



High-Throughput Plant Phenomics (HTTP) for Crop Improvement

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Introduction

Improving plant genetics and breeding is one of the best ways to increase the productivity of major food and feed crops worldwide. However, the discipline of crop improvement must evolve to meet the increasing challenges of climate change. Moreover, some predictions suggest that crop yields must approximately double by 2050 to adequately feed an increasing global population. Modern techniques for crop improvement rely on both DNA sequencing (genotyping) and accurate quantification of plant traits (phenotyping) to characterize germplasm and experimental populations to identify useful genes/QTLs. Despite recent advances in genomics, a lack of suitable phenotyping data (phenomics data) has led to poor results in gene/QTL discovery, limiting progress in genomics-assisted crop improvement programs. Therefore, acquisition of high-throughput, effective and comprehensive trait data needed to understand the genetic contribution to phenotypic variation has become an acute need. Sustaining and increasing crop yields with the advantages afforded by modern genetics tools now depends on rapid advancement of phenomics.

More noticeably, sustainable plant productivity to provide sufficient food for the increasing human population has become a thorny issue to scientists in the era of unpredictable global climatic changes, appearance of more tremendous or multiple stresses, and land restriction for cultivation. Thus high-throughput phenotyping platforms allowed screening of large plant populations, germplasm collections (core collections) breeding material and phenotypic trait acquisition coupled with decreased labour input achieved by automation, remote control and data (image) analysis pipelines amenable to high-throughput. To further these efforts, the International Plant Phenomics Initiative was also launched. Progress has been already made, but more research is required to fully utilize genomics and molecular breeding tools in crop improvement. This review article aims to: i) highlight the importance of phenomics and phenotypic constraints in crop improvement in the genomics era, ii) review the status of phenomics platforms and facilities worldwide, iii) highlight the use of high-throughput phenomics platforms for trait dissection in different crop plants and discovery of genes/QTLs for variety of traits in different crop plants, and (iv) highlight the need for sharing of phenomics databases and phenotypic data for crop improvement.

One of the important highlights in this issue is the review of the benefits brought by high-throughput sequencing technology, which is also known as next-generation sequencing

(NGS). It is not so difficult to recognize that its application has allowed us to carry out biological studies at much deeper level and larger scale.

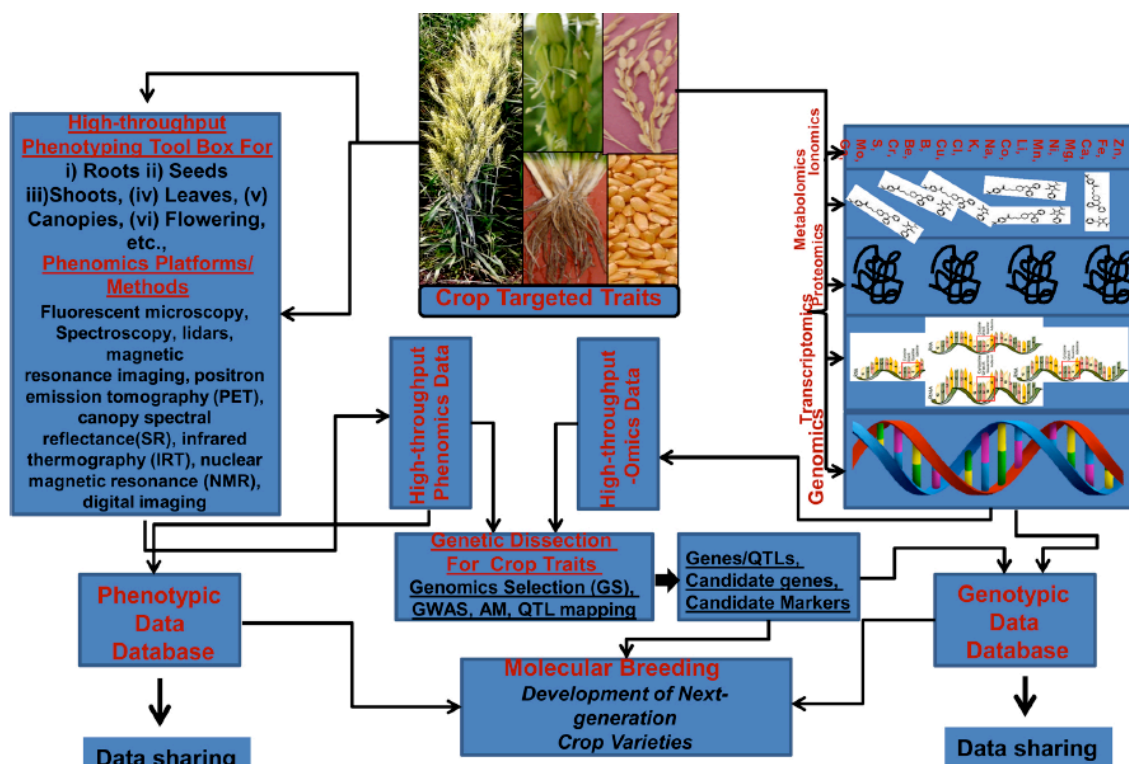


Fig. 1: Holistic view of role of crop phenomics in crop improvement.

The phenomics bottleneck

Unprecedented recent developments in next-generation DNA sequencing technologies have resulted in tremendous progress in unravelling and understanding crop genomes, thus shifting the plant science research bottleneck from genomics to phenomics. This shift has driven the need and efforts for the development of high throughput, non-invasive phenotyping technologies able to capture trait phenotypic data that can be linked to genomics information for crop improvement. Despite these advances, our ability to collect reliable phenotypic data still lags behind the current capacity to generate high-throughput molecular genotyping data resulting in a “phenotypic bottleneck”, which is currently hindering plant breeders from making fast progress.

Effective phenotyping

Effective phenotyping means increasing the accuracy, precision, and throughput of measurements, while reducing costs (cost-efficient/affordable) and minimizing labour through automation, remote sensing, data integration, and experimental design. Cost-efficient phenotyping is developing rapidly due to decreases in cost of equipments such as environmental sensors, and smartphone embedded applications for mobile imaging to capture images and crop performance datasets both under controlled environments and in the field. Too few studies present QTL derived under experimental conditions that are subsequently

also of relevance to crop productivity. For instance, in wheat QTLs/genomic regions have been identified for agronomic and physiological traits in environments encompassing drought, heat, and well-irrigated conditions and efforts have been made in the study to normalize the confounding effects phenology and growth habit.

The phenotyping environment and its control

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. These genetic/environment interactions significantly contribute to the phenotypic variation explained by complex quantitative traits like yield, drought and quality traits like grain protein content. For highly heritable traits such as grain weight, $G \times E$ does not play a major role but main effect genes play major role in controlling grain weight.

Plant phenotyping platforms and initiatives

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;

Phenotyping platforms in trait phenotyping: some examples

The variety of high-throughput phenomics methods/platforms that have been developed over the past decade 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. 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. The approaches range from osmotic balance in hydroponics, to conveyer systems in glass house, to rainout shelters in the field.

Examples of phenomics platforms for trait phenotyping in plants like Growth traits, phenological traits and physiological traits *viz.*, ., Microsoft Excel-based macro, a tool called “LEAF-E”, Light Curtain arrays (LCs), TRiP (Tracking Rhythms in Plants). Biotic and abiotic stresses (drought, heat, cold tolerance, salinity, nutrient-starving, UV light; low N-stress) software’s *viz.*, Hyperspectral imaging (HSI), Hyperspectral absorption-reflectance-transmittance imaging (HyperART) 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

Integrating high-throughput trait phenotyping with genomics

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

Phenotype databases & data sharing:

Advances in analysis of genome sequencing data has benefited greatly due to the publically available genomics databases [11]. Several genomics and proteomics databases are available where DNA sequence and protein sequence data are continuously being stored and shared by researchers world-wide. However, the recording of trait data in highthroughput manner is still not routine and it is important to note that even after collection of effective phenotypic data from hundreds of plants from multiple locations and replications; there is no public repository for the deposition of this raw data. Similarly, crop specific databases have been developed including: (i) Phenome Networks (<http://phnserver.phenomenetworks.com>), and LycoTILL (<http://www.agrobios.it/tilling/index.html>) for tomato, (ii) Oryza Tag Line software (OTL) (<http://urgi.versailles.inra.fr/OryzaTagLine>), Rice Mutant Database software (RMD) (<http://rmd.ncpgr.cn>), Tos17 (<http://pc7080.abr.affrc.go.jp/phenotype>), and OryGenesDB (<http://orygenesdb.cirad.fr/index.html>) for rice, (iii) SCRI Barley Mutants software (<http://bioinf.scri.ac.uk/barley/>) for barley, (iv) Maize GDB software (<http://www.maizegdb.org/rescuemu-phenotype.php>) for maize

Conclusion

In summary, with recent progresses in biological and biotechnological areas, especially rapid development of advanced technologies in biological system modelling, functional genomics, computer-based analysing tools, genetic engineering and molecular



breeding, biological control and biotechnological applications in agriculture have brought about an extraordinary revolution and have been considered the most powerful approaches in maintaining or even increasing crop yield.

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