

High throughput phenotyping and its applications in crop improvement

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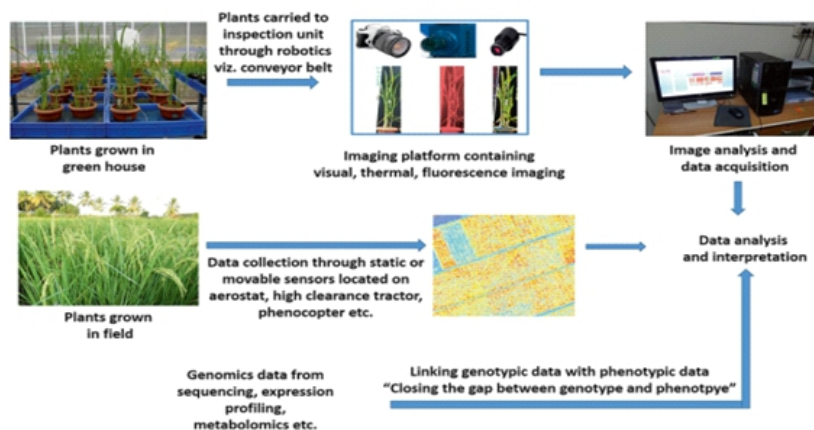


FIGURE 1. Steps involved in High throughput phenotyping (Rahman et al., 2015)

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SUMMARY

The development of high-throughput phenotyping technologies has progressed considerably in the last decade as these technologies provide precise measurements of desired traits among thousands of field-grown plants under diversified environments. Since plant phenotyping is the foundation of crop breeding, high-throughput phenotyping techniques were employed in many crop improvement programs. This allowed breeders to draw on physiological features and mechanistic knowledge to guide their choice of material for crossover and genetic improvement. High-throughput phenotyping techniques and platforms help to unravel the genetic basis of complex traits associated with plant growth and development and targeted traits. The usage of field-based high-throughput approaches will expand with the development of user-friendly data management tools and software for better comprehend phenotyping, which has the potential to revolutionize the breeding strategies and to satisfy future needs.

Introduction

The breeding of high-yielding and stress-tolerant varieties is one of the key challenges in the coming years. Although there is public access to the genomes of many crop plants, there is little known about their phenotypic characteristics since environmental conditions make a significant impact on phenotype. Several morphological, physiological, biochemical, and molecular characteristics can be used to accomplish plant phenotyping as it plays a significant part in crop selection, and choosing the optimal phenotype is the first crucial step in plant breeding (Pieruschka and Schurr 2022).

Phenotype and Phenomics

Phenotype is the combination of genotype and the environment where the plant grows. Phenomics is a way of speeding up phenotyping with the help of high-tech imaging systems and computing power. Phenomics share the advantages of faster evaluation, facilitating a more dynamic who-

-le-of-lifecycle measurement with improved precision, less dependent on periodic destructive assays, and reduced need for replication in the field. It is a comprehensive approach that combines systems biology and statistical correlation. A lot of work has recently been made in creating automated platforms to speed up plant phenotyping *i.e.*, high throughput phenotyping (Kim, 2020).

The field of high-throughput phenotyping (HTP) has undergone a revolution and is expanding quickly as it has sped up plant breeding efforts by allowing the rapid screening of numerous plants at distinct phenological stages. It uses automated sensing, data collecting, and analysis techniques and depends on automated trait analysis to produce phenotypic data (Fig 1). Therefore, it is unnecessary to wait for plant maturity in the field and can quickly test for desirable features at the beginning phases. It can be applied under controlled and natural

circumstances in field and lab conditions.

Types of Phenomics

Forward phenomics and reverse phenomics are two different approaches to phenomics. Forward phenomics employs phenotyping technologies to sieve pools of germplasm for observable and valued features before choosing the best germplasm for a given trait (Furbank and Tester 2011).

Reverse phenomics looks for the causes of the germplasm's improved behavior. In reverse phenomics, the phenotypic or desired trait is already known, for example, drought tolerance present in specific germplasm, and then researchers try to identify the gene(s) responsible for the mechanism controlling the trait through extensive physiological and biochemical investigation (Furbank and Tester, 2010).

Applications of High-Throughput Phenotyping

High-throughput phenotyping has opened an entirely new and improved comprehension of

quantitative characteristics in plants. Determining unknown gene functions and phenotypic changes through conventional phenotyping is still time-consuming. The use of high-throughput phenotyping has significantly contributed to crop improvement by fastening the process. Traits such as photosynthesis, stress tolerance, transpiration rate, and disease tolerance are currently studied using high-throughput phenotyping (Stahl *et al.*, 2020).

Study of Below-Ground Parts

Roots are the basis for plant growth but different physiological activities being performed by roots are still unidentified. Most of the observations carried out on roots are based on controlled conditions or are based on destructive sampling. Non-destructive techniques of studying roots have the advantage of repeated observations. One such technique is the Minirhizotron technique. This technique is capable of studying root distribution, root demography, and interaction between roots and root-soil and soil organisms. The same fine-root segments can be monitored over their lifetime and pictures are stored in a database for processing (Maeght *et al.*, 2013). Wang *et al.*, 2023 developed an X-ray CT technology for the three-dimensional detection of the plant root structure.

Study of Above-Ground Parts

Foliage is an important part of plants responsible for photosynthesis and chlorophyll is the major active component of leaf tissues participating in photosynthesis. Grow screen Fluro is an automated screening device that combines a fluorescence imager with computer-controlled moving stages and automated data analysis routines for high-throughput measurement of relevant photosynthetic parameters and analyses can reveal influences of mutations, transgenes, or environmental factors on the diverse phenotypic properties of the plants (Rascher *et al.*, 2011).

Study of Plant-Insect Interaction

Breeding for resistance to insect pests has been the major area of agricultural research. Non-destructive methods such as fluorescence and hyper spectral imaging can provide great assurance in quantitative scoring. Near-infrared (NIR) reflectance spectroscopy can be used as a screening tool to select/reject cultivars with resistance/tolerance on the basis of different

chemicals being stimulated, overproduced, or underproduced under different biotic stresses. Near-infrared and far-infrared sensors were used to estimate water content, leaf temperature, and stomatal conductance, and in remote sensing by measuring plant reflectance and predicting the degree of stresses (Bhanu *et al.*, 2017).

Assessment of Plant Disease Symptoms

Visible light imaging can be used to assess the leaf area damage and the degree of damage caused by pathogens. Image-based phenotyping offers the potential to improve the reproducibility and sensitivity of disease quantification. Further, image analysis enhances the ability to distinguish between genotypes with different levels of disease severity (Mukta and Bart 2015). Sensors mounted on an unmanned aerial vehicle (UAV) were used to derive various vegetative indices as well as canopy temperatures in a subset of a peanut chromosome segment substitution line (CSSL) for scoring of tomato spotted wilt virus (TSWV) incidence (Gimode *et al.*, 2023)

Application of plant phenomics to trait-based physiological breeding

Phenomics becomes pivotal in physiological breeding since there is a lack of comprehensive knowledge regarding the genetic basis of the differences existing in cultivars. Hence, in the physiological breeding approach, both genomics and phenomics go conjointly. The imaging technique can be used for specific traits of interest like tillering, early vigor, coleoptile length, and biomass at anthesis. The use of phenomic tools (3D models) can increase the efficiency and accuracy of the evaluation of these traits with more accurate and precise information.

High throughput phenotyping and genetic mapping

The size of shoot apical meristem (SAM) in the seedling stage is in positive correlation with early flowering phenotypes in maize (decreasing the number of days to anthesis). Maize SAM morphological traits (shape and size) were extracted in an association panel and a backcross (BC) population using high throughput phenotyping (Leiboff *et al.*, 2016). To identify genetic factors related to the metabolic composition in maize, mass profiles of 198 recombinant inbred lines (RILs) and their parents (B73 and Mo17)

were generated using direct-injection electrospray ionization mass spectrometry (DLI-ESI MS) (Gonzales-Rodriguez *et al.*, 2023).

HTP in Post-harvest technology

During the process of harvesting, the sensors and global positioning system (GPS) could also be integrated with the (combined) harvester to monitor, the yield. Seed and milling industries uses imaging technologies for the quality assessment of seeds. Volpato and Gomez, 2023 developed a Deep Learning (DL) HTP pipeline to capture the sequential behaviour of time series data for estimating days to maturity and to identify target plants in the early growth stage for stand count estimation using field dry bean data obtained from aerial RGB images at the plot-level.

Challenges of High-throughput Phenotyping

Despite the on-going development in computational and imaging systems, extracting quantitative traits reliably from time-series imagery of numerous plants remains a challenge. The traceability and access to trial results and associated metadata were complicated due to the broad range of high-throughput phenotypic data presented in a variety of databases. Furthermore, there was difficulty in accessibility and utilization of facilities due to the high costs involved in purchasing and maintenance.

References

1. Bhanu, A., Bhandari, H., Singh, M. and Srivastava, K. (2017). Introduction to phenomics and its application in physiological breeding. *Advances in Plant Physiology*, Scientific Publishers (India), Jodhpur. 17, 200-226
2. Furbank, R.T. and Tester, M. (2011). Phenomics – technologies to relieve the phenotyping bottleneck. *Trends in plant science*. 16, 635–664
3. Kim, J.Y. (2020). Roadmap to high throughput phenotyping for plant breeding. *Journal of Biosystems Engineering*. 45:43–55
4. Leiboff, S., De Allie, C.K., and Scanlon, M.J. (2016). Modeling the morphometric evolution of the maize shoot apical meristem. *Frontiers in Plant Science*, 7, 1651.
5. Maeght, J.L., Rewald, B. and Pierret A. (2013). How to study deep roots and why it matters. *Frontiers in Plant Science* 4, 1-14.

6. Mutka, A.M. and Bart, R.S. (2015). Image-based phenotyping of plant disease symptoms. *Frontiers in Plant Science*. 5, 1-8
7. Rahaman, M.M., Chen, D., Gillani, Z., Klukas, C. and Chen, M. (2015). Advanced phenotyping and phenotype data analysis for the study of plant growth and development. *Frontiers in Plant Science*. 6, 619.
8. Rascher, U, Blossfeld, S, Fiorani, F, Jahnke, S, Jansen, M, Kuhn, A.J. (2011). Non-invasive approaches for phenotyping of enhanced performance traits in beans. *Functional Plant Biology*, 38, 968–983.
9. Wang, J., Liu, H., Yao, Q., Gillbanks, J. and Zhao, X. (2023). Research on high-throughput Crop Root Phenotype 3D Reconstruction Using X-ray CT in 5G Era. *Electronics*, 12(2), 276.
10. Gonzalez-Rodriguez, T., Perez-Limon, S., Peniche-Pavia, H., Rellan-Alvarez, R., Sawers, R. J., and Winkler, R. (2023). Genetic mapping of maize metabolites using high-throughput mass profiling. *Plant Science*, 326, 111530.
11. Gimode, D., Chu, Y., Holbrook, C. C., Fonseka, D., Porter, W., Dobreva, I., and Ozias-Akins, P. (2023). High-Throughput Canopy and Belowground Phenotyping of a Set of Peanut CSSLs Detects Lines with Increased Pod Weight and Foliar Disease Tolerance. *Agronomy*, 13(5), 1223.
12. Volpato, L., and Gomez, F. E. (2023). Drone-imagery phenotyping using deep learning approaches to estimate plant maturity and stand count at dry beans. *Authorea Preprints*.
13. Stahl, A., Wittkop, B. and Snowdon, R.J. (2020). High-resolution digital phenotyping of water uptake and transpiration efficiency. *Trends in plant science*, 25, 429–433.