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# Phenotyping and other breeding approaches for a New Green Revolution

Editorial

Food security is a global concern in view of the ongoing global (social and climate) change. The challenges to food security from global change, sharply increasing food and feed demands, whereas increasing the frequency and severity of stresses, coupled with degradation and scarcity of natural resources, are indeed urgent and real (Prasanna et al. 2013). Crop production must double by 2050 to meet future production demands (Tilman et al. 2011). The ability to achieve this increase represents a significant challenge to plant breeders. Crop yields must increase at a rate of 2.4% per year, yet the average rate of increase is only 1.3% with yields stagnating in up to 40% of land under cereal production (Ray et al. 2012, 2013). Therefore, crops with higher yield potential and more resistant to abiotic stress conditions are needed to address such challenges. This will be only achievable by speeding up the rate of breeding gains (Hawkesford et al. 2013).

Constraints in phenotyping capability currently limit our ability to dissect the genetics of quantitative traits, especially for those complex traits related to yield and stress tolerance. Development of effective field-based high-throughput phenotyping remains a bottleneck for future breeding advances. While laboratory analyses of key plant parts may complement direct phenotyping in the field, such traits do not always translate well to field conditions (Araus and Cairns 2014).

More efficient crop phenotyping will be pivotal in the future to hasten the breeding pipeline and to take full advantage of the revolution in molecular techniques and bioinformatics (Araus and Cairns 2014). Crop phenotyping is a research area in rapid expansion, in which a wide variety of approaches is being explored, from high-throughput field phenotyping to the evaluation at the cellular level *in vitro* (Masuka et al. 2012; Prasanna et al. 2013).

Increasing emphasis is being given to the high-throughput field phenotyping, which is most often focused on predicting agronomic and physiological performance. Whereas such phenotyping is usually performed at the canopy level (Lobos et al. 2014), it may be also successfully implemented measuring individual organs like leaves (Garriga et al. 2014). High-throughput phenotyping implies usually an empirical approach, allowing breeders to use genome profile or phenotype without necessarily understanding the underlying biology (Cabrera-Bosquet et al. 2012). However, there are particular targets that still require more specialized phenotyping. This is the case, for example, for the roots, which are responsible for water and nutrient uptake (Carvalho et al. 2014), or the green inflorescences that may also contribute as photosynthetic organs to final yield (Sanchez-Bragado et al. 2014). Selecting for more efficient organs may be pivotal, particularly when breeding for a higher performance under abiotic stress conditions. A more efficient phenotyping will be achieved not only through the deployment of more precise, faster and throughput methods but also through a better definition of the target traits for phenotyping. A classical approach in the research for traits increasing yield potential and adaptation to stress are the retrospective studies, where

varieties developed at different ages are cultivated together under common growing conditions (Zhou et al. 2014).

Improved phenotyping techniques will not only help to provide further success of conventional breeding for complex quantitative traits but also to the full implementation of molecular breeding, including mapping and other forward-genetics approaches, as well as transgenic breeding (Gao et al. 2014) and other reverse-genetics methods such as tilling (Chen et al. 2014).

The present special issue of *JIPB* derives from the “EU Workshop on Phenotypic Profiling and Technology Transfer on Crop Breeding” hosted at the University of Barcelona during September 2012, in the context of the EU-project OPTICHINA (Optimizing Chinese Agriculture). This issue represents a continuation from previous special issues of *JIPB* published in the context of the OPTICHINA project (Parry et al. 2012).

## NOVEL PHENOTYPING TECHNOLOGIES FOR THE NEW ERA OF CROP BREEDING

Remote sensing techniques at the canopy and leaf levels together with laboratory-based analysis of the stable carbon signature of the plant are among the most promising phenotyping approaches.

To date, the most usual methodologies deployed to assess the relative importance of different photosynthetic organs during grain filling have been intrusive in nature, dealing with the suppression of photosynthetic activities of specific organs, either through shading, removing organs, or applying herbicides. Such inherent limitations in the existing methodologies have prevented reaching definitive conclusions about the contribution of the ear. The paper of Sanchez-Bragado et al. (2014) investigates the photosynthetic contribution of the ear and the flag leaf to grain filling in durum wheat using a novel methodological approach, based in the constitutive differences in the carbon isotope signatures of the different photosynthetic organs and the mature kernels. The study highlights the importance of the spike as the main organ providing assimilates to grain filling, particularly in pre-Green Revolution genotypes, whereas in the semi dwarf cultivars the relative contribution of the ear was negatively associated with the harvest index.

Remote sensing techniques based on the light reflected by the canopy in the visible and near infrared regions of the spectrum have been proposed in the last decades as a phenotyping approaches. However, the advent in recent years of affordable multiband field spectro-radiometers, reaching up to 2,500 nm  $\lambda$  is making a difference. Lobos et al. (2014) have assessed the genotypic variability in grain yield as well as in carbon isotope discrimination of mature kernels, a well-known indicator of crop water performance. The study has been conducted in a large collection of advanced lines and modern cultivars of spring wheat growing under a wide range of water regimes. A set of 70 spectral reflectance indices were

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tested. Best correlations were attained when these indices were measured during grain filling. The normalized difference moisture index (2,200:1,100) had the best fit to the data for the different growing conditions.

The use of multiband field portable spectro-radiometers together with the support of multilinear regression analysis and cross-validation allows assessing in an empirical manner (i.e., not linked to the formulation of specific reflectance indices) a myriad of traits potentially relevant for crop phenotyping. The paper of Garriga et al. (2014) reports on the evaluation of chlorophyll and anthocyanin contents and gas exchange rates of Chilean strawberry leaves exposed to different levels of salinity, using spectral reflectance data ranging between 350 and 2,500 nm  $\lambda$ . The accuracy of predictions was improved using multilinear regression analysis models and genetic algorithm analysis instead of specific spectral reflectance indices.

## DIFFERENT APPROACHES FOR THE NEW ERA OF CROP BREEDING

Gene validation using transgenic approaches, generation of allelic variability through mutagenesis, or dissecting the traits responsible for recent advances in crop performance under either optimal or limiting conditions are part of the panoply of miscellaneous approaches that are being used in the new era of crop breeding. They will sustain future increases in the world's food productivity.

Durum wheat and barley are the most cultivated cereals in the Mediterranean basin, where water stress is the main abiotic constraint limiting productivity. The study of Carvalho et al. (2014) aimed to compare spring barley and durum wheat root and shoot responses to drought and quantify relationships between root traits and water uptake under terminal drought conditions characteristic of the Mediterranean environments. To that end root architecture, water uptake and plant growth of both species were investigated using soil columns. Durum wheat performed better than barley under drought associated with a higher root plasticity, in terms of increase in the root-to-shoot dry matter ratio, together with a more efficient root length and root volume densities of the former.

Increasing yield potential in wheat is a strategic objective of the Chinese agriculture. In that context it is paramount to define what phenotypic traits may be included in future breeding programs. Zhou et al. (2014) have investigated the traits associated with the increase during the last two decades in yield potential of winter wheat from Henan province, one of the China's grain baskets. Among the agronomical traits, a higher harvest index, kernel number, and aboveground biomass were associated with the increases in grain yield of wheat. Moreover, increases in yield potential were also related to larger sink capacity together with a higher photosynthetic contribution of the ear and higher water use efficiency. However, Rubisco (Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase) and PEP (Phosphoenolpyruvate) carboxylase activities of the ear and the leaves have not changed in spite the breeding advance in grain yield.

Pre-harvest sprouting (PHS) seriously affects grain yield and quality in wheat. ABI3 is a key factor in the activation of seed development and repression of germination in *Arabi-*

*dopsis*. An ABI3-interactin protein (AIP2) could polyubiquitinate ABI3, impair seed dormancy and promote seed germination. Gao et al. (2014) reports higher levels of the transcripts TaAIP2A and TaAIP2B in PHS-susceptible wheat cultivars, and decreased gradually following seed development. Moreover expressing these two wheat AIP2 genes in an *Arabidopsis* aip2-1 mutant resulted in earlier flowering, promotion of seed germination, and reduced ABA (abscisic acid) sensitivity, somehow mimicking the phenotype of the wild type. These results suggested that wheat AIP2s could negatively regulate the ABA signaling pathway playing important roles in seed germination, and thus wheat PHS resistance finally.

TILLING (Targeting Induced Local Lesions in Genomes), which combines traditional chemical mutagenesis with high-throughput genome-wide screening for point mutations in desired genes, offers a powerful way to create novel mutant alleles for both functional genomics and improvement of crops. Moreover, it avoids the regulatory restrictions imposed on genetically modified organisms. The paper of Chen et al. (2014) reviews the recent progress in TILLING, especially on the development in mutation detection technology, application of TILLING in gene functional studies and crop breeding. The potential of TILLING and EcoTILLING for functional genetics and crop improvement is also addressed, being generally applicable to any genome regardless of its size or level of ploidy.

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