices derived from conventional digital cameras as selection criteria for wheat breeding in water-limited environments. *Ann. Appl. Biol.* 150, 227–236
- 52 Mullan, D.J. and Reynolds, M.P. (2010) Quantifying genetic effects of ground cover on soil water evaporation using digital imaging. *Funct. Plant Biol.* 37, 703–712
- 53 Lefsky, M.A. *et al.* (2002) Lidar remote sensing for ecosystem studies. *BioScience* 52, 19–30
- 54 Omasa, K. *et al.* (2007) 3D lidar imaging for detecting and understanding plant responses and canopy structure. *J. Exp. Bot.* 58, 881–898
- 55 Hosoi, F. and Omasa, K. (2009) Estimating vertical plant area density profile and growth parameters of a wheat canopy at different growth stages using three-dimensional portable Lidar imaging. *ISPRS J. Photogram. Remote Sens.* 64, 151–158
- 56 Römer, C. *et al.* (2011) Robust fitting of fluorescence spectra for pre-symptomatic wheat leaf rust detection with support vector machines. *Comput. Electron. Agric.* 79, 180–188
- 57 Montes, J.M. *et al.* (2011) High-throughput non-destructive biomass determination during early plant development in maize under field conditions. *Field Crops Res.* 121, 268–273
- 58 Weiss, U. and Biber, P. (2011) Plant detection and mapping for agricultural robots using a 3D LIDAR sensor. *Robot. Auton. Syst.* 59, 266–273
- 59 Comar, A. *et al.* (2012) A semi-automatic system for high throughput phenotyping wheat cultivars in-field conditions: description and first results. *Funct. Plant Biol.* 39, 914–924
- 60 Rebetzke, G.J. *et al.* (2013) A multisite managed environment facility for targeted trait and germplasm phenotyping. *Funct. Plant Biol.* 40, 1–13
- 61 Montes, J.M. *et al.* (2007) Novel throughput phenotyping platforms in plant genetic studies. *Trends Plant Sci.* 12, 433–436
- 62 Mir, R.R. *et al.* (2012) Integrated genomics, physiology and breeding approaches for improving drought tolerance in crops. *Theor. Appl. Genet.* 125, 625–645
- 63 Rascher, U. *et al.* (2011) Non-invasive approaches for phenotyping of enhanced performance traits in bean. *Funct. Plant Biol.* 38, 968–983
- 64 Losos, J.B. (2013) Evolutionary biology for the 21st century. *PLoS Biol.* 11, e1001466
- 65 Yousfi, S. *et al.* (2012) Combined use of $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ tracks nitrogen metabolism and genotypic adaptation of durum wheat to salinity and water deficit. *New Phytol.* 194, 230–244
- 66 Condon, A.G. *et al.* (2002) Improving intrinsic water-use efficiency and crop yield. *Crop Sci.* 42, 122–131
- 67 Condon, A.G. *et al.* (2004) Breeding for high water-use efficiency. *J. Exp. Bot.* 55, 2447–2460
- 68 Araus, J.L. *et al.* (2013) Comparative performance of $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ for phenotyping durum wheat adaptation to a dryland environment. *Funct. Plant Biol.* 40, 595–608
- 69 Rebetzke, G.J. *et al.* (2008) Quantitative trait loci for carbon isotope discrimination are repeatable across environments and wheat mapping populations. *Theor. Appl. Genet.* 118, 123–137
- 70 Cabrera-Bosquet, L. *et al.* (2009) How yield relates to ash content, $\Delta^{13}\text{C}$ and $\Delta^{18}\text{O}$ in maize grown under different water regimes. *Ann. Bot.* 104, 1207–1216
- 71 Hacisalihoglu, G. *et al.* (2010) Near-infrared reflectance spectroscopy predicts protein, starch, and seed weight in intact seeds of common bean (*Phaseolus vulgaris* L.). *J. Agric. Food Chem.* 58, 702–706
- 72 Ferrio, J.P. *et al.* (2001) Near infrared reflectance spectroscopy as a new surrogate analysis for $\Delta^{13}\text{C}$ in mature kernels of durum wheat. *Austr. J. Agric. Res.* 52, 809–816
- 73 Kleinebecker, T. *et al.* (2009) Prediction of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in plant tissues with near-infrared reflectance spectroscopy. *New Phytol.* 184, 732–739
- 74 Masuka, B. *et al.* (2012) Deciphering the code: successful abiotic stress phenotyping for molecular breeding. *J. Integr. Plant Biol.* 54, 238–249
- 75 Jones, H.G. (2007) Monitoring plant and soil water status: established and novel methods revisited and their relevance to studies of drought tolerance. *J. Exp. Bot.* 58, 119–130
- 76 Gebbers, R. and Adamchuk, V.I. (2010) Precision agriculture and food security. *Science* 327, 828–831
- 77 Lee, W.S. *et al.* (2010) Sensing technologies for precision specialty crop production. *Comput. Electron. Agric.* 74, 2–33
- 78 Weber, V.S. *et al.* (2012) Efficiency of managed-stress screening of elite maize hybrids under drought and low nitrogen for yield under rainfed conditions in Southern Africa. *Crop Sci.* 52, 1011–1020

- 79 Blum, A. (2011) Drought resistance – is it really a complex trait? *Funct. Plant Biol.* 38, 753–757
- 80 Pieruschka, R. and Poorter, H. (2012) Phenotyping plants: genes, phenes and machines. *Funct. Plant Biol.* 39, 813–820
- 81 Fuentes, S. *et al.* (2012) Computational water stress indices obtained from thermal image analysis of grapevine canopies. *Irrig. Sci.* 30, 523–536
- 82 Tardieu, F. and Tuberosa, R. (2010) Dissection and modelling of abiotic stress tolerance in plants. *Curr. Opin. Plant Biol.* 13, 206–212
- 83 Prasanna, B.P. *et al.* (2013) High-throughput and precision phenotyping for cereal breeding programs. In *Cereal Genomics II* (Gupta, P.K. and Varshney, R.K., eds), pp. 341–374, (Chapter 13), Springer
- 84 Gutierrez, M. *et al.* (2010) Association of water spectral indices with plant and soil water relations in contrasting wheat genotypes. *J. Exp. Bot.* 12, 3291–3303
- 85 Walter, A. *et al.* (2012) Advanced phenotyping offers opportunities for improved breeding of forage and turf species. *Ann. Bot.* 110, 1271–1279
- 86 Wasson, A.P. *et al.* (2012) Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. *J. Exp. Bot.* 63, 3485–3498
- 87 Gregory, P.J. *et al.* (2009) Root phenomics of crops: opportunities and challenges. *Funct. Plant Biol.* 36, 922–929
- 88 Trachsel, S. *et al.* (2011) Shovelomics: high throughput phenotyping of maize (*Zea mays* L.) root architecture in the field. *Plant Soil* 341, 75–87
- 89 van Beem, J. *et al.* (1998) Estimating root mass in maize using a portable capacitance meter. *Agron. J.* 90, 566–570
- 90 Zenone, T. *et al.* (2008) Preliminary use of ground-penetrating radar and electrical resistivity tomography to study tree roots in pine forests and poplar plantations. *Funct. Plant Biol.* 35, 1047–1058
- 91 Amato, M. *et al.* (2009) Multi-electrode 3D resistivity imaging of alfalfa root zone. *Eur. J. Agron.* 31, 213–222
- 92 Srayeddin, I. and Doussan, C. (2009) Estimation of the spatial variability of root water uptake of maize and sorghum at the field scale by electrical resistivity tomography. *Plant Soil* 319, 185–207
- 93 Durand, J.L. *et al.* (2007) Ranking of the depth of water extraction by individual grass plants, using natural ^{18}O isotope abundance. *Environ. Exp. Bot.* 60, 137–144
- 94 Lopes, M.S. and Reynolds, M.P. (2010) Partitioning of assimilates to deeper roots is associated with cooler canopies and increased yield under drought in wheat. *Funct. Plant Biol.* 37, 147–156
- 95 Araus, J.L. *et al.* (2003) Breeding cereals for Mediterranean conditions: ecophysiological clues for biotechnology application. *Ann. Appl. Biol.* 142, 129–141
- 96 Elazab, A. *et al.* (2012) Root traits and $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of durum wheat under different water regimes. *Funct. Plant Biol.* 39, 379–393

Plant Science Conferences in 2014

XVI International Congress on Molecular Plant-Microbe Interactions

6–10 July, 2014

Rhodes, Greece

<http://www.mpmi2014rhodes-hellas.gr/index.php>

Plant Biology 2014

12–16 July, 2014

Portland, USA

<http://www.aspb.org/meetings>

GRC: Plant Molecular Biology Decision-Making Pathways, Networks, and Models in Plant Biology

20–25 July, 2014

Holderness, USA

<http://www.grc.org/programs.aspx?year=2014&program=plantmolec>

EMBO Workshop: Intercellular communication in plant development and disease

24–29 August, 2014

Bischoffsheim, France

<http://events.embo.org/coming-soon/index.php?EventID=w14-11>