Plant biotechnology

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From the lab to the field: the age of translational plant biology

The first decade of the 21st century has seen an intense debate of the potential contribution of Plant Biotechnology to meeting present and future world demands of food and biomass. The discussion started in 1997 when the first genetically modified (GM) crops were approved by the EPA for commercial production. The debate has been later stimulated by the increasing awareness of the potential effects of global climate change on agricultural production, as the current crops may be poorly adapted to the additional biotic and abiotic stresses caused by the change. Although cultivation of GM crops now exceeds 120 million hectares, the impact of plant biotechnology on agriculture is, surprisingly, still a matter of debate. While some groups oppose this technology for environmental and food security reasons, farmers are eager to adopt it and the overwhelming majority of scientists is demanding greater investments in plant biology and agricultural research, as well as a greater effort to translate lab results into field applications. Additionally, there is a demand to further extend plant biotechnology to other crops, such as those relevant for developing countries and those related to bioenergy production and green chemical products.

The need for translational plant biology is adding pressure to experimental scientists, as it has been tied to the contribution of public funds to their research. Progress has been clearly limited by excessive regulations on transgenic research and by the increasing cost of introducing novel traits in crops other than the ‘big 4’, maize, wheat, soybean, and rice. However, the most important limitation is the complexity of the plant biological processes and agricultural traits of interest, which has been found to be higher than expected. After 15 years of GM crops, it has become evident that a deeper understanding of plant biological processes is required to facilitate the transfer of novel technologies to the field. This is particularly true with respect to plant development and the responses to abiotic and biotic stresses. The major challenge is to improve crop productivity to get more food/feed and biomass per hectare and to do so with a more efficient use of water, energy, and agrochemicals.

Among the factors that constrain agricultural productivity most dramatically are the abiotic stresses that plants encounter most frequently, such as drought, salinity, flooding, and high and low temperature. The review by J.M. Pardo describes our current knowledge of the molecular and physiological aspects of plants under drought and salinity stresses. Classical breeding approaches have failed to improve crop tolerance to these stresses. This has been due to the multigenic nature of the trait and the scarce genetic and molecular knowledge that we had from the adaptative response of plant to these stresses. Novel genetic determinants governing physiological processes, such as stomata function and seed desiccation, that influence plant performance under water deprivation, showed promising potential application in model plants. Stomata pores regulate gas exchange for photosynthesis and the loss of water by transpiration. The engineering of stomatal closure may contribute to reduce the water loss through evapo-transpiration and to improve the water use efficiency of plants. In principle, the trade-off of diminishing gas exchange will result in decline of photosynthetic activity. Interestingly, examples demonstrating the uncoupling of plant transpiration, stomata patterning, and photosynthesis efficacy have been reported, opening the possibility of unprecedented new approaches to drought tolerance. One of the major recent breakthroughs in the abiotic stress tolerance area was the identification of the receptors of the stress hormone abscisic acid (ABA) and their interplay with regulatory components of this pathway. The review by J.M. Pardo nicely describes the impact of these findings on the design of novel biotechnological approaches, such as the development of ABA agonist or modulator of ABA-signaling response. The excess of salt in the soil affects not only plant water uptake, but also the accumulation of ions in mature tissues that results in progressive damage. Maintenance of K^+/Na^+ balance is crucial for metabolic function as cytotoxic effects of Na^+, that reach a toxic concentration in plants before other ions do, are largely due to competition with K^+ for binding sites. In the review by J.M. Pardo the most relevant conclusions obtained from the in deep characterization of K^+ and Na^+ channels/transporters/exchanger are described. These new data should drive the future directions of plant biotechnology to improve tolerance to salt. For example, we will need to use cell-type specific and stress-inducible promoters instead of constitutive promoters, and to perform fitness field tests with transgenic plants obtained in the lab.

Plants exposed to abiotic or biotic stresses respond with adaptative mechanisms that lead to growth reduction. This occurs even under moderate stress episodes when survival is not threatened. This adaptative response of plants causes major yield losses on agriculture and genetically involves the re-program of plant development by modulating both cell division and cell expansion. The review by Aleksandra Skirycz and Dirk Inzé describes the impact of abiotic stresses, such as water deficit, on plant growth. The mechanisms underlying plant growth reduction under stress situations are not well known, and a better understanding of the physiology and genes controlling this process is an important prerequisite to develop biotechnological application mitigating this response. To reach this goal, the non-destructive monitoring of plant growth dynamics under a range of environmental conditions by using high-throughput phenotyping platforms has to be established to identify relative small differences in a trait as variable as growth. This review describes the most recent advances to optimize plant growth so as to minimize its inhibition by mild stress, as well as the potential biotechnological applications of novel target genes. Among these targets are those related with the GA-biosynthetic pathway and the DELLA proteins, and down-stream effectors, which regulate cell proliferation and expansion. The DELLA-mediated growth regulation is conserved across different stresses and provides a convergence point for classical stress hormones.

Reducing the pre-harvest and post-harvest losses caused by pathogen and pests is essential for increasing agricultural productivity. In this field, plant biotechnology has made significant achievements by generating GM crops with enhanced resistance to insects. However, the contribution of this technology to plant resistance to pathogens has been minor. In the review by Gust *et al.*, the emerging biotechnological concepts for improving crop resistance to pathogens are discussed. Our understanding of plant innate immunity and microbial infection strategies has improved significantly in the past years and several conceptual breakthroughs were published that will drive the design of novel crop protection strategies. Among these novel concepts that can be applied to agriculture are (i) enhancing host recognition capacities of plant pathogens by optimizing the Pathogen Recognition Receptors (PRRs)/Microbe-Associated Molecular Patterns (MAMPs) system; (ii) boosting the executive arsenal of plant immunity; and (iii) interfering with the virulence strategies used by pathogens. One recent example of translational biology in this field is the demonstration that the transgenic overexpression of the bacterial EF-Tu receptor (EFR) in EFR-deficient *Solanacea* species leads to robust resistance to bacterial pathogens. Notably, some of the bacterial pathogens tested in these resistant transgenic plants cause devastating disease in developing countries because effective crop protection strategies are not currently available. Our better understanding at the structure level of the gene for gene resistance also will allow to apply fast-evolutionary process in the lab to generate new broad-spectrum R gene alleles, that could be transferred to crops. In addition the potential to boost plant immunity, by using chemicals or biological agents to enhanced plant resistance, is an example of modulation of the defensive response that may bypass the negative impact on crop growth (yield) of the constitutive activation of broad-spectrum disease resistance. In summary, the need of increasing crop yield and reducing the use of chemicals, that will be mandatory in the next few years, must drive the rapid implementation of all these novel technologies for crop protection.

To respond to the many challenges and to create new environmentally sustainable industries based on photosynthesis (food and fuel production), we need to improve our current knowledge of crop biology, and in particular of grass biology as the 'four big crops' (maize, wheat, rice, and sugarcane) belong to this plant group. In the review of

Michael Bevan and John Vogel, the relevance of using *Brachypodium distachyon* as a model system for grass research is highlighted, and the more recent genomic progress on this plant species and their impact on grass crops are described. Notably, the high level of collinearity in gene organization among grass genomes has been recently supported by the publication of *Brachypodium* genome. This clearly will have an impact on crops like wheat and barley whose genome sizes and complex repetitive sequences make it difficult to perform an in-depth analysis of their genomes. This review describes the unprecedented rapid progress in the development of lab growth conditions and molecular and genetic tools in *Brachypodium*, including plant transformation and virus-induced gene silencing (VIGS), which supports the idea that *Brachypodium* reaches the age for testing agricultural challenges. Interestingly, in the context of biomass crops, the review remarks that *Brachypodium* and the biofuel dedicated species *Myscanthus* have similar cell wall compositions and that the cell wall biosynthetic machinery of *Brachypodium* is quite similar to that of other grasses.

The use of plants for biofuel production is in its initial stage. The review by M. Vega-Sanchez and P. Ronald describes the recent efforts for biofuel production, which are targeted at converting plant biomass into renewable liquid. These efforts are hampered by the lack of biofuel crop domestication, the low oil yields in these crops, and the recalcitrance of plant cell wall to chemical and enzymatic breakdown. Our current genetics and genomics resources for plant breeding of these biofuel crops, and our knowledge of plant lipid metabolism and the synthesis and assembling of cell wall are scarce, limiting further progress of the technology. Although significant advances in the development of draft genomes and transformation protocols for some of these biofuel crops have been achieved in the past years, major breakthroughs on the understanding of lipid metabolism and cell wall structure assembling are still needed to overcome the major limitations in this field. A few gene targets have been demonstrated to be promising through validation by using plant biotechnology tools (transgenic overexpression or knocking down expression) and this number is growing. Last, the impact of modification of lipid metabolism and cell wall structure on crop fitness needs to be considered to avoid negative effects.

One of the postgenomic challenges is to assign functions to the thousands of proteins encoded by plant genomes. Protein activity is an essential functional information for plant biology. In the review by Izabella Kolodziejek and Renier van der Hoorn, the potential application of Activity-Based Protein Profiling (ABPP) technology to plant biology is described. ABPP displays active proteins in proteomes using small-labeled molecules (probes) that irreversibly label proteins in their active site by binding in an activity-dependent manner. This technology has clear advantages with respect to enzyme assays based on measuring substrate conversion rates. ABPP has been successfully used in medical field and in this review the more recently proof-of-concepts studies in plants are described. Among the most relevant achievements of ABPP in the plant field are the identification of protein activities in immunity and development and the finding of unexpected plant targets of commercial inhibitors. On the basis of these results, it could be conclude that ABPP will represent a powerful tool in future plant science and biotechnology with several potential applications such as (i) the identification of targets for herbicides and chemicals used in agriculture; (ii) the measurement of the fruits quality; and (iii) the characterization of plant targets for new leads identified in chemical genomics approaches.