Regulating the Regulators: The Future Prospects for Transcription-Factor-Based Agricultural Biotechnology Products

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It is now more than a decade since the first commercially successful genetically engineered agricultural crops were launched (Castle et al., 2006). These first products were based in large part on simple monogenic traits, such as herbicide tolerance or insect resistance, which did not require manipulation of complex molecular pathways in the transgenic plant. Since then, there has been a growing expectation that the biotechnology industry will deliver a second generation of transgenic products for more challenging traits relating to yield and yield stability, which are under complex polygenic control (Gutterson and Zhang, 2004; Salmeron and Herrera-Estrella, 2006). Advances in plant genomics and systems biology, including the availability of the complete genome sequences of both Arabidopsis (*Arabidopsis thaliana*) and rice (*Oryza sativa*), have offered an unprecedented opportunity to identify regulatory genes and networks that control these important traits. Because transcription factors (TFs) naturally act as master regulators of cellular processes, they are expected to be excellent candidates for modifying complex traits in crop plants, and TF-based technologies are likely to be a prominent part of the next generation of successful biotechnology crops. In this article, we review the prospects for modification of these target traits by TF regulation, including some of the challenges associated with such a strategy.

TFs IN CROP DOMESTICATION AND BREEDING

Although transcriptional regulators are being proposed as the wave of the future for agricultural biotechnology, there is strong evidence that TFs have already played a major role in the origin of agriculture through the domestication of various crop plants. This subject is covered in detail in a review by Doebley et al. (2006), with some important examples for three major crops presented here. Probably the most well-known example of a TF with a critical role in a crop domestication trait is that of *Teosinte branched1* (*Tb1*) of maize (*Zea mays*). *Tb1* is a member of the TCP family of TFs, which are generally involved in the regulation of cell proliferation (Cubas et al., 1999). When *Tb1* is expressed, it represses the outgrowth of lateral branches; maize plants carrying loss of function alleles produce numerous lateral branches (tillers; Doebley et al., 1997). During the domestication of teosinte to produce maize, an allele was selected that altered the regulation of *Tb1*, increasing its expression in primary auxiliary meristems. A survey of maize and teosinte alleles at this locus revealed alterations in the regulatory region of the gene and not in the coding sequence (Wang et al., 1999). Thus, selection for cis-regulatory changes that caused a change in expression of a single TF led to the dramatic shift in architecture that underlies the productivity of the most extensively grown crop in modern day North America.

Shifts in expression patterns and TF activity have generated important characteristics in other grain crops. In rice, a key trait required for domestication is reduced grain shattering, which prevents the seeds from dropping off the panicles and allows for efficient harvesting of the grain. Two TFs have been identified as playing a major role in reducing grain shattering in domesticated rice plants. One of these was isolated as a quantitative trait locus (QTL) in a cross between a shattering-type 'Indica' cultivar and a nonshattering-type 'Japonica' (Konishi et al., 2006). This gene, *qSH1*, encodes a BEL1-type homeodomain protein that is orthologous to Arabidopsis *REPLUMLESS* (*RPL*), which is involved in the formation of an abscission zone in the Arabidopsis silique. The QTL is linked to a single nucleotide polymorphism in the regulatory sequences of the gene, not to any change in the coding sequence itself. *qSH1* from the shattering cultivar is expressed in the developing abscission layer at the base of the seed, but in the nonshattering cultivar it is not expressed in this location. The expression pattern in other tissues is similar, suggesting that the selection was specifically for loss of expression in the abscission layer. This allele is thought to be an early mutation in the Japonica lineage that was selected during domestication. The other TF affecting this trait is *shattering4* (*sh4*), allelic to *sha1* (Li et al., 2006; Lin et al., 2007). *SH4* is a member of the trihelix family of plant-specific TFs and was isolated as a major QTL for shattering in a cross between *O. sativa* and *Oryza rufipogon*. During domestication, a mutant allele was selected with a single amino acid substitution. *Sh4* seems to be necessary for normal

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separation of the abscission layer that releases the grain from the panicle. For a detailed review of the history of rice domestication, including the role of TFs, the reader is referred to Kovach et al. (2007).

Some of the major yield gains achieved by previous generations of conventional crop breeders have subsequently been attributed to alterations in TF activity. Beginning in the 1960s, world wheat (*Triticum aestivum*) grain yields increased dramatically as farmers started using new, semidwarf varieties of wheat along with ''Green Revolution'' cultivation practices that included the application of nitrogen fertilizer. With traditional wheat varieties, the application of fertilizer caused the plant to grow too tall, resulting in lodging. The semidwarf varieties, however, did not grow tall with the application of nitrogen, were resistant to lodging caused by wind and rain, and gave an increased grain yield (Silverstone and Sun, 2000). These wheat varieties are short due to a mutation in at least one of two *Reduced height-1* loci (*Rht-B1* and *Rht-D1*), which causes the plant to respond abnormally to the hormone GA. Peng et al. (1999) elegantly demonstrated that *Rht-B1* and *Rht-D1* were orthologs of the Arabidopsis *GIBBERELLIN INSENSITIVE* (*GAI*) gene, a member of the GRAS family of TFs, which function as transcriptional repressors of growth that are themselves regulated through inhibition by GAs (Peng et al., 1997; Pysh et al., 1999). The mutations stabilize the protein, causing a semidominant trait for GA insensitivity. Peng et al. (1999) transferred the mutant Arabidopsis *gai* allele into Basmati rice (normally tall and prone to lodging) and produced dwarf plants. These results confirm that TFs involved in GA insensitivity were selected during conventional wheat breeding and suggest that similar mechanisms can be used to create dwarf varieties of other cereals.

There are several further examples of TFs playing major roles in crop improvement via domestication and breeding, generally by way of increasing intrinsic yield through modification of plant architecture (for review, see Doebley et al., 2006; Kovach et al., 2007; Pourkheirandish and Komatsuda, 2007). The question for future biotechnology crops, therefore, is not whether TFs can play an important role, but rather how we can best exploit our current knowledge about molecular pathways regulated by TFs to produce crops with improved agricultural traits. Traditional breeding is limited by the amount of genetic diversity in the germplasm of a particular crop; transgenic technologies bypass genetic barriers, allowing for the possibility of modifying regulatory pathways in one plant using TFs from another plant.

THE CASE FOR TFs

Even before the onset of the genomics era, by the early 1990s there was ample evidence that TFs act as master regulators that coordinate the expression of entire response networks of target genes, including numerous examples where a change in activity of a single TF was observed to have a profound effect on an important aspect of plant biology. Well-known examples include the MADS proteins that were shown to control flower development in both Arabidopsis and snapdragon (*Antirrhinum majus*; Coen and Meyerowitz, 1991), the KNOX class homeodomain proteins that control shoot apical meristem development (Vollbrecht et al., 1991; Long et al., 1996), and the *Delila* and *R* genes (HLH family TFs) that control pigmentation in snapdragon and maize, respectively (Goodrich et al., 1992).

The availability of the complete Arabidopsis genome sequence and other genomics tools has enabled new reverse genetics strategies for identifying candidate genes for future agricultural biotechnology products. Although there are other gene classes that could be pursued as sources for potentially useful loci for engineering (e.g. signal transduction molecules such as kinases or receptors), TFs in general make particularly attractive targets. These regulators can be broadly defined as proteins that bind DNA and activate or repress the expression of target genes, either directly themselves, or as part of a larger protein complex. There are estimated to be upwards of 1,500 TFs encoded by the Arabidopsis, genome comprising more than 5% of the genes of this plant (Riechmann et al., 2000; Riechmann and Ratcliffe, 2000; Riechmann, 2002; Qu and Zhu, 2006). These TFs can be grouped into different gene families based on DNA-binding domains and other conserved features. Studies of individual TFs have revealed that genes from the same family often regulate similar physiological functions even among very different plant species, and that overall regulation of most biological processes in the plant cell can be linked to one or more TF families (Riechmann and Ratcliffe, 2000; Zhang, 2003; Qu and Zhu, 2006). For these reasons, several research groups in both the public and private sectors have used systematic functional genomics strategies to identify TFs with commercial potential. Nonetheless, although a substantial number of biotechnology product opportunities clearly exist, particularly when all of the different types of crops are considered, many of these represent relatively niche markets, which would not justify the very high costs that are currently associated with biotechnology product development. For this reason, significant commercial efforts are primarily focused on increasing intrinsic yield potential and stabilizing yield in the face of environmental pressures in large-acre row crops (Crews and Padgette, 2008). These two general trait areas offer high-value returns on products, and are currently major targets for TF-based genetic improvements. The status of and future predictions for research in these areas are assessed below.

IMPROVED INTRINSIC YIELD POTENTIAL

Yield potential can be defined as ''the yield of a cultivar when grown in environments to which it is adapted, with nutrients and water non-limiting and

with pests, diseases, weeds, lodging, and other stresses effectively controlled'' (Evans and Fischer, 1999, p. 1544). The Green Revolution saw tremendous yield increases in the major crop plants, due to both genetic improvement and changes in cultivation practices; however, data suggest that the rate of yield improvement is tapering off for major food crops (Lee, 1998). Raising the ''yield ceiling'' for staple crops such as maize, soybean (*Glycine max*), rice, and wheat, could be considered the Holy Grail of crop biotechnology. Other traits (especially tolerance to abiotic and biotic stresses) affect the overall yield of a given crop, but only within the limits of the intrinsic yield potential.

There are a number of approaches that might be taken to boost intrinsic yield, including increasing photosynthetic capacity, modifying plant architecture, and enhancing the plant's rate of growth. Zhu et al. (2007) recently described the use of an evolutionary algorithm to identify key points of regulation for enhancing the rate of photosynthesis. The prospects for controlling plant photosynthetic capacity are reviewed by Horton (2000) and Long et al. (2006b). Some of the specific opportunities identified are excellent targets for TF-based genetic manipulation. Modifying plant architecture to improve the efficiency of light capture is one such area. As described in the previous section, the selection of dwarf wheat varieties and adoption of maize with reduced tillering were pivotal events in the Green Revolution, both of which relied on modification of native developmental processes through mutation or altered regulation of TFs. It is likely, therefore, that other TFs involved in plant development could be utilized to produce a leaf canopy that is more efficient at light capture. A possible improved morphology would be one in which the upper leaves intercepted less light, allowing for increased photosynthesis in the lower leaves (Long et al., 2006b). A summary of genes involved in the development of plant architecture, including several key TFs, is provided in a recent review by Wang and Li (2006).

Similarly, using TFs to limit the shade avoidance response could result in a beneficial change in plant architecture in some species. In nature, plants have to compete for light when they grow close together. Obviously it is disadvantageous for a plant to be positioned in the lower part of the canopy where light availability is severely limited. In response to the proximity of neighboring vegetation many plant species have evolved mechanisms to dramatically alter their architecture to avoid shading by competitors. During a typical shade avoidance response, resources are essentially redirected from leaves and storage organs into increased extension growth and decreased branching. There is a penalty to this mode of growth in that it can result in accelerated flowering and is often associated with lowered seed set, truncated fruit development, and a reduction in seed germination efficiency (Morelli and Ruberti, 2002). Indeed, plants often initiate shade avoidance at very early stages of development, well before restricted light availability becomes a growth-limiting factor, which can be a particular problem in row crops. This response is well adapted to mixed species natural or weedy agricultural environments, but it depresses total yield when expressed in modern monoculture production. Two HD-Zip TF genes, *ATHB-2* (also known as *HAT4*) and *ATHB-4*, are specifically regulated by light-quality changes and play an apparent role in auxin-mediated regulation of shade-induced growth responses (Carabelli et al., 1993, 1996; Steindler et al., 1999). In addition, *PIL1* (a TF from the bHLH family) has been found to be required for the normal shade avoidance response in Arabidopsis (Salter et al., 2003). Shade avoidance responses are transmitted through a complex web of regulatory networks, much of which remains to be elucidated, and currently there are no published examples of specific components of these networks having been modified so as to enhance crop yield under field conditions. Nonetheless, it is clear that transcriptional regulation has a critical role within these pathways and they therefore represent a useful target for future engineering strategies.

Further strategies for improving intrinsic yield include the possibility of modifying cell-cycle regulation to enhance plant growth rates (Beemster et al., 2005; Van Camp, 2005). There is some evidence that altering expression patterns of the E2F TF genes from Arabidopsis can benefit cell division and cell size, potentially increasing biomass and yield (Beemster et al., 2005; Van Camp, 2005). In addition, it has been reported that *HERCULES1* (*HRC1*), an AT-hook family TF, increases plant organ size and yield when overexpressed in Arabidopsis, with associated increases in cell size and number (Jiang, 2004); similar phenotypes have been observed when *HRC1* is overexpressed in a number of additional species such as tomato (*Solanum lycopersicum*; C. Jiang, N. Gutterson, O. Ratcliffe, R. Creelman, and F. Hempel, Mendel Biotechnology, unpublished data). There is also evidence that some NF-Y (nuclear factor Y) family TFs and the *GOLDEN2*-like TFs regulate chloroplast development (Hall et al., 1998; Rossini et al., 2001; Fitter et al., 2002; Miyoshi et al., 2003), which points toward these families as potential targets for regulating photosynthesis. Delaying leaf senescence represents yet another approach for increasing photosynthetic capacity by maintaining photosynthesis for a longer period of time. A number of TFs are involved in leaf senescence, including many from the WRKY, AP2/EREBP, and Myb families (Eulgem et al., 2000; Chen et al., 2002), and these could be useful for engineering prolonged photosynthetic output through delayed senescence. Many of these TFs are also involved in the response to pathogens and other stresses and will be discussed further in a later section.

YIELD STABILITY

In contrast to intrinsic yield potential, yield stability refers to maintenance of yield under nonideal growth conditions. The main areas of focus are abiotic stress

tolerance, disease resistance, and nutrient use efficiency. All of these traits represent excellent targets for improvement through transgenic TF technology and are addressed individually below.

Abiotic Stress Tolerance

Abiotic stresses, including drought, salt, heat, and cold, cause extensive crop losses worldwide, a situation that is worsening as water resources become more scarce and soil salinity becomes more widespread (Vinocur and Altman, 2005). The need for improved abiotic stress tolerance in crop plants is great, but engineering these traits is particularly challenging because multiple complex pathways are involved in controlling the native stress responses in plants. Abiotic stress tolerance in plants has been an area of intense study, with the advent of genomics in model species shedding light on the regulatory networks required for abiotic stress tolerance. This subject has been comprehensively addressed in numerous recent reviews (Zhang et al., 2004; Vinocur and Altman, 2005; Agarwal et al., 2006; Kim, 2006; Tuberosa and Salvi, 2006; Umezawa et al., 2006; Valliyodan and Nguyen, 2006; Van Buskirk and Thomashow, 2006; Bhatnagar-Mathur et al., 2008), however, some specific examples of using TFs to engineer abiotic stress tolerance bear further mention. Many different TFs have been implicated in abiotic stress tolerance, mostly from large TF families including AP2/EREBP, bZIP, NAC, MYB, MYC, and WRKY (Umezawa et al., 2006; Bhatnagar-Mathur et al., 2008). Probably the most well-studied group of TFs involved in drought and cold tolerance are the *CBF* (C-repeat binding factor) genes (also known as *DREB1* [dehydration-responsive element-binding protein] genes). As reviewed by Zhang et al. (2004) and Umezawa et al. (2006), ectopic expression of these genes in Arabidopsis, as well as in heterologous systems such as wheat, tomato, tobacco (*Nicotiana tabacum*), strawberry (*Fragaria* spp.), rice, oilseed rape (*Brassica napus*), and (most recently) potato (*Solanum tuberosum*; Behnam et al., 2007; Pino et al., 2007) has produced enhanced tolerance to one or more types of abiotic stress. However, a common undesirable side effect of constitutive overexpression of the *CBF* genes is plant growth retardation. Kasuga et al. (1999), however, reported significant stress tolerance without strong growth retardation by expressing *DREB1A/CBF3* in Arabidopsis under the control of the promoter from the stress-inducible *rd29a* gene; Pino et al. (2007) described similar results in potato. Oh et al. (2005) also reported enhanced drought tolerance in rice plants that constitutively overexpressed either *CBF3* or *ABF3* (a bZIP TF from Arabidopsis), with no obvious negative side effects. Additionally, the rice *CBF3* overexpression lines showed increased salt tolerance and slightly enhanced tolerance to low temperature. The *CBF* genes, therefore, have a proven track record for engineered abiotic stress tolerance in a number of plants, demonstrating the utility of Arabidopsis as a model system and as a source of useful TF leads. However, it still remains to be seen whether the CBF technology will be durable in a commercial agricultural setting.

The *CBF* genes apparently produce abiotic stress tolerance by up-regulating a suite of native stress-responsive pathways that together produce physiological adaptations that enable the plant cells to cope with osmotic stress (Fowler and Thomashow, 2002; Maruyama et al., 2004). Interestingly, *WXP1*, another AP2/EREBP TF from alfalfa (*Medicago truncatula*), has been found to produce enhanced drought tolerance in transgenic alfalfa plants by increasing cuticular wax, presumably by improving the water retaining capacity of the plant (Zhang et al., 2005). The same gene and a closely related paralog (*WXP2*) also provided enhanced drought tolerance in transgenic Arabidopsis plants, as well as enhanced freezing tolerance in the *WXP1* overexpressing lines (Zhang et al., 2007). Similarly, the *WIN1/SHN1* gene from Arabidopsis (also an AP2/EREBP family member) increased cuticular wax and provided enhanced drought tolerance in transgenic Arabidopsis plants (Aharoni et al., 2004; Broun et al., 2004; Kannangara et al., 2007). Using TFs to modify biochemical properties of leaves, such as increased wax production, is clearly one potential path for producing enhanced abiotic stress tolerance in crop plants.

The Arabidopsis *HARDY* gene (*HRD*) is yet another example of an AP2/EREBP TF that was recently found to provide enhanced drought tolerance in transgenic Arabidopsis and rice plants (Karaba et al., 2007). *HRD* was isolated by activation tagging in Arabidopsis; the activation-tagged line had smaller but thicker leaves and a robust root system with increased numbers of secondary and tertiary roots. Microscopy revealed elevated numbers of mesophyll cells in the leaf and cortical cells in the root. The activation-tagged line and reconstructed overexpressing lines showed drought and salt tolerance. Rice lines transformed with the *HRD* gene had a deep green color, more bundle sheath cells, and more tillers. They also showed better water use efficiency and better drought tolerance, parameters that were correlated with a lower transpiration rate and a higher net carbon assimilation rate. *HRD* might therefore be considered as a candidate gene for manipulating photosynthetic efficiency and intrinsic yield as well as drought tolerance.

The recently described *SNAC1* gene represents another example of a rice TF that can be used to manipulate abiotic stress tolerance in transgenic rice. *SNAC1* is a NAC family TF that was isolated as a drought-responsive gene and overexpressed in rice (Hu et al., 2006). The researchers report increased drought tolerance in the transgenic lines grown in dry fields and in a controlled drought experimental system. Increased stomatal closure and abscisic acid sensitivity may be at least partially responsible for the enhanced drought tolerance in the transgenic plants.

In a final example, Nelson et al. (2007) enhanced drought tolerance in transgenic maize plants through

overexpression of a member of the NF-Y family of TFs. The Arabidopsis gene *AtNF-YB1* was first identified as a drought lead in a systematic genomics screen in Arabidopsis. Maize plants transformed with the orthologous maize TF, *ZmNF-YB2*, showed improved tolerance to drought, as indicated by chlorophyll content, stomatal conductance, leaf temperature, reduced wilting, and maintenance of photosynthesis under water-limiting conditions. Most importantly, in field trials, the transgenic lines gave higher grain yields than control lines under drought conditions. Interestingly, microarray analysis suggested that the pathways regulated by AtNF-YB1 were different from the CBF-regulated pathways, indicating that drought tolerance can be achieved through multiple different molecular pathways.

Disease Resistance

Another major limitation to worldwide agricultural productivity is plant disease. Pathogens reduce yield by damaging host plant tissues and by diverting resources to pathogen growth. Initial strategies for engineering resistance to plant pathogens (for review, see Gurr and Rushton, 2005a) have focused on single genes with known antimicrobial properties (downstream components of the defense response) or, more recently, highly specific pathogen recognition and signal transduction genes (resistance genes). Although some level of resistance to specific pathogens may be obtained, the most important goal—that of broad-spectrum disease resistance to multiple unrelated pathogens—is difficult if not impossible to produce with these strategies. However, a significant number of TFs have been found to play a role in conserved pathogen response pathways in multiple plants, and are thus useful components for engineering enhanced disease resistance.

Members of the ERF subfamily of the AP2/EREBP family in particular have been implicated in the plant pathogen response (for review, see Gutterson and Reuber, 2004). The first indication that members of the ERF group might be involved in regulation of plant disease resistance pathways was the identification of Pti4, Pti5, and Pti6 as interactors with the tomato disease resistance protein Pto in yeast 2-hybrid assays (Zhou et al., 1997). Since that time, many ERF genes have been shown to enhance disease resistance when overexpressed in Arabidopsis or other species, including: *ERF1* (Berrocal-Lobo et al., 2002; Berrocal-Lobo and Molina, 2004), *AtERF1* and *TDR1* of Arabidopsis (Heard et al., 2003; T.L. Reuber, K. Century, and K. Jakob, Mendel Biotechnology, unpublished data); *Pti4* and *Pti5* of tomato (He et al., 2001; Gu et al., 2002); *Tsi1*, *NtERF5*, and *OPBP1* of tobacco (Park et al., 2001; Shin et al., 2002; Fischer and Droge-Laser, 2004; Guo et al., 2004); *CaERFLP1* and *CaPF1* of hot pepper (*Capsicum annuum*; Lee et al., 2004; Yi et al., 2004); *GbERF2* of cotton (*Gossypium barbadense*; Zuo et al., 2007); *HvRAF* of barley (*Hordeum vulgare*; Jung et al., 2007); and *TaERF1* of wheat (Xu et al., 2007). Encouragingly, in several cases, the overexpressed ERF TF provided enhanced resistance to multiple unrelated pathogens, which would be essential for a viable commercial product.

Although ERF TFs are primarily recognized for their role in biotic stress responses, some ERFs have also been characterized as being responsive to abiotic stress. For example, Fujimoto et al. (2000) have shown that *AtERF1*, *AtERF2*, *AtERF3*, *AtERF4*, and *AtERF5* can respond to various abiotic stresses, including cold, heat, drought, abscisic acid, cycloheximide, and wounding. In addition, several ERF TFs that enhance disease resistance when overexpressed also enhance tolerance to various types of osmotic stress. The first published example of this phenomenon was the tobacco gene *Tsi1*, which was isolated as a salt-inducible gene, and found to enhance salt tolerance and resistance to *Pseudomonas syringae* pv *tabaci* when overexpressed in tobacco (Park et al., 2001), and resistance to several other pathogens when overexpressed in hot pepper (Shin et al., 2002). A number of other ERFs have now been shown to confer some degree of disease resistance and osmotic stress tolerance when overexpressed, including: *OPBP1*, which enhances salt tolerance (Guo et al., 2004); *CaPF1*, which produces freezing tolerance (Yi et al., 2004); *CaERFLP1*, which enhances salt tolerance (Lee et al., 2004); *HvRAF*, which enhances salt tolerance (Jung et al., 2007); and *TaERF1*, which enhances drought, salt, and cold tolerance (Xu et al., 2007). Taken together, these results indicate that ERF TFs offer the exciting potential for engineering both biotic and abiotic stress tolerance in the same plant.

Other TF families strongly implicated in pathogen defense include the WRKYs, bZIPs, and MYBs (Eulgem et al., 2000; Singh et al., 2002; Gurr and Rushton, 2005a; Eulgem and Somssich, 2007). These families provide a large genetic resource for engineering broad-spectrum disease resistance in crop plants because they may be used to manipulate native plant defense response pathways in a pathogen nonspecific manner. One argument against constitutively activating the pathogen defense response is that it often results in negative side effects such as growth retardation (similar to what has been observed with the *CBF* genes used in abiotic stress tolerance). A way around this problem is through the use of alternative (i.e. nonconstitutive) promoters, including tissue-specific and inducible promoters. This strategy for engineering plants with enhanced disease resistance is reviewed by Gurr and Rushton (2005b), and represents a similar approach to that which can be utilized for other traits such as abiotic stress tolerance, as with the previously described abiotic stress-inducible promoter used with *DREB1A* (Kasuga et al., 1999; Pino et al., 2007).

Nutrient Use Efficiency

Nitrogen is a critical limiting nutrient for plants and has to be exogenously supplied to many annual crops. The addition of nitrogen fertilizer to crops greatly

increases the yield, but it also represents a significant fraction of grower input costs and can have negative effects on the environment (Good et al., 2004). Nitrogen fertilizer that is not taken up by plants is generally lost as runoff or converted to nitrogen gases by microbial action, contributing to water and air pollution. Improving the nitrogen use efficiency of crop plants has the potential to reduce fertilizer application rates, providing both cost savings and environmental benefits.

A review by Good et al. (2004) summarizes recent attempts to genetically manipulate nitrogen use efficiency in plants. Most efforts have focused on overexpressing enzymes in the nitrogen uptake and assimilation pathways, with varying degrees of success, but two TFs were also targeted in such studies. Zhang and Forde (1998) described *ANR1*, an Arabidopsis MADS family TF that showed inducibility by nitrate in Arabidopsis root cultures. Transgenic plants in which *ANR1* was down-regulated exhibited an altered sensitivity to nitrate and did not display the lateral root proliferation normally induced by localized nitrate treatment. A later publication by the same laboratory (Gan et al., 2005) reported the opposite expression pattern (repression by nitrate and induction by nitrogen starvation) in mature hydroponically grown plants, although the lack of lateral root growth in nitrate rich zones was again observed in *ANR1* knockout lines. Although the exact mechanism remains unclear, this gene apparently plays a role in the response to nitrogen and could be useful for manipulating nutrient response pathways. An example of the successful engineering of enhanced nitrogen uptake using a TF was reported by Yanagisawa et al. (2004); the authors overexpressed the maize *Dof1* gene, which was known to be involved in organic acid metabolism, to create transgenic Arabidopsis plants that showed increases in free amino acid content and total nitrogen uptake, as well as improved growth under low nitrogen conditions.

Although nitrogen is one of the most expensive nutrients in fertilizer applications and is therefore the main target for enhanced nutrient use efficiency in plants, it is possible that TFs will be used to engineer tolerance to other nutrient deficiencies as well. As an example, Yi et al. (2005) describe the engineering of tolerance to phosphate starvation in rice, using the rice bHLH family TF gene *OsPTF1*. Under conditions of low inorganic phosphate, the transgenic lines showed improved root and shoot biomass, phosphorus content, and panicle weight.

THE KEY CHALLENGE: DEVELOPING LEADS INTO PRODUCTS

It is clear that plant TFs are a useful source of candidate leads for new agricultural biotechnology products. Identifying leads, however, is only the first step in the lengthy and costly process of developing a new commercial genetically engineered crop. In a review of the application of genomics to biotechnology traits, Gutterson and Zhang (2004) outlined a typical development process for an agricultural biotechnology product, which can take up to 12 years or more from gene discovery to a commercial product. Over the course of the development process, there are numerous challenges that must be faced to produce a commercially viable end product. The first important issue to consider, especially when a lead has been identified in a model system such as Arabidopsis, is whether the target pathways of that TF are present in the engineered crop. However, given the many examples that have now been cited for Arabidopsis TFs functioning in heterologous systems and the evidence of conserved pathways from orthology among different species (Xiong et al., 2005), this issue is likely to be less of a problem than might have been previously anticipated.

As was indicated in the preceding section, TF technologies often require optimization, either to reduce unwanted side effects such as growth retardation or to enhance the desired trait to the level at which it is of commercial value. Optimization is frequently approached by modifying expression of the TF transgene; tissue-specific, developmental, or inducible promoters (Kasuga et al., 1999; Gurr and Rushton, 2005b), rather than the usual constitutive promoters, can be utilized to limit expression of the transgene to the appropriate tissues or environmental conditions. Another strategy for optimizing the phenotype is by protein modification. Sakuma et al. (2006) demonstrate an example of this approach by converting the Arabidopsis DREB2A protein to a constitutively active form through deletion of negative regulatory domain. When overexpressed in transgenic Arabidopsis plants, the modified DREB2A version produced enhanced drought tolerance whereas the native form did not.

Another major hurdle for commercializing a genetically modified crop is securing approvals from regulatory authorities. Typically, each transgenic event (i.e. independent insertion of a transgene into a crop genome) that is to be commercialized in the United States has to be ''deregulated'' or approved by a number of government agencies, which, depending upon the specific trait and species, may include the U.S. Department of Agriculture, the U.S. Food and Drug Administration, and the U.S. Environmental Protection Agency. Similar approvals are required from regulatory authorities in other countries. In the United States, current restrictions on field trials put in place by the U.S. Department of Agriculture were designed specifically for the first round of biotechnology crops, which expressed transgenes from exogenous (i.e. nonplant) sources for herbicide tolerance, insect resistance, or virus resistance. Strauss (2003) makes a compelling case for lower restrictions on field testing of biotechnology crops containing transgenes from plant sources, which modify native molecular pathways in the host plant. Thus, the deregulation of such transgenic crops would logically be expected to be more straightforward than for the first-generation biotechnology crops. However, it will

clearly be necessary to gain a thorough understanding of the molecular mode of action for new technologies to prove that a transgenic plant engineered with an enhanced trait poses no new environmental or health risks when compared to the nonengineered plant from which it was derived.

SYSTEMS BIOLOGY: PAVING THE WAY FOR THIRD-GENERATION CROPS

Based on the examples discussed in this article, it seems reasonable to expect that TFs will be a significant component of the next round of agricultural biotechnology products, conferring enhanced intrinsic yield and yield stability, which will hit the market during the next decade. These second-generation products, derived from discoveries made during the genomics era that began in the late 1990s, are expected to deliver significant gains in yield compared to those achieved through conventional breeding approaches. Additionally, it is likely that further incremental improvements will come through refinements of these technologies based on knowledge of their molecular mode of action. However, what are the prospects for the longer term future and what will it take to deliver truly dramatic yield increases? Evidence suggests that there is substantial potential to increase primary productivity in crop plants. Long et al. (2006b) describe possible improvements in photosynthesis that could achieve a 50% increase in yield potential. In a more recent article, Zhu et al. (2007) use an evolutionary algorithm to identify changes in the distribution of resources among enzymes of carbon metabolism that are modeled to increase C_3 photosynthesis by 76%. Theoretically, improvements in photosynthesis combined with other crop optimizations such as enhanced yield stability and increased harvest index could potentially double the average yield of major row crops in the next 25 years. To achieve such goals, however, we will need to understand the intricacies of plant gene regulation at a global rather than a local level.

A decade ago, the plant biology community was engaged in reverse genetics screens to identify and test the function of individual genes in the emerging genome sequence. A survey of current publications reveals that many research groups are now focusing their efforts toward ''systems biology'' projects aimed at assembling all of the genes in the genome into transcription networks (of which TFs form the hubs) or protein interaction networks underpinning major biological processes, based on genetic, expression, and interaction data (Gutierrez et al., 2007; Keurentjes et al., 2007; Ma et al., 2007; Michael et al., 2008). Such a task is truly massive in a plant system because it has to represent global molecular relationships and also take into account both cell-type and environmental variables; as such it is a vastly more challenging problem than for a single cell system such as yeast. Nonetheless, the breakthroughs that have been made in single cell systems (Gasch et al., 2004; Harbison et al., 2004; Kim et al., 2006) should serve as a paradigm for developing such approaches for complex multicellular organisms. Recent advances in wet-laboratory technology and computing power are helping to resolve these problems, and are making network inference a reality for multicellular organisms (Sachs et al., 2005; Lee et al., 2008). For example, there are now several companies offering ultra-high-throughput sequencing capabilities, and advances in protein biochemistry have enhanced the potential to identify the protein partners of TFs on a large scale, both through in vivo and in vivo methods (Johnson et al., 2007; Robertson et al., 2007). Armed with these new technologies, the effort to produce integrated systems biology maps for plants is being tackled by both the public and private sector, and includes major academic centers for systems biology such as New York University Center for Genomics and Systems Biology (http://biology.as.nyu.edu/object/facilities.gsb.html) and the Centre for Systems Biology at Edinburgh (http://csbe.bio.ed.ac.uk/).

Another potentially important approach for determining the best crop intervention points and pathway optimization is dynamic modeling of local regulatory circuits informed by the various systems biology tools. As a paradigm, Millar and colleagues (Locke et al., 2005, 2006) have built dynamic models of the circadian clock, which is largely built up of TF-regulated regulatory loops. This modeling approach has been used to reveal previously unidentified components of clock function. Such a strategy may ultimately prove useful to predict the impact of specific alterations in TF-regulated networks on crop performance.

As the information obtained from these integrated systems biology maps grows in resolution, it will be possible to choose optimal intervention points in the networks and identify sets of genes that can be coregulated to produce synergistic or additive effects on intrinsic yield or yield stability. In addition, network maps will very likely lead us to components that can be used to engineer new traits, such as enhanced plant performance in a changing global environment (see Long et al. [2006a] for a discussion of the predicted effects of climate change on crop yield). Metabolic engineering to improve nutrient profiles is another target third-generation biotechnology crop (for review, see Kinney, 2006), which we expect to be accelerated by newly available network maps. Thus, even while second-generation biotechnology crops are still under development, a new focus toward building high-resolution systems biology maps will provide the discoveries that deliver later generations of engineered biotechnology crops that will likely be commercialized in the second quarter of the century.

CONCLUSION

Considering that the human population is expected to total 9 billion by 2050 (Cohen, 2003), there is a clear

need to sustain and even accelerate the rate of improvement in crop productivity, simply to be able to feed, clothe, and provide energy and building materials for such a large populace. Enhancing intrinsic yield and plant stress tolerance through genetic engineering will be a critical part of this effort, building on the achievements of conventional breeding. Because of their nature as master switches for major regulatory networks and their prior role in the domestication of many crop species, TFs are predicted to be among the best and safest candidate loci for engineering these traits. Rapid advances in systems biology are expected to drive the third-generation biotechnology products, including targeted pathway regulation, enhanced nutrient quality, and stacked traits, all of which are likely to have TF components useful for engineering. The manipulation of native plant regulatory networks therefore represents a new era for genetically modified crops, and we are optimistic that commercial and consumer acceptance of this strategy will be high.

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LITERATURE CITED

Agarwal PK, Agarwal P, Reddy MK, Sopory SK (2006) Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. Plant Cell Rep **25:** 1263–1274

Aharoni A, Dixit S, Jetter R, Thoenes E, van Arkel G, Pereira A (2004) The SHINE clade of AP2 domain transcription factors activates wax biosynthesis, alters cuticle properties, and confers drought tolerance when overexpressed in *Arabidopsis.* Plant Cell **16:** 2463–2480

Beemster GT, Mironov V, Inze D (2005) Tuning the cell-cycle engine for improved plant performance. Curr Opin Biotechnol **16:** 142–146

Behnam B, Kikuchi A, Celebi-Toprak F, Kasuga M, Yamaguchi-Shinozaki K, Watanabe KN (2007) *Arabidopsis rd29A:DREB1A* enhances freezing tolerance in transgenic potato. Plant Cell Rep **26:** 1275–1282

Berrocal-Lobo M, Molina A (2004) Ethylene response factor 1 mediates *Arabidopsis* resistance to the soilborne fungus *Fusarium oxysporum.* Mol Plant Microbe Interact **17:** 763–770

Berrocal-Lobo M, Molina A, Solano R (2002) Constitutive expression of *ETHYLENE-RESPONSE-FACTOR1* in *Arabidopsis* confers resistance to several necrotrophic fungi. Plant J **29:** 23–32

Bhatnagar-Mathur P, Vadez V, Sharma KK (2008) Transgenic approaches for abiotic stress tolerance in plants: retrospect and prospects. Plant Cell Rep **27:** 411–424

Broun P, Poindexter P, Osborne E, Jiang CZ, Riechmann JL (2004) WIN1, a transcriptional activator of epidermal wax accumulation in *Arabidopsis.* Proc Natl Acad Sci USA **101:** 4706–4711

Carabelli M, Morelli G, Whitelam G, Ruberti I (1996) Twilight-zone and canopy shade induction of the *Athb-2* homeobox gene in green plants. Proc Natl Acad Sci USA **93:** 3530–3535

Carabelli M, Sessa G, Baima S, Morelli G, Ruberti I (1993) The *Arabidopsis Athb-2* and *-4* genes are strongly induced by far-red-rich light. Plant J **4:** 469–479

Castle LA, Wu G, McElroy D (2006) Agricultural input traits: past, present and future. Curr Opin Biotechnol **17:** 105–112

Chen W, Provart NJ, Glazebrook J, Katagiri F, Chang HS, Eulgem T, Mauch F, Luan S, Zou G, Whitham SA, et al (2002) Expression profile matrix of *Arabidopsis* transcription factor genes suggests their putative functions in response to environmental stresses. Plant Cell **14:** 559–574

Coen ES, Meyerowitz EM (1991) The war of the whorls: genetic interactions controlling flower development. Nature **353:** 31–37

Cohen JE (2003) Human population: the next half century. Science **302:** 1172–1175

Crews T, Padgette S (2008) R&D pipeline review. Monsanto Investors Presentations & Financial Reports. http://www.monsanto.com/pdf/investors/2008/01-08-08.pdf (April 8, 2008)

Cubas P, Lauter N, Doebley J, Coen E (1999) The TCP domain: a motif found in proteins regulating plant growth and development. Plant J **18:** 215–222

Doebley J, Stec A, Hubbard L (1997) The evolution of apical dominance in maize. Nature **386:** 485–488

Doebley JF, Gaut BS, Smith BD (2006) The molecular genetics of crop domestication. Cell **127:** 1309–1321

Eulgem T, Rushton PJ, Robatzek S, Somssich IE (2000) The WRKY superfamily of plant transcription factors. Trends Plant Sci **5:** 199–206

Eulgem T, Somssich IE (2007) Networks of WRKY transcription factors in defense signaling. Curr Opin Plant Biol **10:** 366–371

Evans LT, Fischer RA (1999) Yield potential: its definition, measurement, and significance. Crop Sci **39:** 1544–1551

Fischer U, Droge-Laser W (2004) Overexpression of *NtERF5,* a new member of the tobacco ethylene response transcription factor family enhances resistance to tobacco mosaic virus. Mol Plant Microbe Interact **17:** 1162–1171

Fitter DW, Martin DJ, Copley MJ, Scotland RW, Langdale JA (2002) *GLK* gene pairs regulate chloroplast development in diverse plant species. Plant J **31:** 713–727

Fowler S, Thomashow MF (2002) *Arabidopsis* transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. Plant Cell **14:** 1675–1690

Fujimoto SY, Ohta M, Usui A, Shinshi H, Ohme-Takagi M (2000) *Arabidopsis* ethylene-responsive element binding factors act as transcriptional activators or repressors of GCC box-mediated gene expression. Plant Cell **12:** 393–404

Gan Y, Filleur S, Rahman A, Gotensparre S, Forde BG (2005) Nutritional regulation of ANR1 and other root-expressed MADS-box genes in Arabidopsis thaliana. Planta **222:** 730–742

Gasch AP, Moses AM, Chiang DY, Fraser HB, Berardini M, Eisen MB (2004) Conservation and evolution of *cis*-regulatory systems in ascomycete fungi. PLoS Biol **2:** e398

Good AG, Shrawat AK, Muench DG (2004) Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? Trends Plant Sci **9:** 597–605

Goodrich J, Carpenter R, Coen ES (1992) A common gene regulates pigmentation pattern in diverse plant species. Cell **68:** 955–964

Gu YQ, Wildermuth MC, Chakravarthy S, Loh YT, Yang C, He X, Han Y, Martin GB (2002) Tomato transcription factors pti4, pti5, and pti6 activate defense responses when expressed in *Arabidopsis.* Plant Cell **14:** 817–831

Guo ZJ, Chen XJ, Wu XL, Ling JQ, Xu P (2004) Overexpression of the AP2/EREBP transcription factor OPBP1 enhances disease resistance and salt tolerance in tobacco. Plant Mol Biol **55:** 607–618

Gurr SJ, Rushton PJ (2005a) Engineering plants with increased disease resistance: what are we going to express? Trends Biotechnol **23:** 275–282

Gurr SJ, Rushton PJ (2005b) Engineering plants with increased disease resistance: how are we going to express it? Trends Biotechnol **23:** 283–290

Gutierrez RA, Lejay LV, Dean A, Chiaromonte F, Shasha DE, Coruzzi GM (2007) Qualitative network models and genome-wide expression data define carbon/nitrogen-responsive molecular machines in *Arabidopsis.* Genome Biol **8:** R7

Gutterson N, Reuber TL (2004) Regulation of disease resistance pathways by AP2/ERF transcription factors. Curr Opin Plant Biol **7:** 465–471

Gutterson N, Zhang JZ (2004) Genomics applications to biotech traits: a revolution in progress? Curr Opin Plant Biol **7:** 226–230

Hall LN, Rossini L, Cribb L, Langdale JA (1998) GOLDEN 2: a novel transcriptional regulator of cellular differentiation in the maize leaf. Plant Cell **10:** 925–936

Harbison CT, Gordon DB, Lee TI, Rinaldi NJ, Macisaac KD, Danford TW, Hannett NM, Tagne JB, Reynolds DB, Yoo J, et al (2004) Transcriptional regulatory code of a eukaryotic genome. Nature **431:** 99–104

He P, Warren RF, Zhao T, Shan L, Zhu L, Tang X, Zhou JM (2001) Overexpression of *Pti5* in tomato potentiates pathogen-induced defense gene expression and enhances disease resistance to *Pseudomonas syringae* pv. tomato. Mol Plant Microbe Interact **14:** 1453–1457

Heard J, Broun P, Riechmann JL, Keddie J, Pineda O, Adam L, Samaha R, Zhang J, Yu GL, Ratcliffe O, et al, inventors. December 16, 2003. Transgenic plants comprising polynucleotides encoding transcription factors that confer disease tolerance. U.S. Patent No. 6,664,446

Horton P (2000) Prospects for crop improvement through the genetic manipulation of photosynthesis: morphological and biochemical aspects of light capture. J Exp Bot **51:** 475–485

Hu H, Dai M, Yao J, Xiao B, Li X, Zhang Q, Xiong L (2006) Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. Proc Natl Acad Sci USA **103:** 12987–12992

Jiang CZ, inventor. April 6, 2004. Method for modifying plant biomass. U.S. Patent No. 6,717,034

Johnson DS, Mortazavi A, Myers RM, Wold B (2007) Genome-wide mapping of in vivo protein-DNA interactions. Science **316:** 1497–1502

Jung J, Won SY, Suh SC, Kim H, Wing R, Jeong Y, Hwang I, Kim M (2007) The barley ERF-type transcription factor HvRAF confers enhanced pathogen resistance and salt tolerance in *Arabidopsis.* Planta **225:** 575–588

Kannangara R, Branigan C, Liu Y, Penfield T, Rao V, Mouille G, Hofte H, Pauly M, Riechmann JL, Broun P (2007) The transcription factor WIN1/SHN1 regulates Cutin biosynthesis in *Arabidopsis thaliana.* Plant Cell **19:** 1278–1294

Karaba A, Dixit S, Greco R, Aharoni A, Trijatmiko KR, Marsch-Martinez N, Krishnan A, Nataraja KN, Udayakumar M, Pereira A (2007) Improvement of water use efficiency in rice by expression of *HARDY,* an *Arabidopsis* drought and salt tolerance gene. Proc Natl Acad Sci USA **104:** 15270–15275

Kasuga M, Liu Q, Miura S, Yamaguchi-Shinozaki K, Shinozaki K (1999) Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. Nat Biotechnol **17:** 287–291

Keurentjes JJB, Fu J, Terpstra IR, Garcia JM, van den Ackerveken G, Snoek LB, Peeters AJM, Vreugdenhil D, Koornneef M, Jansen RC (2007) Regulatory network construction in *Arabidopsis* by using genome-wide gene expression quantitative trait loci. Proc Natl Acad Sci USA **104:** 1708–1713

Kim H, Hu W, Kluger Y (2006) Unraveling condition specific gene transcriptional regulatory networks in *Saccharomyces cerevisiae.* BMC Bioinformatics **7:** 165

Kim SY (2006) The role of ABF family bZIP class transcription factors in stress response. Physiol Plant **126:** 519–527

Kinney AJ (2006) Metabolic engineering in plants for human health and nutrition. Curr Opin Biotechnol **17:** 130–138

Konishi S, Izawa T, Lin SY, Ebana K, Fukuta Y, Sasaki T, Yano M (2006) An SNP caused loss of seed shattering during rice domestication. Science **312:** 1392–1396

Kovach MJ, Sweeney MT, McCouch SR (2007) New insights into the history of rice domestication. Trends Genet **23:** 578–587

Lee I, Lehner B, Crombie C, Wong W, Fraser AG, Marcotte EM (2008) A single gene network accurately predicts phenotypic effects of gene perturbation in *Caenorhabditis elegans.* Nat Genet **40:** 181–188

Lee JH, Hong JP, Oh SK, Lee S, Choi D, Kim WT (2004) The ethylene-responsive factor like protein 1 (CaERFLP1) of hot pepper (*Capsicum annuum* L.) interacts *in vitro* with both GCC and DRE/CRT sequences with different binding affinities: possible biological roles of CaERFLP1 in response to pathogen infection and high salinity conditions in transgenic tobacco plants. Plant Mol Biol **55:** 61–81

Lee M (1998) Genome projects and gene pools: new germplasm for plant breeding? Proc Natl Acad Sci USA **95:** 2001–2004

Li C, Zhou A, Sang T (2006) Rice domestication by reducing shattering. Science **311:** 1936–1939

Lin Z, Griffith ME, Li X, Zhu Z, Tan L, Fu Y, Zhang W, Wang X, Xie D, Sun C (2007) Origin of seed shattering in rice (*Oryza sativa* L.). Planta **226:** 11–20

Locke JC, Kozma-Bognar L, Gould PD, Feher B, Kevei E, Nagy F, Turner MS, Hall A, Millar AJ (2006) Experimental validation of a predicted feedback loop in the multi-oscillator clock of *Arabidopsis thaliana.* Mol Syst Biol **2:** 59

Locke JC, Southern MM, Kozma-Bognar L, Hibberd V, Brown PE, Turner MS, Millar AJ (2005) Extension of a genetic network model by iterative experimentation and mathematical analysis. Mol Syst Biol **1:** 2005.0013

Long JA, Moan EI, Medford JI, Barton MK (1996) A member of the KNOTTED class of homeodomain proteins encoded by the *STM* gene of *Arabidopsis.* Nature **379:** 66–69

Long SP, Ainsworth EA, Leakey ADB, Nosberger J, Ort DR (2006a) Food for thought: lower-than-expected crop yield stimulation with rising CO2 concentrations. Science **312:** 1918–1921

Long SP, Zhu XG, Naidu SL, Ort DR (2006b) Can improvement in photosynthesis increase crop yields? Plant Cell Environ **29:** 315–330

Ma S, Gong Q, Bohnert HJ (2007) An *Arabidopsis* gene network based on the graphical Gaussian model. Genome Res **17:** 1614–1625

Maruyama K, Sakuma Y, Kasuga M, Ito Y, Seki M, Goda H, Shimada Y, Yoshida S, Shinozaki K, Yamaguchi-Shinozaki K (2004) Identification of cold-inducible downstream genes of the *Arabidopsis* DREB1A/CBF3 transcriptional factor using two microarray systems. Plant J **38:** 982–993

Michael TP, Mockler TC, Breton G, McEntee C, Byer A, Trout JD, Hazen SP, Shen R, Priest HD, Sullivan CM, et al (2008) Network discovery pipeline elucidates conserved time-of-day-specific *cis*-regulatory modules. PLoS Genet **4:** e14

Miyoshi K, Ito Y, Serizawa A, Kurata N (2003) *OsHAP3* genes regulate chloroplast biogenesis in rice. Plant J **36:** 532–540

Morelli G, Ruberti I (2002) Light and shade in the photocontrol of *Arabidopsis* growth. Trends Plant Sci **7:** 399–404

Nelson DE, Repetti PP, Adams TR, Creelman RA, Wu J, Warner DC, Anstrom DC, Bensen RJ, Castiglioni PP, Donnarummo MG, et al (2007) Plant nuclear factor Y (NF-Y) B subunits confer drought tolerance and lead to improved corn yields on water-limited acres. Proc Natl Acad Sci USA **104:** 16450–16455

Oh SJ, Song SI, Kim YS, Jang HJ, Kim SY, Kim M, Kim YK, Nahm BH, Kim JK (2005) Arabidopsis CBF3/DREB1A and ABF3 in transgenic rice increased tolerance to abiotic stress without stunting growth. Plant Physiol **138:** 341–351

Park JM, Park CJ, Lee SB, Ham BK, Shin R, Paek KH (2001) Overexpression of the tobacco *Tsi1* gene encoding an EREBP/AP2-type transcription factor enhances resistance against pathogen attack and osmotic stress in tobacco. Plant Cell **13:** 1035–1046

Peng J, Carol P, Richards DE, King KE, Cowling RJ, Murphy GP, Harberd NP (1997) The *Arabidopsis GAI* gene defines a signaling pathway that negatively regulates gibberellin responses. Genes Dev **11:** 3194–3205

Peng J, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE, Beales J, Fish LJ, Worland AJ, Pelica F, et al (1999) 'Green revolution' genes encode mutant gibberellin response modulators. Nature **400:** 256–261

Pino MT, Skinner JS, Park EJ, Jeknic Z, Hayes PM, Thomashow MF, Chen TH (2007) Use of a stress inducible promoter to drive ectopic *AtCBF* expression improves potato freezing tolerance while minimizing negative effects on tuber yield. Plant Biotechnol J **5:** 591–604

Pourkheirandish M, Komatsuda T (2007) The importance of barley genetics and domestication in a global perspective. Ann Bot (Lond) **100:** 999–1008

Pysh LD, Wysocka-Diller JW, Camilleri C, Bouchez D, Benfey PN (1999) The GRAS gene family in Arabidopsis: sequence characterization and basic expression analysis of the *SCARECROW-LIKE* genes. Plant J **18:** 111–119

Qu LJ, Zhu YX (2006) Transcription factor families in Arabidopsis: major progress and outstanding issues for future research. Curr Opin Plant Biol **9:** 544–549

Riechmann JL (2002) Transcriptional regulation: a genomic overview. *In* CR Somerville, EM Meyerowitz, eds, The Arabidopsis Book. American Society of Plant Biologists, Rockville, MD, doi: 10.1199/tab.0085, http://www.aspb.org/publications/arabidopsis/

Riechmann JL, Heard J, Martin G, Reuber L, Jiang C, Keddie J, Adam L, Pineda O, Ratcliffe OJ, Samaha RR, et al (2000) Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes. Science **290:** 2105–2110

Riechmann JL, Ratcliffe OJ (2000) A genomic perspective on plant transcription factors. Curr Opin Plant Biol **3:** 423–434

Robertson G, Hirst M, Bainbridge M, Bilenky M, Zhao Y, Zeng T, Euskirchen G, Bernier B, Varhol R, Delaney A, et al (2007) Genome-

wide profiles of STAT1 DNA association using chromatin immunoprecipitation and massively parallel sequencing. Nat Methods **4:** 651–657

Rossini L, Cribb L, Martin DJ, Langdale JA (2001) The maize *Golden2* gene defines a novel class of transcriptional regulators in plants. Plant Cell **13:** 1231–1244

Sachs K, Perez O, Pe'er D, Lauffenburger DA, Nolan GP (2005) Causal protein-signaling networks derived from multiparameter single-cell data. Science **308:** 523–529

Sakuma Y, Maruyama K, Osakabe Y, Qin F, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2006) Functional analysis of an *Arabidopsis* transcription factor, DREB2A, involved in drought-responsive gene expression. Plant Cell **18:** 1292–1309

Salmeron J, Herrera-Estrella LR (2006) Fast-forward genomics for improved crop production. Curr Opin Plant Biol **9:** 177–179

Salter MG, Franklin KA, Whitelam GC (2003) Gating of the rapid shade-avoidance response by the circadian clock in plants. Nature **426:** 680–683

Shin R, Park JM, An JM, Paek KH (2002) Ectopic expression of *Tsi1* in transgenic hot pepper plants enhances host resistance to viral, bacterial, and oomycete pathogens. Mol Plant Microbe Interact **15:** 983–989

Silverstone AL, Sun T (2000) Gibberellins and the green revolution. Trends Plant Sci **5:** 1–2

Singh K, Foley RC, Onate-Sanchez L (2002) Transcription factors in plant defense and stress responses. Curr Opin Plant Biol **5:** 430–436

Steindler C, Matteucci A, Sessa G, Weimar T, Ohgishi M, Aoyama T, Morelli G, Ruberti I (1999) Shade avoidance responses are mediated by the ATHB-2 HD-zip protein, a negative regulator of gene expression. Development **126:** 4235–4245

Strauss SH (2003) Genetic technologies. Genomics, genetic engineering, and domestication of crops. Science **300:** 61–62

Tuberosa R, Salvi S (2006) Genomics-based approaches to improve drought tolerance of crops. Trends Plant Sci **11:** 405–412

Umezawa T, Fujita M, Fujita Y, Yamaguchi-Shinozaki K, Shinozaki K (2006) Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future. Curr Opin Biotechnol **17:** 113–122

Valliyodan B, Nguyen HT (2006) Understanding regulatory networks and engineering for enhanced drought tolerance in plants. Curr Opin Plant Biol **9:** 189–195

Van Buskirk HA, Thomashow MF (2006) Arabidopsis transcription factors regulating cold acclimation. Physiol Plant **126:** 72–80

Van Camp W (2005) Yield enhancement genes: seeds for growth. Curr Opin Biotechnol **16:** 147–153

Vinocur B, Altman A (2005) Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. Curr Opin Biotechnol **16:** 123–132

Vollbrecht E, Veit B, Sinha N, Hake S (1991) The developmental gene *Knotted-1* is a member of a maize homeobox gene family. Nature **350:** 241–243

Wang RL, Stec A, Hey J, Lukens L, Doebley J (1999) The limits of selection during maize domestication. Nature **398:** 236–239

Wang Y, Li J (2006) Genes controlling plant architecture. Curr Opin Biotechnol **17:** 123–129

Xiong Y, Liu T, Tian C, Sun S, Li J, Chen M (2005) Transcription factors in rice: a genome-wide comparative analysis between monocots and eudicots. Plant Mol Biol **59:** 191–203

Xu ZS, Xia LQ, Chen M, Cheng XG, Zhang RY, Li LC, Zhao YX, Lu Y, Ni ZY, Liu L, et al (2007) Isolation and molecular characterization of the *Triticum aestivum* L. ethylene-responsive factor 1 (*TaERF1*) that increases multiple stress tolerance. Plant Mol Biol **65:** 719–732

Yanagisawa S, Akiyama A, Kisaka H, Uchimiya H, Miwa T (2004) Metabolic engineering with Dof1 transcription factor in plants: improved nitrogen assimilation and growth under low-nitrogen conditions. Proc Natl Acad Sci USA **101:** 7833–7838

Yi K, Wu Z, Zhou J, Du L, Guo L, Wu Y, Wu P (2005) *OsPTF1*, a novel transcription factor involved in tolerance to phosphate starvation in rice. Plant Physiol **138:** 2087–2096

Yi SY, Kim JH, Joung YH, Lee S, Kim WT, Yu SH, Choi D (2004) The pepper transcription factor *CaPF1* confers pathogen and freezing tolerance in Arabidopsis. Plant Physiol **136:** 2862–2874

Zhang H, Forde BG (1998) An Arabidopsis MADS box gene that controls nutrient-induced changes in root architecture. Science **279:** 407–409

Zhang JY, Broeckling CD, Blancaflor EB, Sledge MK, Sumner LW, Wang ZY (2005) Overexpression of *WXP1*, a putative *Medicago truncatula* AP2 domain-containing transcription factor gene, increases cuticular wax accumulation and enhances drought tolerance in transgenic alfalfa (*Medicago sativa*). Plant J **42:** 689–707

Zhang JY, Broeckling CD, Sumner LW, Wang ZY (2007) Heterologous expression of two *Medicago truncatula* putative ERF transcription factor genes, *WXP1* and *WXP2*, in *Arabidopsis* led to increased leaf wax accumulation and improved drought tolerance, but differential response in freezing tolerance. Plant Mol Biol **64:** 265–278

Zhang JZ (2003) Overexpression analysis of plant transcription factors. Curr Opin Plant Biol **6:** 430–440

Zhang JZ, Creelman RA, Zhu JK (2004) From laboratory to field. Using information from Arabidopsis to engineer salt, cold, and drought tolerance in crops. Plant Physiol **135:** 615–621

Zhou J, Tang X, Martin GB (1997) The Pto kinase conferring resistance to tomato bacterial speck disease interacts with proteins that bind a *cis*-element of pathogenesis-related genes. EMBO J **16:** 3207–3218

Zhu XG, de Sturler E, Long SP (2007) Optimizing the distribution of resources between enzymes of carbon metabolism can dramatically increase photosynthetic rate: a numerical simulation using an evolutionary algorithm. Plant Physiol **145:** 513–526

Zuo KJ, Qin J, Zhao JY, Ling H, Zhang LD, Cao YF, Tang KX (2007) Overexpression *GbERF2* transcription factor in tobacco enhances brown spots disease resistance by activating expression of downstream genes. Gene **391:** 80–90