NEXT-GENERATION PHENOTYPING IN PLANTS: OLD PROBLEMS, NEW PROMISES

FENOTIPIFICACIÓN DE PRÓXIMA GENERACIÓN EN PLANTAS: VIEJOS PROBLEMAS, NUEVAS PROMESAS

Breccia G. [1,2], Nestares G. [1]

[1] Facultad de Ciencias Agrarias, Universidad Nacional de Rosario, Campo Experimental Villarino, CC14 S2125ZAA, Santa Fe, Argentina.
[2] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

gbreccia@unr.edu.ar

ABSTRACT

Current progress in DNA sequencing technology has enabled to gather a large amount of genomic information. Genotyping has become a routine activity with lower costs as new technology emerges. However, genomic information cannot provide much insight into how a given genotype performs. In fact, improvement in understanding phenotypes and the ability to generate good phenotypic data became essential for better utilization of genotypic data and understanding the effect of environment. In this post-genomic era, phenomics emerge as a new discipline that aims the acquisition of high-throughput phenotypic information at all levels of biological organization integrating different "omics" data. New technology in plant phenotyping comprises a wide range of complexity including image analyzing softwares, large-scale platforms and field-phenotyping systems. The increasingly number of tools for accurate phenotyping as well as the advantages and opportunities for plant sciences are being discussed.

Key words: crop breeding, high-throughput phenotyping, image analysis

RESUMEN

Los rápidos avances en la tecnología de secuenciación de ADN han permitido obtener una gran cantidad de información a nivel genómico. Actualmente, el principal interés se ha desplazado hacia el estudio del fenotipo con la expectativa de que los progresos en los sistemas de fenotipado puedan aumentar nuestro conocimiento de las relaciones genotipo-fenotipo-ambiente. En esta era post-genómica, la fenómica emerge como una nueva disciplina que integra las demás "ómicas" y busca la adquisición de información en todos los niveles de la organización biológica. En este sentido, las nuevas tecnologías abarcan un amplio rango de complejidad incluyendo programas para el análisis digital de imágenes, plataformas de fenotipado a gran escala y sistemas de fenotipado a campo. En el presente trabajo se discute la importancia de contar con herramientas para la determinación precisa de fenotipos y las oportunidades que las mismas ofrecen para el mejoramiento vegetal.

Palabras clave: mejoramiento vegetal, fenotipado de alto rendimiento, análisis de imágenes

Genotyping and sequencing technologies were developed a few decades ago and now are available in a high-throughput manner at relatively low cost. Genomes of several organisms have been sequenced and genomic information is publicly accessible though different databases. Genomic analyses have been successful but considerable efforts are still necessary to make sense and exploit genetic information.

Phenotyping methods have lagged behind genomics. Most of phenotypic information lacks automation and precision. The large amount of genomic information needs to be put into phenotypic and breeding context in order to understand gene function and to be useful in plant breeding. For that reason, a fundamental change in research is needed to address the deficiency of phenotypic information. In this way, phenotyping has become the major bottleneck (Furbanck and Tester, 2011; Cobb *et al.*, 2013).

Nowadays, it is considered that the discovery of new genes and the revelation of complex interactions will be possible thanks to the development of accurate phenotyping technology. The search in PubMed database for *plant phenotyping* (accessed on September 2013) retrieved more than 400 results and, from those, 300 were studies published in the last five years. A new paradigm with focus on phenotyping has emerged in plant research, particularly in the area of genomics, physiology and breeding. Currently, advances have been made in high-throughput and non-invasive techniques at whole-plant level while phenotyping at lower levels such as organ, tissue or cell is still an artisanal work and often destructive to the sample.

The interest in high-throughput phenotyping has led to a new discipline known as phenomics, which involves the acquisition of high-dimensional phenotypic data on an organism-wide scale (Houle *et al.,* 2010). Phenomics involves the integration of other omics (transcriptomics, epigenomics, proteomics and metabolomics) and it is enabled by advances in high-throughput technology. The aim of plant phenomics is to characterize all the possible phenotypes under different environmental conditions of a given genotype. For that purpose, phenomics includes phenotyping at multiple levels of organization (ranging from cellular components to whole plants and canopy) and comprises structural, physiological, and performance-related traits (Dhondt *et al.*, 2013).

Imaging technology is a fundamental tool for gathering of high-throughput phenotype data. Digital image analysis enables the accurate acquisition of morphometric parameters that can quantify growth and shape of plant organs. Similarly, cell production and expansion can be measured from microscope images. Automatic or semiautomatic methods are necessary to replace standard manual methods to increase precision and enable population genetic studies (Spalding and Miller, 2013). Digital image analysis can be a powerful alternative to achieve a more precise phenotyping than traditional visual ranking that depends on the subjectivity of the operator. Furthermore, automation enables large-scale studies. An updated list of available image-analyzing tools can be found in www.plant-image-analysis.org (Lobet, 2013). From our experience, the implementation of this technology improved the assessment of morphology, color and enhanced accuracy in the evaluation of growth variables without increasing costs. For example, Tomato Analyzer software (Rodríguez *et al.*, 2010) provided an objective and reliable evaluation of size and color variation of sunflower leaves under herbicide treatment (Breccia *et al.*, 2012). SmartRoot software (Lobet *et al.*, 2011) was efficient in analysing root architecture of sunflower genotypes under different herbicide treatments. Primary and lateral root growth parameters were key components in characterizing root growth under herbicide application and to discriminate different degrees of herbicide resistance.

Hardware, imaging, software, and analysis tools are used in the development of phenotyping platforms. There are various examples of successful automated systems in controlled conditions for measuring aboveground traits (e.g. Pereyra-Irujo *et al.*, 2012; Tisné *et al.*, 2013), root architecture (e.g. Famoso *et al.*, 2010; Clarck *et al.*, 2011) and both shoot and root growth (e.g. Ruts *et al.*, 2013). Field-phenotyping initiatives are also developed using proximal sensing devices (e.g. Busemeyer *et al.*, 2013). Infrared imagery, stereo image analysis, acoustic-based distance sensing, non-contact measurement of chlorophyll fluorescence, laser distance sensing and near infrared spectroscopy are potential tools to obtain phenomic data in field conditions (White *et al.*, 2012). On the other hand, large-scale platforms for tissue or cell level-phenotyping are still undeveloped. Advances in robotic sampling of plants grown in field or greenhouse-platforms combined with automatic analysis and proper conservation of the

sample for further analysis will facilitate biochemical and histological characterization in large-scale phenotyping.

The correct determination of phenotype is of outstanding importance to unravel gene function and plant responses to several biotic and abiotic stresses. Will high-throughput technologies meet that purpose? Most of the greenhouse and laboratory-based phenotyping platforms were developed for screening abiotic (e.g. Shi *et al.*, 2013) and biotic (e.g. Chen *et al.*, 2012) stress tolerance. On the other hand, high-throughput phenotyping in mutational analysis enables the identification and characterization of gene function (Sozzani and Benfey, 2011).

A recent review describes how phenotyping tools in combination with known breeding strategies will empower crop improvement (Cobb *et al.*, 2013). In this sense, 3D-phenotyping and quantitative trait *locus* mapping allowed the identification of core regions of the rice genome controlling root architecture (Topp *et al.*, 2013). Similarly, genomic regions associated with endosperm hardness, grain density and size in barley were detected using a phenotyping platform under controlled conditions (Walker *et al.*, 2013). Field-based phenotyping allowed the dissection of the genetic architecture of biomass accumulation by a genome-wide association study in triticale (Busemeyer *et al.*, 2013).

Most of phenotypic data are virtually lost, particularly for crop performance traits. Less than 1% of 5,000 publications that report on Quantitative Trait Loci (QTL) mapping publicly provide the raw data (Zamir, 2013). The availability of phenomic data through repository public databases is an essential requirement for increasing crop yields and food production.

In conclusion, great advances in phenotyping technology give us tools to accurately measure plants characters in large-scale and will narrow the differences between genomics, plant function and agricultural traits. As Cobb *et al.* (2013) have pointed out the next generation phenotyping *will allow geneticists and breeders to productively interrogate the complex ménage à trois between genotype, phenotype and the environment*. However, will this be possible? Will a new bottleneck emerge? What else will be needed for understanding genotype-phenotype-environment relationships? We will probably not know the answers until these technologies become accessible and a large amount of phenotypic data becomes available. How to manage the complexity of phenotypic information at different organizational levels? Concerted efforts among different areas of technology, statistics and biology will be required to address this and other coming issues.

Under this new scenario, it is necessary for plant breeders to take into account these new technologies that can precisely assess the phenotypic variability of the traits of interest in breeding programs.

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