HIGH THROUGHPUT PHENOTYPING IN PLANTS

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Global food demand is increasing rapidly. Several studies have shown that global crop production needs to double by 2050 to meet the projected demands from rising population. 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%.Development of improved varieties relies on both molecular approach (DNA sequencing or genotyping) and accurate quantification of plant traits (phenotyping) to identify useful genes/QTLs. Despite recent advances in genomics a lack of suitable phenotypic data has led to poor results in gene/ QTLs discovery, ultimately limiting the progress of genomic assisted crop improvement. Therefore, acquisition of high throughput, effective and comprehensive trait data needed to understand the genetic contribution to phenotypic variation has become an acute need.

High-throughput image-based plant phenotyping facilitates the computation of phenotypes by analyzing a large number of plants in short time interval with precision, nullifying the need for time-consuming physical human labour. The process is generally non-destructive. It allows the same traits to be quantified repeatedly at multiple times and scales during a plant's life cycle. It is an interdisciplinary research field involving computer science, biology, remote sensing, statistics, and genomics in the effort to link intricate plant phenotypes to genetic expression in order to ensure future food security under dwindling natural resources and projected climate variability and change.

In recent years, there has been increased interest in high-throughput phenotyping platforms (HTPPs). Most HTPPs are operated by the big transnational seed companies and the most advanced public plant research institutions around the world, such as the Australian Plant Phenomics Facility, the European Plant Phenotyping Network and the USDA. They have 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 starting to be developed.

High throughput phenotyping can be divided into three different approach

i) Technology to collect imaging or other remote sensing data

ii) Computational data approach to convert the image data into numerical phenotypes

iii) Use of numerical phenotypes to map genes or QTL to built genomic prediction model and to integrate them in breeding program

Environmental monitoring

Earlier HTPP initiatives have largely focused on phenotyping and little emphasis has been placed on environmental monitoring and reducing error variances. The environment plays a crucial role in plant phenomics since most of the important traits in plants are quantitative in nature and highly influenced by environmental factors. Further, plant breeders aim to develop crop varieties with good buffering and stability that perform well under different environmental conditions. Therefore, appropriate documentation of the experimental environmental conditions (e.g. rainfall, temperature, photoperiod and soil characteristics) is essential for any crop phenomics strategy. Even under controlled environment phenotyping, detailed environmental metadata is preferred. New technologies allow the systematic collection and integration of meteorological data at different spatio-temporal scales. Moreover, the low cost of these sensors permits the deployment of many of them in the field, enabling detailed characterization of the effects of fluctuations in the environment. Certainly controlled environments (including growth chambers, walk-in rooms, and research greenhouses) can provide high quality and highly reproducible data. Plant breeders and agronomists usually prefer field phenotyping especially for complex traits like yield and drought tolerance. On the other hand, phenotyping under controlled conditions can provide a better understanding of trait response to stress factors that cannot be reliably controlled or are difficult to measure in the field.

Plant phenotyping platforms

The area of plant phenotyping is continuously progressing, with invasive, low throughput phenotyping methods being replaced by high-throughput, non-destructive methods. Rapid developments in non-destructive inexpensive sensors and imaging techniques over the last decade have revolutionized crop phenomics. Current implementations of non-destructive high-throughput phenotyping platforms include the use of sophisticated technologies such as: i) infrared thermography and imagery to scan temperature profiles/transpiration; ii) fluorescent microscopy/spectroscopy to assess photosynthetic rates; iii) 3D reconstruction to assess plant growth rate and structure; iv) light detection and ranging (LIDAR) to measure growth rates; v) magnetic resonance imaging and positron emission tomography to measure growth patterns, root/leaf physiology, water relations, and/or assimilate translocation properties; vi) canopy spectral reflectance for monitoring dynamic complex traits; vii) nuclear magnetic resonance for monitoring the structure of tissues, mapping water movements, and monitoring sucrose allocation; and viii) digital RGB imaging for recording data on various attributes of roots, shoots, leaves, seeds, and grains.

A variety of high-throughput phenotyping platforms exist and are currently used to phenotype different traits in almost all crop species. Some examples of these software /platforms include the Microsoft Excel-based macro, a tool called “LEAF-E” developed for analysing leaf growth parameters, “Zeppelin NT aircraft” as an experimental sensor platform used for remote aerial phenotyping “Phenovator” and “GROWSCREEN FLUORO” for phenotyping large samples for photosynthesis and growth, and “TRiP (Tracking Rhythms in Plants)” for determining circadian period.

Traits for phenotyping

The variety of high-throughput phenomics methods/platforms that have been developed over the past decade (some discussed above) have been used for phenotyping of a variety of plant traits including growth, phenology, physiology, disease incidence, insect damage, drought tolerance and for recording data on different plant organs such as roots, seeds and shoots. For example using different phenotyping platforms, data has been recorded in high-throughput and automated manner for plant height, leaf growth parameters including leaf area, area of canopies, photosynthesis, photosynthesis efficiency, chlorophyll content, leaf nitrogen content and canopy height in different plant species including wheat, maize, barley, rice, pea, Arabidopsis, potato, canola, and soybean among others.. Similarly, phonological traits like flowering (spikelet anthesis) in rice that is considered one of the most important but difficult to capture phenotypic characteristics and circadian period could be also estimated using high-throughput phenotyping platforms.

High-throughput methods have been also used to study plant responses to various types of abiotic stresses (drought, heat, cold tolerance, salinity, Nitrogen limitation, and UV light. Drought tolerance is considered one of the most important complex quantitative traits and many phenomics approaches have been used to understand the nature of drought tolerance. Different high throughput techniques have been successfully used for drought tolerance. These methods are based on imaging, robotics and computers which allow for automatic measurement of phenotypic traits for thousands of plants in a day in non-destructive manner. For biotic stress like insect pests, phenomics platform based on automated video tracking has been developed such as quantifying aphid feeding behaviour on leaf discs to assess the level of plant resistance. Such automated video tracking platforms can be used to screen large plant populations for resistance to aphids and other piercing-sucking insects in plants, as has been already tested on Arabidopsis and lettuce. Phenotyping for disease resistance in plants by visual assessment and rating is often time consuming and expensive. Therefore high-throughput platforms /methods developed like hyperspectral imaging (HSI) are considered promising non-invasive sensor techniques in order to accelerate and to automate classical phenotyping methods. The use of hyperspectral microscopy to determine spectral changes on the leaf and cellular level in plants during resistance reactions has been used in barley (*Hordeumvulgare*)-powdery mildew interaction studies.

Several high-throughput platforms and computer software programs are available and being used for collecting and analysing root images. Typically, root phenotyping platforms are divided into two main groups: ex situ where samples or the entire root system are evaluated outside of the growth environment, and in situ also called non-invasive evaluation of the entire root system is done non-destructively under the natural growth environment.

Field-based phenotypingsystems

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. 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 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

Remote sensing phenotyping methods are non-destructive, non-invasive approaches 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. 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. 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: (i) VIS-NIR spectroradiometry (ii) infrared thermometry and thermal imaging, and (iii) conventional digital photography (RGB colour cameras). There are several software programs available to process information to extract phenotype data from images.

Cameras for crop monitoring

Different categories of imaging systems used for remote sensing evaluation of vegetation are RGB/CIR cameras, Multispectral cameras, Hyper spectral visible and near-infrared imager, Long-wave infrared cameras or thermal imaging cameras and Conventional digital cameras (NDVI type work).

Integrating high-throughput trait phenotyping with genomics for genetic dissection of traits

To understand the genetic makeup of a complex character, we need both genotypic and phenotypic data of that character. The effectiveness of genetic dissection and reliability of marker-trait associations identified largely depends on the accuracy of trait data. The evolution of high-throughput phenomics platforms during the last decade has revolutionized trait phenotyping by helping in recording data effectively and cost-efficiently. The data generated was used in trait dissection using a variety of approaches like QTL interval mapping, association mapping, candidate gene association studies, genome-wide association studies, QTLseq. and genomic selection leading to discovery of genes/QTLs for important targeted traits. The rice automatic phenotyping platform (RAP) was used for high-throughput screening of recombinant inbred line (RIL) mapping population combined with genome-wide association studies (GWAS) that led to the identification of 141 associated loci, 25 of which were previously known genes such as the Green Revolution semi-dwarf gene, SD1. Thus high-throughput phenotyping has the potential to replace traditional phenotyping techniques and can provide valuable for gene identification.

Conclusion

Phenomics is a field of science at the junction of biology and informatics which solves the problems of rapid, accurate estimation of the plant phenotype. At present, plant phenomics is rapidly developing. It is characterized by wide approbation of new phenotyping methods for the solution of diverse problems and involvement of the newest computer and engineering technologies in the creation of these methods. Field phenotyping of the appropriate traits, using low cost, easy-to-handle tools, should become an integral and key component in the breeding pipeline. 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. The successful development of the methods of automation and computerization will open lot of new things in our understanding of the functioning of the genome and its interrelation with the phenotype and environment.

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