

REVIEW ARTICLE

Perspectives on high throughput phenotyping in developing countries

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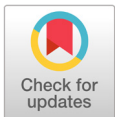
Abstract

The demand for crop production is increasingly becoming steeper due to the rapid population growth. As a result, breeding cycles should be faster than ever before. However, the current breeding methods cannot meet this requirement because traditional phenotyping methods lag far behind even though genotyping methods have been drastically developed with the advent of next-generation sequencing technology over a short period of time. Consequently, phenotyping has become a bottleneck in large-scale genomics-based plant breeding studies. Recently, however, phenomics, a new discipline involving the characterization of a full set of phenotypes in a given species, has emerged as an alternative technology to come up with exponentially increasing genomic data in plant breeding programs. There are many advantages for using new technologies in phenomics. Yet, the necessity of diverse man power and huge funding for cutting-edge equipment prevent many researchers who are interested in this area from adopting this new technique in their research programs. Currently, only a limited number of groups mostly in developed countries have initiated phenomic studies using high throughput methods. In this short article, we describe the strategies to compete with those advanced groups using limited resources in developing countries, followed by a brief introduction of high throughput phenotyping.

Keywords: automated system, environmental factors, genomics, image analysis, phenomics

Introduction

Crop production must be doubled by 2050 to meet the predicted production demands of the population worldwide (Ray et al., 2013). Consequently, breeding methods for crop improvement as well as new technologies have to be enhanced and developed in order to meet food requirements accordingly (Barker et al., 2016). However, it will be a huge challenge for plant breeders to meet this demands since crop yields would have to increase at a rate of 2.4% per year at best and the



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capacity of agricultural production achieved by plant breeding will be maximized due to limited genetic resources (Ray et al., 2013). However, extensive breeding efforts over the past 50 years have been responsible to increase cereal yields about three times and continuing advances in the techniques available to breeders offer the potential to increase the rate of genetic improvement. In addition, utilizing new molecular tools reached their full potential to dissect the genetics of quantitative traits. Nonetheless, all the valuable information is limited by the availability of phenotypic data for maximum utilization to improve crops much further. Hence, phenotyping is a key component to ensure genetic improvement of crops for future food security at this moment. Consequently, there has been increased interest in high throughput phenotyping (HTP) in recent years (De Souza, 2010; Cabrera-Bosquet et al., 2012; Araus and Cairns, 2014; Gehan and Kellogg, 2017). In this mini-review, the current technologies as well as the difficulties of HTP, especially in the field conditions, will be addressed and discussed for those researchers who are interested in HTP in developing countries.

Rationale to use HTP

Data in agricultural sciences have been changed from genomics to proteomics; now, to phenomics, HTP (Fig. 1). The integration of those different types of data is indispensable for future research and now the integrated data is called as multi-dimensional agricultural omics information. In most cases, high throughput generation of data becomes common; however, phenotypic data collection is still in its beginning. HTP is defined as automated methods to score phenotypes in target organisms that continue to develop; consequently, it permits previously inaccessible areas of biology, such as plant growth by fine scale, to be probed. For breeding purpose, collecting phenotypic data is focused on decoding the genetic basis of complex traits such as yield and drought stress tolerance, and predicting these traits from genetic composition of lines or cultivars to be combined with the genomic information enabled by genomics revolution and advances in genomic technology (Furbank and Tester, 2011).

Genotyping methods have become highly mechanized, uniform across organisms, and relatively at low cost, with cost decreasing every year (Cobb et al., 2013). However, the methods for collecting plant trait data or phenotyping have been barely improved in the concourse, especially for collecting data in field-based conditions (White et al., 2012), which is a huge bottle-neck of genetic association analysis (Furbank and Tester, 2011; Cobb et al., 2013). To enhance complex traits through any breeding methods such as genomic selection, phenotypic data from thousands of plant varieties

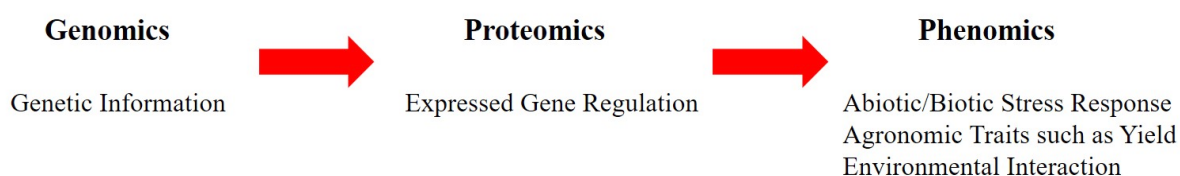


Fig. 1. Trend of data in agricultural sciences.

grown in replications under various environmental conditions is required, and measurements must be repeatedly taken throughout different developmental stages in order to observe the interaction between the expression of plant traits and environments (Montes et al., 2007; Cabrera-Bosquet et al., 2012). However, the current methods for collecting phenotypic data require researchers to visit each plot and manually measure specific parameters, which may be subjective and prone to human error. With larger trials of thousands of plots, manual phenotyping would become laborious, time consuming, and costly; even impossible in some cases. Consequently, many breeding programs only collect limited kinds of phenotypic data such as yield at only one point, in general, at the end of the growing season, missing phenotypic information before the data collection point (Furbank and Tester, 2011). Therefore, reliable and automated systems which are a flexible, robust, mobile, rapid, and efficient multi-sensor platform are in need.

Indoor vs Outdoor

HTP can be categorized into two types; indoor and outdoor trials. Indoor HTP can collect phenotypes in the controlled environments which would offer valuable information for biological questions rather than for the practical information for the breeding purposes (Zhang et al., 2017). On the other hand, outdoor HTP (in other word, field-based HTP) can phenotype traits to obtain more practical information for breeding in the natural environments, which is far more challenging due to the notoriously heterogeneous condition of fields and the inability to control environmental factors (Tanger et al., 2017). Nonetheless, these obstacles of outdoor HTP should be dealt with, considering the urgent increasing requirements of a large-scale phenotypic data to integrate multi-dimensional agri-information so that the outcome could be used for genomics-assisted breeding programs for many crop species.

Current technologies for acquiring and analyzing data

In very recent years, a large number of HTP related research papers are published using diverse traits and various techniques such as visible light imaging (Ikeda et al., 2010), infrared/hyperspectral imaging (Kastberger and Stachl, 2003), 3-dimensional structural tomography/functional imaging (Yang et al., 2011). The majority of these studies are based on the image analysis from the wide-wavelength spectrum of light and the main idea was well written by Fahlgren et al. (2015). Various types of cameras are available to capture signals as images of wide ranges of light spectrum; different range of light spectrum shows the different aspects of biological implication. These image data can be acquired by optic devices such as camera attaching to stationary poles, ground vehicles such as tractors, and unmanned aerial vehicles (drones) in order to capture the target wave-length. However, the most challenging part is the analyses of collected data. There are two major issues for the analytical procedures; one is to handle the vast amount of data and the other is to transform the image data to numeric data. Each component needs expertise from engineering area mostly in computer sciences and statistics, indicating that plant breeding is the area not only for the breeders, but also for biologists, computer scientists, engineers, and statisticians. Depends on the target trait,

the method/technology to phenotype, and the goal, the process of data analysis is different; hence, it is hard to describe the analysis process in a simply manner. However, the research paper titled “Infra-red thermography for high throughput field phenotyping in *Solanum tuberosum*” can be a good example to explain how complex the process for data analysis (Prashar et al., 2013). This paper used thermal imaging for the high throughput field phenotyping of *Solanum tuberosum* to see the differences in stomatal behavior. Digital data was image data using computer science technique. Then, statistical methods were applied. Normalizing data by expressing genotype temperatures as differences from image means and overlapping images for obtaining the enhanced replication, and a Monte Carlo simulation approach were used to confirm the magnitude of genotypic differences that it is possible to discriminate. Based on the analysis via these complicated processes, the results showed that a clear negative association between canopy temperature and final tuber yield for this population. This paper indicates that a multi-disciplined team must work together in the harmonious manner to achieve the goal which is, in this case, to make improved cultivars using the integrated power of different research fields. Furthermore, large sized research funds are crucial to support the expensive equipment and the payroll for a large group of researchers.

Strategies to compete with leading groups under the situations of developing countries

Major components in HTP are developing, operating, and analyzing in HTP (Fig. 2). They are covering a wide range of research fields such as machine learning, electronic engineering, statistics, genetics, and breeding. With all of these requirements satisfied, vigorous investigation by many research teams have already been in the process using such huge man power pool in the developed countries in Europe and United States. However, the infra structure and the culture for collaborations inter-/intra research fields in developing countries are not well paved as much as those world-leading groups in HTP. Besides, the research fund is not sufficient as well. Under these unfavorable

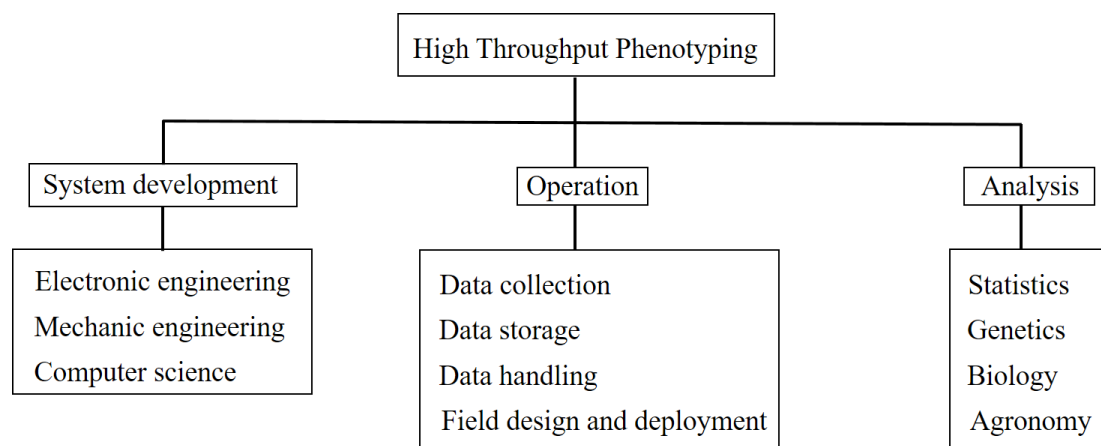


Fig. 2. Major components in high throughput phenotyping.

circumstances, how can we compete with them?

First, excavating and screening expertise from each field necessary to fill each component for HTP have to be prioritized. Those screened pool must be aware of the potential of HTP to be integrated into the HTP research system. This integration will have synergic effects on the new possibilities on expanding research focuses by exchanging information that each individual or group has. To make it happen, the collaboration among different groups is the key, which needs time and effort because the jargon and the way to think in each groups are different one another. Second, sufficient research fund has to be provided. Although the immense scales causing huge bulk of research funding is not necessary, large funding is crucial since the scale of the HTP due to the nature of large-sized data set. Thus, it is ideal to be invested by both government and private sectors at once. Fortunately, the growing interests in HTP in developing countries would be helpful on this matter. For example, Korean government is planning to provide some financial support to automate field data collections in the near future. Third, breeding population with unique germplasm indigenous to developing countries should be developed and utilized. New population using native germplasms in developing countries having unique traits would be powerful to be competitive with other research groups that were initiated ahead because there would be higher chance to find something new and noble in different resources.

Practical applications of HTP to developing countries

The most important thing is novel research ideas based on the biological background. In this sense, the good example would be a field-based high throughput phenotyping rapidly identifies genomic regions controlling yield components in rice by Tanger et al. (2017). In their study, QTL analysis were performed with five manually collected phenotypic data (days to heading, biomass, height, grain yield, and Harvest Index) and five traits by HTP method (Canopy Temperature Depression (CTD), HTP Height, Normalized Difference Vegetation Index (NDVI), Normalized Difference Red Edge Index (NDRE), and Chlorophyll-a (Chl-a)). Notably, each phenotypic trait shared the same QTLs, suggesting that each trait is positively correlated one another. This implies that a trait that is difficult to obtain with current technology can be studied with a trait that can be collected with easy method if those traits are correlated each other. The biological background and knowledge on target traits and other phenotypic traits that are associated with the target traits would lead us to the new approach to obtain the information about the target phenotype indirectly, which would be faster and easier with less cost than collecting the original target trait. This could be called “phenome association study”. In the case with not sufficient funds and not enough man-power to analyze complex digital image data, utilization of free software with a novel idea could be the alternative. The paper, titled “Case study: Estimation of sorghum biomass using digital image analysis with Canopeo”, published by Chung et al. (2017) used a free software, called Canopeo (<http://www.canopeoapp.com>) to estimate biomass of sorghum. This tool can measure the percentage of green color in a given picture frame in a semi-automated manner. Authors used this function to measure

biomass of sorghum and proved that this method could be applicable in specific situations where research budget is tight. If budget is more available, one could use tools for measuring fluorescence, which is not highly expensive. Fluorescence in plant is correlated with plant physiologic traits including nitrogen deficiency, photosynthesis, and more (Lu and Zhang, 2000; Shangguan et al., 2000; Huang et al., 2004; Netto et al., 2005). As mentioned the paper by Tanger et al. (2017), these traits could be correlated with yield, biomass, and even other agronomically important traits. Equipment to measure fluorescence could be attached to tractors or bicycle which is modified depending on the purpose of the given research.

There could be some other case studies which can be applied to circumstances in developing countries. However, there are not many examples to represent here since this HTP is quite new and hard to access even for developed countries, We highly encourage researchers in developing countries to benchmark those small-scale studies to come up with HTP leading groups in developed countries for their future HTP research.

Conclusion

HTP is developing fast due to its necessity in the accordance with genotype data. However, it is yet challenging to perform with limited budget and experience. The situations in developing countries are not favorable to compete with world-leading groups due to several reasons such as insufficient research fund. However, it could be overcome with unique resources such as breeding populations using germplasm indigenous to developing countries and novel research ideas although there would be unexpected obstacles in the way. For this, the active discussions and organization of multi-discipline groups for HTP has to be initiated as soon as possible, nationwide.

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