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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**



*Abbreviations:* FAA, Federal Aviation Administration; FBP, field-based phenotyping; GIS, geographic information system; IRT, infrared thermometer; LAI, leaf area index; LED, light-emitting diode; NAM, nested-associated mapping; NDVI, normalized difference vegetation index; NIR, near infrared; QTL, quantitative trait loci; RTK, real time kinematic.

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## 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

**Table 1**
Scenarios for high-throughput phenotyping based on existing or proposed sets of mapping populations in barley, maize, wheat and cotton.

| Crop | Populations | Number of families | Total number of lines[a] | Reference |
|---|---|---|---|---|
| Barley | From ten U.S. barley breeding programs | – | 3840[b] | http://www.BarleyCAP.org |
| Cotton | Candidates being assembled from US public breeding programs (*G. hirsutum*) | 25 | 5000 | Proposed |
| Maize | Nested association mapping (NAM) based on crosses to B73 | 25 | 5000 | http://www.panzea.org |
| Wheat | Contrasting parents from many US spring and winter wheat programs | 17 | 3315 | http://maswheat.ucdavis.edu |

[a] Number of lines for each population.
[b] Breeding lines identified as important by breeding programs.

using GPS with a positional accuracy under 2 cm. With the help of GPS-based auto-pilot systems, we can get closer to fully automating the deployment of instruments at the field-scale, reducing unintended human error by the driver or differences between drivers when using multiple vehicles.

## 2. An integrated FBP platform

Previous experience in characterizing crop responses to water and nutrients, and to a lesser extent for breeding and genetics, has established that numerous plant traits can be measured via remote sensing of the crop canopy (Wiegand and Namken, 1966; Idso et al., 1980; Blum et al., 1982; Ball and Konzak, 1993; Li et al., 2001; Moran et al., 2003; Babar et al., 2006a,b,c). A core challenge of FBP is to adapt these techniques for small-plot evaluations of diverse crops and traits. The system needs to be rapid, flexible and reliable. Ideally, it should work at spatial scales less than 1 m, permit the evaluation of hundreds to thousands of plots in a few hours – regardless of time of day or field conditions – and characterize multiple traits in a single pass. The system should permit measurements to be made repeatedly throughout the season and as needed, including intensive sampling through a diurnal cycle. Complementary high-throughput analyses of leaf, seed or other samples at key phases of crop growth may also be required (Fig. 2).

A FBP platform requires six components:

1. Instruments for acquiring raw data from field plots.
2. Physical systems for integrating different instruments including providing power, data logging or transmission, partial or complete shading, and protection from dust, vibration and adverse weather.
3. Vehicles for positioning the instrument rapidly and accurately in a field.
4. High-throughput analytic capabilities to complement field measurements (e.g., of leaf or seed samples).

**Fig. 1.** High-clearance tractor in operation over young cotton plants at Maricopa, AZ. Replicated sets of sensors allow simultaneous measurement of canopy height, temperature, and spectral reflectance at three bandwidths. Real time kinematic GPS provides positional accuracy under 2 cm.

5. Software systems for managing and analyzing potentially large and complex datasets.
6. Integrated management protocols to maximize reliability and efficiency of the phenotyping.

We emphasize that use of field-based systems does not exclude complementary phenotyping in controlled environments or rapid screening for specific traits such as shoot or root architecture.

### 2.1. Instruments for acquiring raw data

The range of plant traits that can be evaluated directly and nondestructively through measurements of reflectance or emissions is increasing rapidly with advances in our understanding of plant biology, sensor and imaging technologies, and data analysis. Example traits range from levels of specific leaf pigments, to plant biomass, to phenology (Table 2). While single types of measurements often show relations with yield or biomass, FBP requires instruments that can help to characterize multiple, interacting plant processes. This likely will require deploying several types of instruments at once and measuring plant responses at both diurnal and seasonal time scales.

Instrument options are evolving rapidly, and the ability to position instruments and sources of illumination or shading near the foliage further increases the choices. Examples of potentially enabling technologies include:

- Photodiodes that allow construction of low-cost sensors at specific bandwidths (Garrity et al., 2010), including versions that incorporate built in signal conditioning and optical filters.
- High intensity light emitting diodes, which deliver continuous or pulsed photon fluxes, while drawing relatively little power (Yeh and Chung, 2009), thus allowing novel options for active sensing.
- Infrared imagery using commercial digital cameras and accurate infrared thermometers (IRT, French et al., 2007).
- Stereo image analysis, which shows potential for characterizing plant height, leaf shape and leaf angle distribution (Biskup et al., 2007; Yu et al., 2007).
- Acoustic-based distance sensing (Ruixiu et al., 1989; Andrade-Sanchez et al., 2012)
- Systems for non-contact measurement of chlorophyll fluorescence as an indicator of photosynthetic performance (Kolber et al., 2005).
- Other examples include laser distance sensing and near infrared spectroscopy.

Airplane and satellite-based systems are invaluable sources of information at field to regional scales, but proximal (close-range) sensing is often the only approach that can provide adequate resolution for phenotyping studies. Besides allowing higher resolution sensing, an FBP system can provide multiple view-angles, control illumination and regulate the distance from the target to the sensors. In remote measurement of plant water content, atmospheric water vapor and aerosols introduce a significant background signal (Gao, 1996) but with proximal sensing, this constraint is greatly

**Table 2**
Examples of proximal sensing methods that show promise for field-based phenomics. IR = Infrared; NIR = near infrared.

| Trait class | Target trait | Index or method | Applications or relevant traits | Point (P) or image-based (I) | Wavelengths | References |
|---|---|---|---|---|---|---|
| Pigment constituents | Chlorophyll | Normalized difference vegetation index (NDVI) | | P | Red, NIR | Tucker (1979) |
| | | Canopy chlorophyll content index (CCCI) | | | 720 and 790 nm | Barnes et al. (2000) |
| | Carotenoids | Green atmospherically resistant vegetation index (GARI) | Chlorophyll concentration, rate of photosynthesis | P/I | 550 and 860 nm | Gitelson et al. (2006) |
| Non-pigment constituents | Cellulose | Cellulose absorption index (CAI) | Bioenergy potential. | P | 2100 nm | Daughtry (2001); Kokaly et al. (2009) |
| | Nitrogen | NDVI & CCCI | Plant nitrogen status, especially under stress | P | 670, 720, 790 nm<br>670 and 770 nm; 590 and 880 nm | Tilling et al. (2007)<br>Bronson et al. (2011) |
| | Lignin | Cellulose absorption bands | Stress responses. Bioenergy potential. | P | | Kokaly et al. (2009) |
| Photosynthesis | Photosystem II activity | Photochemical reflectance index (PRI) | Diurnal radiation use efficiency | P | 531 and 570 nm | Gamon et al. (1997) |
| | Photosystem II activity | Chlorophyll fluorescence | Stress effects on photosynthesis | P/I | | Baker and Rosenqvist (2004) |
| Water relations | Transpiration or canopy conductance | Canopy temperature (CT)<br>Crop water stress index (CWSI) | Instantaneous transpiration and hence crop water status. | P/I | Thermal IR | Jackson et al. (1981); Blum et al. (1982); Wanjura et al. (1984); Chaudhuri et al. (1986) |
| | | Normalized water index (NWI) | Crop water status | P | 850, 900 and 970 nm | Babar et al. (2006c); Gutierrez et al. (2010) |
| | Canopy water content | Normalized difference water index (NDWI) | Crop water status | P | 860 and 1240 nm | Gao (1996) |
| | Water content | Leaf water thickness (LWT) | | P | 1300 nm and 1450 nm<br>1500–1700 nm | Seelig et al. (2008)<br>Li et al. (2001) |
| Plant growth | Leaf area index | NDVI | Overall growth | P | Red, NIR | Babar et al. (2006a) |
| | Plant biomass | NDVI | Overall growth | P | 590 and 880 nm; 670 and 770 nm | Bronson et al. (2011) |
| | | NWI | Overall growth | P | 850, 880, 920 and 970 nm | Prasad et al. (2009) |
| Plant architecture | Canopy height | Close-range photogrammetry | Light interception, overall growth, lodging resistance | I | Visible or NIR | Biskup et al. (2007); Frasson and Krajewski (2010) |
| | | Ultrasonic | Canopy height and width | P | (Ultrasonic) | Ruixiu et al. (1989) |
| | | Depth camera | Canopy height and width; leaf orientation and size | I | Infrared | Chéné et al. (2012) |
| Phenology | Maturity | Time series of index | Tracking leaf senescence | I | Green, red | Idso et al. (1980) |
| | | Time series of fluorescence | Anthocyanin levels | P | | Ghozlen et al. (2010) |
| | Flower number | Image analysis | Plant development | I | Visible | Adamsen et al. (2000); Thorp and Dierig (2011) |
| | Multiple stages | Analysis of time series of indices | Seedling emergence, onset of grain-filling, senescence | P + I | 400–900 nm | Viña et al. (2004) |

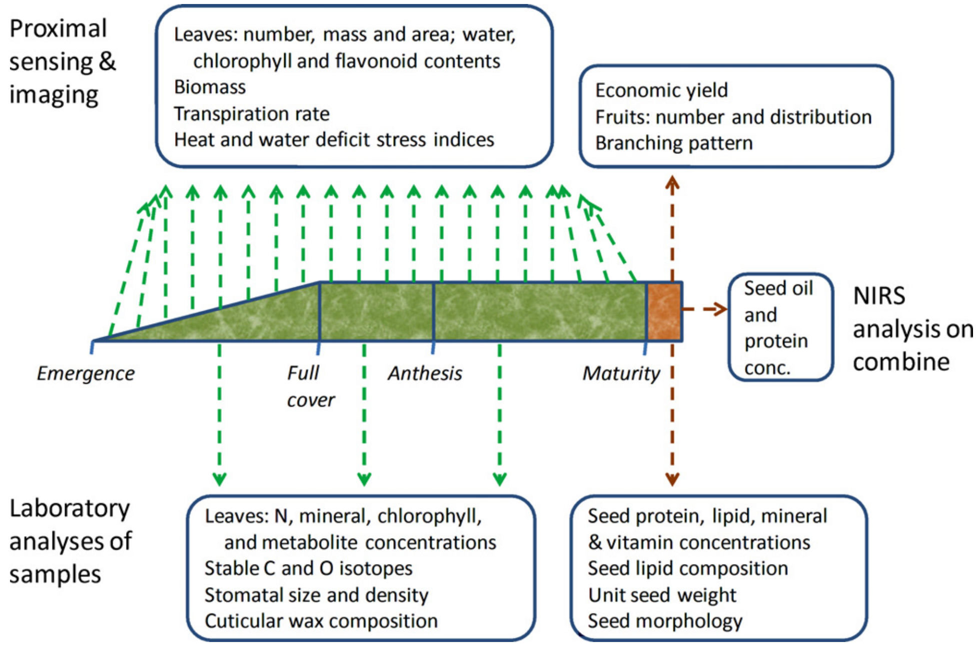


**Fig. 2.** Diagram of possible flows of data in relation to traits measured over the life-cycle of an annual seed crop. Types of data acquisition include: proximal sensing and imaging at frequent intervals, laboratory analyses of samples taken at specific intervals, and near-infrared spectroscopy (NIRS) of seed for oil or protein content during combine harvesting.

reduced. For many crops, close-range field access may permit positioning of sensors or sources of illumination at the base or side of the canopy, allowing measurement of transmittance rather than reflectance (Fig. 3).

Canopy reflectance from visible to near infrared is measured at either a few selected wavelengths (i.e., multi-spectral; Bronson et al., 2003) or as hyper-spectral data (1–2 nm bandwidths ranging from 270 to 1100 nm; K.R. Thorp, personal communication). Reflectance data are often used to calculate an index, such as the normalized difference vegetative index (NDVI). Tucker (1979) was one of the first to propose red NDVI as $(R_{NIR} - R_{red})/(R_{NIR} + R_{red})$, where $R_{NIR}$ and $R_{red}$ are reflectance in the near infrared (NIR) and red regions, respectively. Hyperspectral reflectance data have been subject to principal components regression in order to estimate plant parameters such as cotton lint yield, biomass and plant nitrogen content (Bronson et al., 2005). K.R. Thorp (personal communication) combined hyperspectral data with radiative transfer and crop simulation models to estimate wheat yield, leaf area index (LAI), biomass and nitrogen content.

Because sensor and imaging technologies are evolving rapidly, an initial research objective should be to assemble and evaluate a suite of sensors and imaging tools that compare well-established and novel instruments. Testing should initially focus on the biological utility of data, requiring “ground truth” comparisons with conventional field (e.g., hand-held instruments) or laboratory measurements. It is also essential to develop well-defined, readily implemented methods of standardization to ensure reliability of data collection from multiple instruments located at different geographical sites. For dynamic plant responses such as canopy temperature or leaf angle, the appropriate temporal scale and time

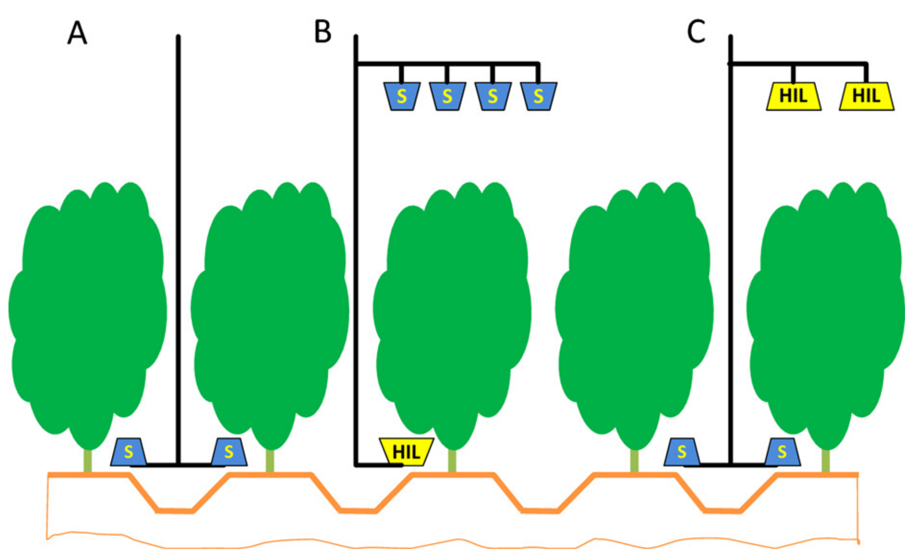


**Fig. 3.** Examples of possible locations of sensors or cameras (S) and high-intensity illumination (HIL) suspended above or below the crop canopy to measure transmittance and thus infer light interception or canopy architecture at specific wavelengths.

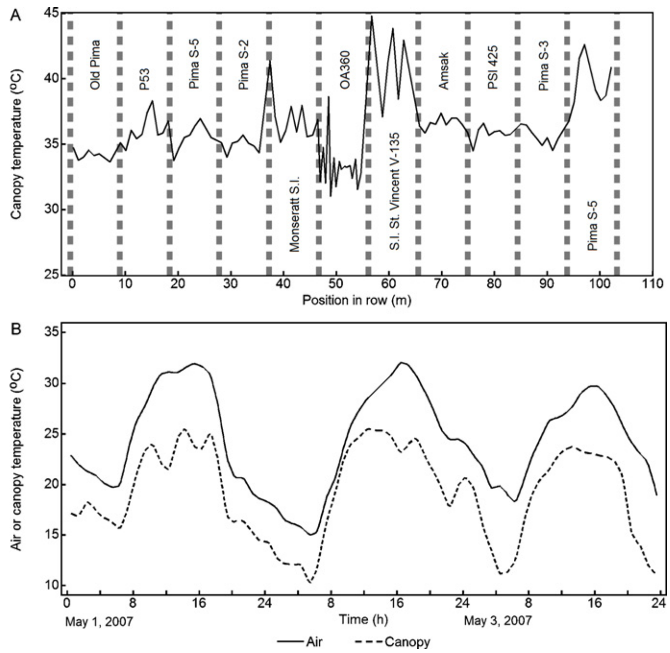


**Fig. 4.** Variation in canopy temperatures recorded at different temporal scales. (A) Temperatures measured at 10:11 AM along a 104 m long plot containing 11 Pima cotton lines. Vertical lines indicate positions of 0.9 m-wide walkways. (B) Canopy and air temperatures measured over 3 days on a well-watered plot of the spring wheat Yecora Rojo.

of measurement should be carefully evaluated. For instance, canopy temperatures recorded as a sensor passes along a series of plots might reveal important plant-to-plant variation at a sub-second time scale, whereas temperature variation over a diurnal cycle would largely reflect effects of daytime transpiration vs. nighttime radiative cooling.

As a specific example of the challenges in acquiring data, we present the case of canopy temperature measured at different spatial and temporal scales. Canopy temperatures of Pima cotton (*Gossypium barbadense* L.) plants measured at 10:00 AM along a 100 m row (transect) containing 11 plots of different cotton lines (Fig. 4A) showed expected differences in mean canopy temperature among the cultivars (e.g., cv. Old Pima vs. P53). Less expectedly, it also revealed differences that were likely related to variation in plant stand and soil texture. The higher and more variable temperatures for cv. S.I. St. Vincent V-135 were associated with the lower stand density (2 plants $m^{-2}$) compared to 7 plants $m^{-2}$ for the other plots, suggesting that the IRT registered emissions from soil as well as plant canopy. Temperatures for the plot of Pima S-5 centered at 99 m along the transect were warmer and more variable than for the plot at 23 m. Comparison of plot positions with soil texture (from a geospatial analysis) indicated that the plot at 99 m was on a sandier soil with lower water holding capacity. These measurements were taken at mid-morning when canopy temperatures were rising with air temperature and solar radiation. Measurements at other times of day will reflect different balances of ecophysiological processes and thus may provide information on different plant processes or traits. Thus, canopy temperatures measured from a well-watered plot of wheat (*Triticum aestivum* L.) over 72 h showed large variations in the difference between canopy and air temperature (Fig. 4B).

Additional concerns for sensor testing include how the field of view, vertical and horizontal position, and speed of travel affect measurements. Kimes (1981) demonstrated that inverse modeling of IRT measurements obtained with multiple view angles can improve estimation of the effective canopy temperature. Another

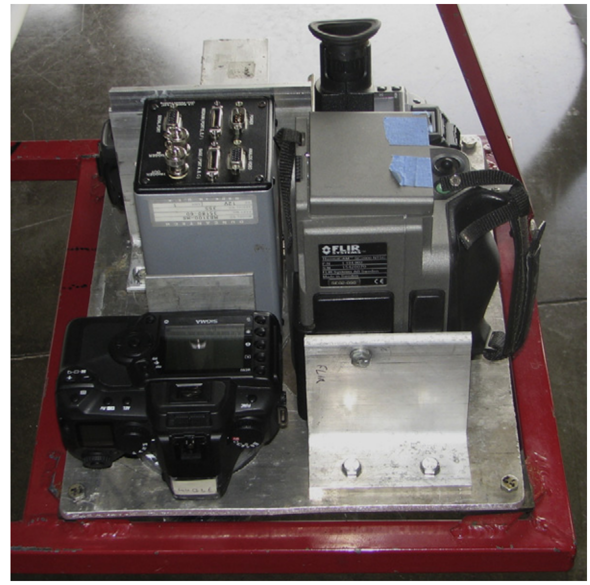

**Fig. 5.** Helicopter-mounted integrated instrument system carrying three types of cameras: multispectral, infrared and conventional digital for visible light. Note the backplate of the multispectral imager (upper left) which has seven connectors, four of which require connection during field use.

criterion is how sensitive the instruments are to environmental conditions, especially temperature, humidity and dust. The overall goals for sensor research would not be to only identify promising initial sets of sensors but to develop testing protocols that help accelerate the adoption and effective use of future instruments.

### *2.2. Systems for integrating instruments*

Electronic instruments require robust but flexible physical mounting, electrical power, connections to data loggers and in some cases, controlling software. The Duncan Technologies MS3100 imager, as deployed on our helicopter instrument system, is illustrative (Fig. 5). The camera required four cable connections for field use: one for control, two for data transfer and one for electrical power.

To link observations to individual plots, plants or positions within plants, position data (coordinates) from on-board real time kinematic (RTK) GPS with 2-cm or better accuracy need to be linked to the data acquired from instruments. The electrical supply should support artificial illumination for active sensing and/or night operation, stepper motors used to vary instrument view angles, temperature controllers, robotic devices (e.g., calibration targets, shade screens and samplers) and data logging or transmission.

Data acquisition per se involves integrating the data from multiple instruments, typically with individual, proprietary communication protocols. Digital memory devices may prove either too slow or too sensitive to vibration. Sensor outputs often require conversion to compatible digital formats (e.g., millivolt outputs from infrared thermometers and photodiodes). The logging system must also provide reliable mechanisms to transfer data.

### *2.3. Vehicles for positioning instruments*

FBP requires a means to rapidly and accurately position instruments over field plots, or even individual plants. Vehicle options include high-clearance tractors (Schleicher et al., 2003; Andrade-Sanchez et al., 2012), linear-move or central-pivot irrigation systems (Kostrzewski et al., 2003; Colaizzi et al., 2003; Haberland

**Table 3**
Comparison of five vehicle options for field-based phenomics.

| Criteria | High-clearance tractor | Crane or linear move | Cable robot | Helicopter[a] | Aerostat[b] |
|---|---|---|---|---|---|
| Maximum payload | 200 kg | Over 500 kg | 100 kg | 400 kg | 10 kg |
| Portable | Yes | Limited | No | Yes | Limited |
| All weather operation | No | Yes | Yes | No | No |
| Potential for damage to plants, soil compaction, or transmission of pathogens | Yes | No | No | No | No |
| Type of operator required | Driver | Technician | Technician | Pilot plus assistant | Technician |
| Power supply | On-board | On-board or cable | Battery or cable | Battery | Battery or cable |
| Random access to field positions, with start/stop | No | Yes | Yes | Yes[c] | Yes |
| Potential for high-frequency vibration | Yes | No | No | Yes | No |
| Minimum sensor distance to canopy | 10 cm | 10 cm | 10 cm | 160 m[c] | 1 m |
| Maximum vertical clearance | 2 m | 5 m | 5 m | > 100 m | > 10 m |
| Can support sensors spaced for multiple rows | Yes | Yes | Weight limited | No | Weight limited |
| Based on well-established engineering | Yes | Yes | No | Yes | Yes |
| Base cost (exclusive of fuel, operators, maintenance, etc.) | $100,000 | ? | ? | $1,000 per 1 h flight | $3,000 |

[a] Based on contracted research flights at Maricopa, AZ and assumes a Hiller UH-12E helicopter.
[b] Based on a 6 $m^3$ capacity with helium as the lifting gas.
[c] US FAA regulations limit helicopter flights to a minimum ceiling of 160 m (500 ft) in rural areas.

et al., 2010), manned fixed- and rotary-wing aircraft (French et al., 2007), unmanned aircraft (Hunt et al., 2005; Hakala et al., 2010) and tethered aerostats (Jensen et al., 2007; Ritchie et al., 2008). Each option has strengths and weaknesses. For instance, tractors offer close proximity to plants but can compact the soil, damage leaves and stems, and propagate diseases and pests. Aircraft can cover large areas rapidly but cannot be used in inclement weather, have high operating costs and may not permit adequate resolution. Five vehicle options are discussed further below, with various criteria tabulated in Table 3.

#### 2.3.1. *High-clearance tractors*

High-clearance tractors are readily available, reliable and transportable. However, even with wheels powered by electric or hydraulic drive, their vertical clearance and maneuverability have limits, and they may not be able to enter fields after irrigation or precipitation events. The requirement for an experienced operator increases the cost of operation, especially for studies that require continuous measurements over 24 h or longer periods. Nevertheless, because of their availability and ease of use, high-clearance tractors will likely play a central role in FBP.

There also is scope for developing lighter-weight, unmanned field vehicles. For research fields where vehicle traffic can be restricted to designated strips or berms, lower-clearance vehicles equipped with cantilever booms that extend over plots can be used.

#### 2.3.2. *Crane-like vehicles*

Numerous configurations of crane-like vehicles can be envisaged, but most options appear constrained by the cost of spanning distances of 50 m or more. A modified linear-move irrigation system appears to be the only system that has been deployed over large field areas (Haberland et al., 2010). At the Maricopa Agricultural Center (MAC), a linear-move was adapted to carry reflectance and infrared sensors mounted on a trolley that ran parallel to the main span, which measured 100 m and traveled the length of a 100 m-long field. The system, called the Agricultural Irrigation Information System (AgIIS, pronounced “Ag Eyes”), was used to characterize water and nitrogen stress for plots within a field of a single cotton cultivar. AgIIS obtained multi-spectral data for vegetation, nutrient and water status indices at a spatial resolution of 1 m (Kostrzewski et al., 2003; Colaizzi et al., 2003; Haberland et al., 2010). The linear-move system retained its capabilities for irrigation and was also used to apply fertilizers and pesticides. Discussions with vendors of linear moves indicate that the speed, control and positional accuracy can be improved through use of variable speed motors coupled with RTK GPS.

#### 2.3.3. *Cable robots*

Cable-suspended robots, pioneered at the National Institute of Standards and Technology (NIST) in the 1980s (Albus et al., 1993), provide an option for a vehicle that can operate continuously over a field without physical contact of the vehicle with the soil or plants. An integrated set of instruments would be suspended from cables supported by towers at the outside corners of the field. Position within the field would be controlled by cable winches and software. Cable robots are widely used for positioning cameras in the entertainment industry (e.g., for filming sporting events and action movies). Tests with a manually operated 1:17 scale prototype demonstrated the feasibility of the approach for large land areas but highlighted the difficulty of maintaining a constant instrument height, especially if sensors must remain level (White and Bostelman, 2011). However, alternative cabling configurations can reduce these problems. Besides refining the basic design, safety and maintenance are also concerns for continuous operation under field conditions.

#### 2.3.4. *Helicopters*

Manned helicopters represent a mature technology capable of carrying a large payload and supplying power to an instrument system. The foremost constraint is not being able to hover close to the crop due to rotor downwash and regulations on minimum safe altitudes. Thus, to resolve plots, only imaging is appropriate. For a helicopter flying at a height of 150 m, our experience suggests resolutions of 10–50 cm are feasible. Cost of operation is also high. In Arizona, helicopter rentals were roughly US$1000 per hour.

Unmanned helicopters are a promising alternative to manned aircraft. They allow flying at much lower altitudes and cost far less to operate (Berni et al., 2009; Merz and Chapman, 2011). The system of Merz and Chapman (2011) carried a 2.1 kg payload for a 30 min flight. Zarco-Tejada et al. (2009) imaged 0.6 ha of citrus orchards using hyperspectral and infrared cameras. There is uncertainty over how regulations will affect unmanned flights. Current US regulations require that flights for experimental and commercial purposes have Federal Aviation Authority (FAA) approval (FAA, 2012). However, tethered vehicles that maintain a height less than 45 m appear not to require approval (Pratt et al., 2008), and a commercial tethered, quad-rotor platform reportedly can maintain a 20 kg payload aloft indefinitely, receiving electrical power via its tether (Israel Aerospace Industries Ltd., 2002).

#### 2.3.5. *Aerostats*

Helium-filled tethered aerostats (balloons) are available commercially for surveillance applications. An aerostat measuring 2 m in diameter by 1.8 m high can carry a 2 kg payload (Aerial Products, 2008). Concerns with aerostats include accurate positioning and orientation, especially under windy conditions, and providing safe storage when not in use. Jensen et al. (2007) described use of a 1.8 m aerostat to monitor response of wheat to nitrogen using color and near-infrared images acquired with digital cameras. Ritchie et al. (2008) used a similar two-camera system to monitor NDVI in a cotton irrigation study. In the USA, tethered balloons are also subject to FAA regulation (FAA, 2012).

### *2.4. High-throughput analysis of plant samples*

Proximal sensing is best suited to characterize traits that are directly linked to externally detectable features of the crop or to traits that can be estimated indirectly from statistical or ecophysiological modeling. However, situations likely will arise where plant tissues will be sampled in the field and then taken to a laboratory for chemical, anatomical or other analysis. Robotic stations adapted for grinding, weighing and analysis of plant tissue (Gomez et al., 2010; Santoro et al., 2010) facilitate high throughput for chemical analyses. Measurements of leaf traits such as leaf venation pattern or stomatal density may require robotic sample preparation combined with image analysis. Perhaps the biggest challenge for field sampling of plant tissue is how to obtain, identify and conserve the samples prior to analysis.

The harvest per se requires automation through use of small plot yield monitors. Combine-mounted near-infrared spectroscopy can measure protein or oil content of seed (Long et al., 2008). Automated systems for subsampling harvested material for subsequent analyses would facilitate further characterizations.

### *2.5. Management of data flow and analysis*

After data are logged and transferred to a computing facility, subsequent processing and management present further challenges. Data should be processed rapidly to check for errors and to guide subsequent measurements, plant sampling or pollinations. Further analyses can enhance the information value of the measurements (Fig. 6). Basic post-processing might involve extraction of values for traits such as canopy structure (row height and width) or calculation of spectral indices. Reflectance-based indices such as NDVI may be converted to LAI values through simple equations (Wiegand et al., 1992).

Analyzing time series data from crop research is challenging because many observed traits are autocorrelated and integrate the effects of multiple, underlying physiological processes that operate at different time scales. Mixed model analysis can account for autocorrelation (Piepho et al., 2004) but may require that plots be sampled using common, fixed time intervals. Moreover, the anticipated large numbers of plots and samples may require exceptional computational resources. Conventional analyses of time series also ignore knowledge of underlying physiological processes. Zhao et al. (2004) proposed a method for estimating quantitative trait loci (QTL) for parameters of the Richards growth curve.

A more efficient approach may be to model time series data as a function of known ecophysiological responses using simulation. Through "inverse modeling", cultivar-specific parameters such as for photoperiod sensitivity, root–shoot portioning or representative unit grain weight would be estimated through comparison of simulation outputs with "measured" data, where the measured data are estimated from proximal sensing. The model parameters should represent more biologically fundamental and meaningful traits (White and Hoogenboom, 1996; Messina et al., 2006; Reymond et al., 2003; Baret et al., 2007). Because the modeling process partially filters out effects of environment and management, such traits should have relatively high heritabilities and strong associations with genetic loci or molecular markers (Bertin et al., 2010). Incorporation of simulation modeling in the research process can facilitate estimation of performance landscapes and integration with tools such as QU-GENE that simulate plant breeding options (Podlich and Cooper, 1998; Messina et al., 2011).

Image analysis can improve separation of background (usually soil) from the plant canopy and open myriad options for quantifying plant architecture (Biskup et al., 2007), flower or fruit numbers (Adamsen et al., 2000; Thorp and Dierig, 2011) and other traits. The iPlant Collaborative's PhytoBisque project seeks to provide a high-throughput platform for image analysis via the iPlant Discovery Environment (Goff et al., 2011).

Considering the scenario for the maize NAM populations (Table 1), the quantity of data to be managed requires careful review in terms of storage and processing requirements. A single pass of 20,000 plots with four 10 mega-pixel images per plot (e.g., three in the visible spectrum to infer canopy architecture and one thermal infrared image for canopy temperature) stored with a 1:10 data compression would generate 80 GB of data. For routine crop improvement, researchers might only store data on derived traits but for more basic research, the entire dataset would be archived. If file transfer, merging and processing each set of images required 10 s $plot^{-1}$, the net processing time for all plots would be approximately 60 h.

Data representation and communication standards have recognized value in promoting efficient analysis of large datasets, but there is a staggering array of proposed standards (Brazma et al., 2006). Examples potentially relevant to FBP include the International Consortium for Agricultural Systems Applications standard for describing field experiments (Hunt et al., 2001), the Minimum Information About a Microarray Experiment standard (MIAME; Brazma et al., 2001) and the NetCDF standard developed for remote sensing datasets (Rew et al., 1997). We expect that given the breadth of data acquired in FTP that elements of multiple standards should be incorporated.

### *2.6. Integrated management of FBP*

Converting a prototype FBP system into an efficient tool for screening thousands of plots is far more complex than simply deploying prototype vehicles with sensors and data loggers. Trained personnel are required to operate and maintain the vehicles, instruments and software. Standard operating procedures are needed to ensure reliable performance throughout an experiment, including crop management, instrument calibration, data transfer and initial analysis, and vehicle maintenance.

Field management per se should seek to minimize or control within-field sources of variation. This can involve characterizing fields for variability in soil texture and at the onset of each experiment, characterizing within field variability in initial soil nitrogen and moisture. Drip or low pressure aerial irrigation can minimize variability from irrigations. In-field portable weather stations capable of recording solar radiation, air temperature and humidity, wind speed and direction, and precipitation are required to analyze crop performance in relation to growing season conditions. Vehicle-mounted solar radiation, humidity and air temperature sensors may be required for optimal analysis of IRT data.

## 3. Challenges

The challenges for plant science to enable requisite increases in crop productivity while conserving the natural resource base for agriculture are daunting, but there are promising avenues to greatly enhance our capabilities to exploit genetic diversity through integration of phenomics with genomics. By

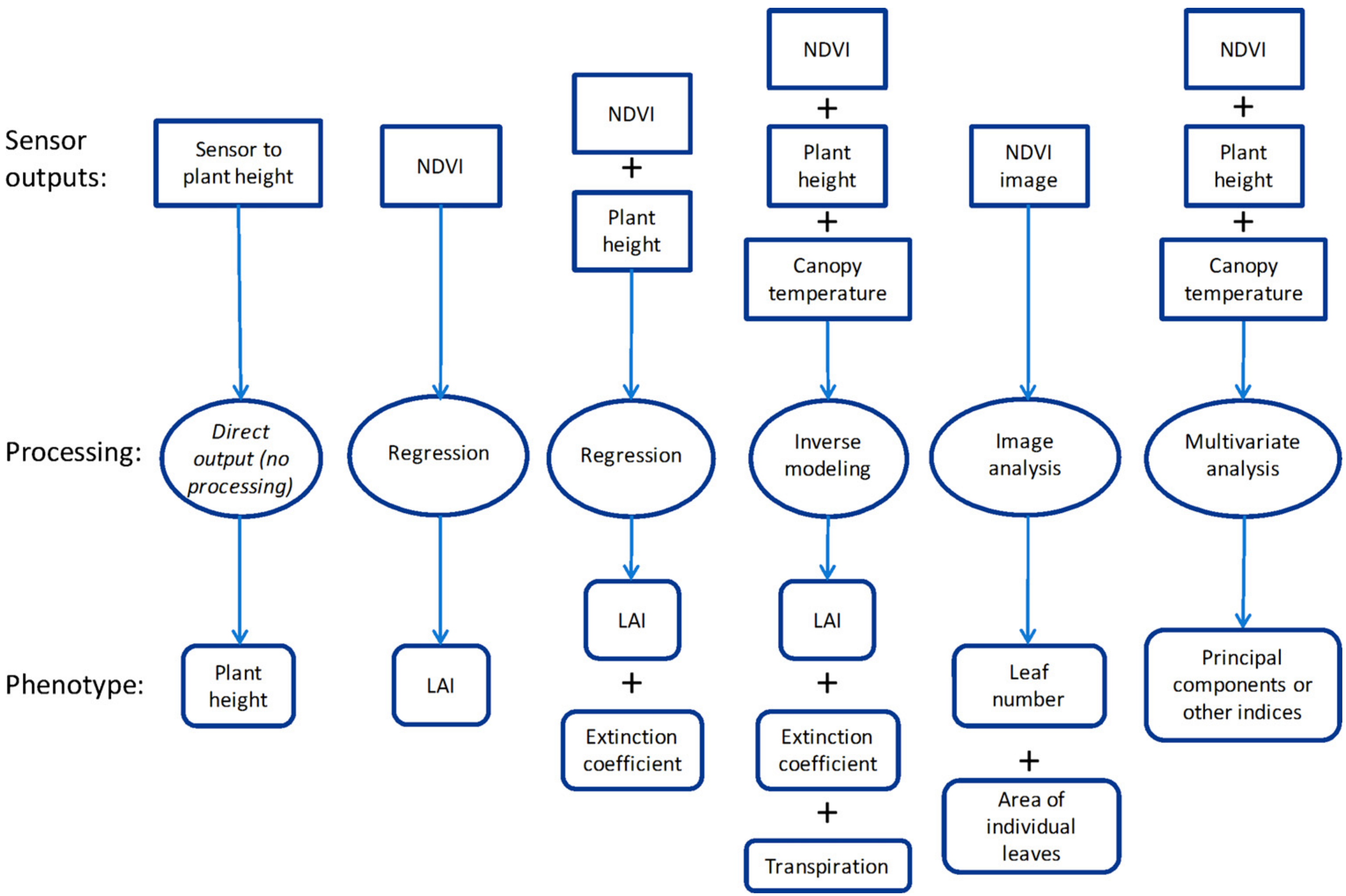


**Fig. 6.** Examples of possible paths of data analysis whereby field measurements are processed to provide more biologically meaningful data. Field data usually would be recorded as time series, allowing estimation of growth or developmental rates.

providing powerful new capabilities for phenotyping large numbers of field plots, there is a clear research path that can transform FBP. While not all of the components of an FBP system are in place, the research problems appear tractable, having numerous potential solutions. However, due to the need for highly integrative approaches, efforts to advance FBP cannot rely solely on individual researchers or small groups iteratively pursuing local solutions. Attaining the phenotyping capability that will allow agriculture to address climate change, food security, and bioenergy requires coordinated and sustained efforts with adequate resources to test and develop the necessary infrastructure and procedures. Recently established Australian and European phenomics centers (e.g., http://www.plantphenomics.org.au and http://www2.fz-juelich.de/icg/icg-3/jppc) are indicative of the growing interest in high-throughput technologies and of the potential for developing the relatively large, integrated research infrastructure required for FBP.

Throughout this discussion we have avoided emphasizing specific traits. This reflects our conviction that searching for single indices that correlate strongly with yield is unlikely to provide more information than simply analyzing yield differences. A given yield level is often attainable through multiple mechanisms, and the optimal combination of traits for one environment often differs from that required in another. The challenge of phenotyping is to provide data on those underlying mechanisms.

Our FBP experiences to date suggest priority research needs. Foremost is effectively managing the data streams, starting with field acquisition and ending with genetic analysis or application in plant breeding. Accumulating large volumes of unprocessed data is remarkably easy. However, simply combining data from different sensors and from a GPS receiver can require extensive custom processing with a geographic information system (GIS) and other software tools. A second priority is to develop protocols for testing promising instruments. While demonstrating that a given instrument provides potentially useful descriptions on plant traits, the demanding conditions for FBP require testing for features including ease of calibration, temperature stability and ease of integration in the overall data stream. Better algorithms for analyzing proximal sensing data are also needed. Inverse modeling appears especially promising but to date has seen limited application. Implicit in the efforts to integrate the FBP components lies the challenge of maintaining balance among numerous potentially exciting lines of research.

In modern crop improvement, management of intellectual property is a continuous concern. There are surprisingly broad US patents relating to methods for inferring plant characteristics. US patent 5,764,819 awarded to Orr et al. (1998) describes “methods for classifying plants for evaluation and breeding programs by use of remote sensing and image analysis technology.” While patents can allow inventors to recover their research investments through royalties, Heller (2008) emphasized that patenting component technologies can inhibit innovation in systems that require assembling multiple components, such as the instruments and software required for FBP. The basic principles and applications of proximal sensing appear to be well-established and to lack the novelty required to justify patents. Innovation is needed, however, in design of specific instruments. If sufficiently novel, these might justify application for patents. Outputs from specific instruments often are in proprietary formats, which also complicate integration of components. Obviously, for public research, instruments are preferred whose outputs and controls are readily accessed without use of additional proprietary hardware or software, and an overall philosophy of open architectures and software using “off the shelf” electronic components will help stimulate collaborative development. The Cubesat program, which promotes development of low cost satellites that conform to an open design standard (Woellert et al., 2010), may provide a useful model.

Advances in instruments, computers, software or other components will undoubtedly impact FBP in the coming 10–20 years. Readily predicted trends include decreased costs of hyperspectral sensors, increased resolution of imaging devices, greater capacity for data storage, faster processing in computers and improved algorithms for image analysis. The impact of novel instruments is less easily anticipated. A recent example is the infrared time-of-flight camera developed by PrimeSense (2012). The single chip system measures the distance to the target with a resolution of 640 by 480 pixels. Although primarily sold in the Microsoft Kinect video game controller, the system is also marketed to software developers for other platforms and applications. Chéné et al. (2012), in what appears to be the first published application with plants, showed that this distance camera can resolve individual leaves, allowing automated measurement of leaf orientation.

Tools developed in FBP should also find applications in other fields including crop and range management, weed science, plant pathology, and insect pest management. In principle, these disciplines all seek to describe plant phenotypes. FBP differs primarily in its emphasis on characterizing the large numbers of plots required for genetic studies and crop improvement. Many of the techniques that show progress for FBP were developed for nitrogen or water management (e.g., Pinter et al., 1979; Jackson et al., 1981), and there are numerous other examples of remote and proximal sensing in agriculture (e.g., Nilsson, 1991, 1995; Riley, 1989; Chaerle et al., 2006; Oerke et al., 2006).

## 4. Conclusion

Advances in crop improvement were largely responsible for the first green revolution, which doubled crop yields in less than 50 years. If yields are to double again over the next 50 years, crop improvement must achieve unprecedented increases in productivity and resource use efficiency. Next generation genotyping tools for characterizing sequence variation appear capable of providing the requisite throughput and resolution. However, modern phenotyping technology currently lags that of genotyping. The FBP approach described here appears capable of attaining the requisite high levels of throughput. Reflecting the complex, dynamic nature of plant responses to the environment, FBP requires integrative, interdisciplinary teamwork and meticulous attention to quality control at all stages, starting with field preparation and experimental design, followed by timely processing and analysis of data, and ending with direct application toward finding solutions to major problems currently limiting crop production.

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