Review

Field-based phenomics for plant genetics research

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ABSTRACT

A major challenge for crop research in the 21st century is how to predict crop performance as a function of genetic architecture. Advances in "next generation" DNA sequencing have greatly improved genotyping efficiency and reduced genotyping costs. Methods for characterizing plant traits (phenotypes), however, have much progressed more slowly over the past 30 years, and constraints in phenotyping capability limit our ability to dissect the genetics of quantitative traits, especially those related to harvestable yield and stress tolerance. As a case in point, mapping populations for major crops may consist of 20 or more families, each represented by as many as 200 lines, necessitating field trials with over 20,000 plots at a single location. Investing in the resources and labor needed to quantify even a few agronomic traits for linkage with genetic markers in such massive populations is currently impractical for most breeding programs. Herein, we define key criteria, experimental approaches, equipment and data analysis tools required for robust, high-throughput field-based phenotyping (FBP). The focus is on simultaneous proximal sensing for spectral reflectance, canopy temperature, and plant architecture where a vehicle carrying replicated sets of sensors records data on multiple plots, with the potential to record data throughout the crop life cycle. The potential to assess traits, such as adaptations to water deficits or acute heat stress, several times during a single diurnal cycle is especially valuable for quantifying stress recovery. Simulation modeling and related tools can help estimate physiological traits such as canopy conductance and rooting capacity. Many of the underlying techniques and requisite instruments are available and in use for precision crop management. Further innovations are required to better integrate the functions of multiple instruments and to ensure efficient, robust analysis of the large volumes of data that are anticipated. A complement to the core proximal sensing is high-throughput phenotyping of specific traits such as nutrient status, seed composition, and other biochemical characteristics, as well as underground root architecture. The ability to "ground truth" results with conventional measurements is also necessary. The development of new sensors and imaging systems undoubtedly will continue to improve our ability to phenotype very large experiments or breeding nurseries, with the core FBP abilities achievable through strong interdisciplinary efforts that assemble and adapt existing technologies in novel ways.

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Contents

1. Introduction

Ensuring that agricultural production will be sufficient to satisfy the needs of a human population likely to exceed 9 billion by 2050 (http://www.unpopulation.org) presents a tremendous challenge for plant science and crop improvement in the 21st century. A fundamental step forward is to dramatically improve phenotypic prediction based on the genetic composition of lines or cultivars. By connecting genotype to phenotype, high yielding, stress-tolerant plants can be selected far more rapidly and efficiently than is currently possible. Spectacular advances in "next generation" DNA sequencing are rapidly reducing the costs of genotyping (Shendure and Ji, 2008; Jackson et al., 2011). In contrast, plant phenotyping has improved only slowly over the past 30 years, and obtaining sufficient, relevant phenotypic data on a single plot or plant-by-plant basis remains problematic. This is especially true for complex traits such as abiotic stress tolerance and yield potential, which have particular relevance for crop improvement and ultimately, commercial production. However, dissecting complex traits requires an examination of thousands of lines (Myles et al., 2009). Practical application through genomic selection (Goddard and Hayes, 2007; Jannink et al., 2010) or genome-wide association studies (Myles et al., 2009) will similarly involve phenotyping thousands of genetically distinct lines (reference or association populations) grown in replication across multiple environments in order to assess differential expression of multiple genes (i.e., detection of genotype-by-environment interactions). Research to improve phenotyping techniques is termed "phenomics" (following the terminology of "-omics" from plant sciences; Furbank, 2009).

Recognition of the limits of current approaches in phenomics has stimulated interest in high-throughput phenotyping methods that can be used to characterize large numbers of lines or individual plants accurately and that require a fraction of the time, cost and labor of current techniques (Montes et al., 2007; Furbank, 2009). Much of the discussion of phenotyping systems has focused on intensive measurement of individual plants using platforms that combine robotics and image analysis with controlled-environment systems (e.g., Arvidsson et al., 2011). While acknowledging the value of these systems for certain targeted applications, the use of greenhouses and controlled environments to represent field environments has well-known limitations. Limited greenhouse space or chamber volumes often preclude allowing plants to flower and set seed, making it impossible to assess effects of stresses during reproductive growth. The soil volume that is provided for plants in controlled environments usually is far less than that available to plants in the field, affecting nutrient and water regimes and altering normal patterns of growth and development. Enclosed aerial environments are also problematic for characterizing responses relevant to field situations. In greenhouses and chambers, solar radiation, wind speed and evaporation rates typically are lower than under open-air conditions. Mechanical vibration can induce physiological artifacts in plant growth (Biddington, 1986; Chehab et al., 2009). Not surprisingly, researchers focusing on demonstrable, field-level improvements in yield potential or abiotic stress tolerance favor field-based phenotyping. Drought is a climatological event, and Campos et al. (2004) argued that "drought tolerance that impacts crop yield can only be assessed reliably in the field".

Field-based phenotyping (FBP) is increasingly recognized as the only approach capable of delivering the requisite throughput in terms of numbers of plants or populations, as well as an accurate description of trait expression in real-world cropping systems. However, to date, most field-based phenotyping systems have focused on rapid assessment of individual suites of traits such as vegetation indices (Babar et al., 2006a,b) or root morphology (Trachsel et al., 2011).

Through use of vehicles carrying multiple sets of sensors, a FBP platform can transform the characterization of plant populations for genetic research and crop improvement. An example of FBP requirements for maize (*Zea mays* L.) is instructive. The maize nested association mapping (NAM) population consists of 25 biparental crosses, each represented by 200 lines (Buckler et al., 2009; McMullen et al., 2009), giving a total of 5000 lines. Specialized experimental designs combined with spatial analysis permit two replicates, thus requiring 10,000 plots for a single treatment (e.g., well-watered or water-limited). Adding just one additional treatment doubles the count to 20,000 plots. Using single-row, 1-m wide by 4-m long plots and ignoring the need for walkways or borders, the net row-length would be 80 km (roughly 50 miles), occupying 8 ha (20 acres). A person walking 3 km h^{-1} (2 mph) would need about 27 h to visually score traits, assuming no stopping. Halting at each plot for 30 s (e.g., to measure leaf conductance or chlorophyll concentration) would require an additional 165 h. Existing and planned mapping populations for other crop species are of similar scale (Table 1), so without even considering direct applications in crop improvement, the need for high throughput is apparent.

Accomplishing FBP in a cost-effective manner will require breakthroughs in techniques and research infrastructure. FBP approaches will likely use wheeled or aerial vehicles to deploy multiple types of instruments that can measure plant traits on a time scale of a few seconds per plot. However, even this sampling rate will likely require multiple vehicles and/or multiple sets of sensors on a single vehicle. Returning to the maize NAM example, a vehicle measuring traits on single rows and traveling 2 km h^{-1} would require over 40 h to cover the entire field. Using three vehicles with eight sets of sensors per vehicle would reduce the required time to less than 2 h, allowing up to 12 visits per day to any plot. Fig. 1 shows a prototype FBP vehicle carrying sensors that measure plant height, canopy temperature and spectral reflectance at three wavelengths (Andrade-Sanchez et al., 2012). Observations are geo-referenced