High-Throughput Phenotyping for Crop Improvement

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One of the most effective strategies for boosting the productivity of the main food and feed crops in the world is through breeding and genetic improvements in plants. Nonetheless, the field of crop improvement needs to change to meet the expanding challenges concerning climate change. In order to classify germplasm and experimental populations and find relevant genes or QTLs, modern crop improvement approaches rely on both precise quantification of plant attributes (phenotyping) and DNA sequencing (genotyping). Even with recent improvements in genomics, the development of genomics-assisted crop improvement initiatives has been hindered by inadequate findings in gene/QTL discovery due to inadequate phenotyping data (phenomics data). Thus, there is an urgent need to obtain the high-throughput, efficient, and thorough trait data required to comprehend the genetic contribution to phenotypic variation.

The quick development of phenomics is now necessary to maintain and boost crop yields while utilizing the benefits of modern genetics methods. With the use of contemporary phenomics instruments, hundreds or even thousands of plants can have their characteristics, including growth, biomass, photosynthesis, and architecture, measured in a single day. Large plant populations, breeding material, germplasm collections (core collections), and population mapping could all be screened using high-throughput phenotyping platforms with greater accuracy and precision in the acquisition of phenotypic traits and less labour input thanks to automation, remote control, and data (image) analysis pipelines that support high-throughput. Progress has been already made, but more research is required to fully utilize genomics and molecular breeding tools in crop improvement (Fig. 1).

Networks and programs for plant phenotyping

Plant phenotyping is an ever-evolving field, with high-throughput, non-destructive techniques gradually replacing invasive, low-throughput approaches. Crop phenomics has been transformed in the last ten years by the quick advancement of low-cost, non-destructive sensors and imaging methods. Utilizing advanced technologies, non-destructive high-throughput phenotyping platforms are currently being implemented with the following capabilities:

i) scanning temperature profiles and transpiration using infrared thermography and imagery;

ii) measuring photosynthesis rates using fluorescent microscopy/spectroscopy;

iii) assessing plant growth rate and structure using 3D reconstruction;

iv) measuring growth rates using light detection and ranging (LIDAR);

v) measuring growth patterns, root/leaf physiology, water relations, and/or assimilate translocation properties using magnetic resonance imaging and positron
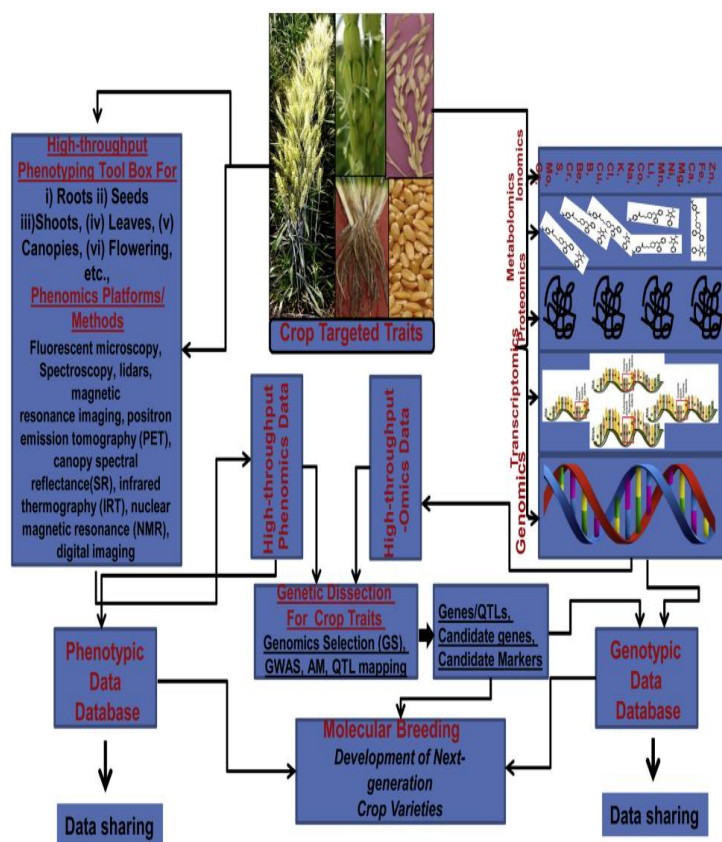

Fig. 1. Holistic view of role of crop phenomics in crop improvement.

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emission tomography and magnetic resonance imaging;

vi) canopy spectral reflectance for monitoring dynamic complex traits;

vii) scanning temperature profiles and transpiration using infrared thermography and imagery;

viii) measuring photosynthesis rates using fluorescent microscopy/spectroscopy;

ix) assessing plant growth rate and structure using 3D reconstruction;

x) measuring growth rates using light detection and ranging (LIDAR);

xi) measuring growth patterns, root/leaf physiology, water relations, and/or assimilate translocation properties using magnetic resonance imaging and positron emission tomography and magnetic resonance imaging;

xii) canopy spectral reflectance for monitoring dynamic complex traits;

xiii) nuclear magnetic resonance for monitoring the structure of tissues, mapping water movements, and monitoring sucrose allocation; and

xiv) digital RGB imaging for recording data on various attributes of roots, shoots, leaves, seeds and grains.

Phenotyping platforms in trait phenotyping: some examples

With the development of various high-throughput phenomics platforms and methods over the last ten years, a wide range of plant traits, including growth, phenology, physiology, disease incidence, insect damage, drought tolerance, and data on various plant organs like roots, seeds, and shoots, have been phenotyped. For instance, high-throughput and automated data collection has been conducted on various phenotyping platforms concerning plant height, leaf growth parameters (leaf area, canopy area, photosynthesis, photosynthesis efficiency, chlorophyll content, leaf nitrogen content, and canopy height) in various plant species, such as wheat, maize, barley, rice, pea, Arabidopsis, potato, canola, and soybean.

Similarly, one of the most significant yet challenging to capture phenological features in rice is flowering, or spikelet anthesis.

Table 1. Details of use of selected phenomics platforms for trait phenotyping in plants.

Platform /Technique/Software	Traits recorded/ Approach used	Crop
Growth traits, phonological traits and physiological traits		
Light Curtain arrays (LCs)	Rapid determination of plant height and leaf area	Maize, barley, rapeseed and tomato
Microsoft Excel-based macro, a tool called "LEAF-E"	Analysing leaf growth parameters	Maize, *Miscanthus* spp. and *Brachypodium distachyon*
Dual-mode microwave resonator	Water content of leaves and the ionic conductivity of the leaf	Potato, maize, canola and wheat
Phenoplant	Chlorophyll fluorescence parameters impacted on leaves by a stress	Arabidopsis
GROWSCREEN FLUORO	Growth and photosynthesis (PSII) efficiency	Arabidopsis
Imaging based methods	Chlorophyll-a fluorescence (ChlF)	Arabidopsis

Reverse phenomics utilizes a suite of new tools applied to a limited set of germplasm to elucidate common strategies responsible for stress tolerance or yield potential. It involves in-depth dissection of physiological traits down to the level of deducing underlying biochemical or biophysical processes. This helps to identify traits like yield components underpinning a superior crop variety through a hypothesis-driven (rather than a descriptive) screening approach. Thus, reverse phenomics can be used, for example, when the phenotype or target trait

is already known and the goal is to determine the mechanism(s) responsible for controlling the trait and identify the responsible gene(s). Reverse phenomics tools and technology are already being used by the Australian Plant Phenomics Facility (http://www.plantphenomics.org.au/) and the Australian National University (http://www.garnetcommunity.org. uk/resources/phenomics). Both forward and reverse phenomics approaches are being used at CIMMYT in wheat to develop advanced lines with complementary physiological traits for drought tolerance as well as yield potential and heat tolerance. In summary, reverse phenomics is used to better understand the trait constellations that contribute to yield. while forward phenomics is used to identify better sources of those traits.

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

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. For instance, QTLs/genes/associated markers have been identified for different traits including root traits, seed traits, morphology, biomass, yield (rice), osmotic tolerance (wheat), boron tolerance (barley), biomass (brassica), plant growth traits, and biomass (in maize). 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.

The combination of the multifunctional phenotyping tools HRPF and GWAS provides deep insights into the genetic architecture of important traits. Similarly, in maize by combining the high-throughput maize phenotyping platform and the high-density linkage map, 988 QTLs including three QTL hotspots were identified that provided the opportunity to investigate QTL distribution at the genome wide level.

Summary

In summary, combining the high-throughput phenotyping technology and large-scale QTL analysis not only greatly expanded our knowledge of the maize dynamic development process but also provided a new strategy for breeders to optimize plant architecture toward ideotype breeding in maize. It is important to mention here that the genetic dissection approaches routinely used for trait dissection like genome-wide association studies (GWAS) and linkage mapping typically use measurements at the whole organ or organismal scale that results in generation of too complex to dissect out the biological processes information. Therefore, use of effective phenotyping data with omics approaches including transcriptomics data that changes as a consequence of climate has offered new perspectives for genetic integration of several levels of molecular regulation of phenotypic trait variation.

Reference

R. R. Mir, 2019. High-throughput phenotyping for crop improvement in the genomics era *Plant Science* 282. 60–72.

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