Drought tolerant crops: Assessing 20 years' plant biotechnology efforts in crop improvement

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Since the dawn of modern biotechnology, public and private enterprises have pursued the development of a new breed of drought tolerant crop products. However, only a few such products have reached the market due to technical and market constraints. Advances in the biology of plant water management reveal new opportunities to improve crop response to water deficit and new genome-based tools promise to usher in the next era of crop improvement.

Biotechnology is considered one of the most promising ways to develop new cultivars with a substantially improved tolerance to water deficit. In this context, biotechnology encompasses the introduction of transgenes that directly affect plant water use. This is also known as genetic engineering, genetic modification (GM), and the creation of genetically modified organisms (GMOs).

Defining drought

Drought or water deficit is a consequence of the environment which varies across place and time. No two environments are exactly alike, and weather conditions change throughout growing seasons. The impact of water deficit on crop productivity varies with respect to crop, when it occurs in the crop cycle, and its duration and intensity.

Genetics that provide durable resistance to water deficit are likely already fixed in commercial germ plasm. Many approaches have been developed to simulate water deficit. These included imposing osmotic stress with chemicals such as sodium chloride or polyethylene glycol (PEG), among others.

The challenge is to define a practical water deficit problem that is compelling enough to initiate a product development project. The initial hypothesis needs to be granular enough to connect metabolism to the desired phenotype.

One approach hypothesises that there is a class of genes that confers drought tolerance when expressed using the CaMV 35 promoter in plants exposed to water deficit, but it does not impact productivity in well-watered environments. The bacterial cold shock protein in Monsanto's DroughtGard® trait is an example, and given the intensity of their drought research programme, the evidence suggests these genes are extremely rare.

Another approach only considers the impact of water deficit during early reproductive development. Most crop production incorporates a package of technologies to make it as efficient and profitable as possible.

Trait gene identification

A basic outline of the drought tolerant trait development process can be seen in *Figure 1*. Much research has been focussed on the discovery of genes that might form the basis for drought trait development.

Early genes were identified in forward/reverse genetic screens in model organisms such as *Arabidopsis* and rice. Genomics opened entire genomes to evaluation with respect to drought.

Large-scale differential expression analysis using micro-arrays and DNA sequencing technology identified thousands of genes that respond to water deficit. This began with direct sequencing of cDNA libraries to assemble large-scale transcriptome databases that formed the basis for micro-array technology. The draft rice genome extended the ability to probe for drought tolerant genes on a genome scale.

The role of microRNAs in drought response is also being studied on a genome scale. The accumulated data enable assembly and interrogation of drought responsive gene regulatory networks. This body of work led to the identification of candidate genes for the development of drought tolerant traits.

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While the data provide insight into genes involved in plant response to water deficit, their application to drought response in production environments is not firmly established. Examples of drought responsive candidate genes include transcriptional regulators such as dehydration-responsive element-binding (DREB) protein, the feast/famine signalling kinase (SnRK1), and ABA receptors.

The DREB1/CBF transcription factor was identified for its ability to bind a drought responsive regulatory element in response to water deficit and its ability to bind C-repeats in cold responsive promoters. It was an early candidate for drought tolerant trait development, but overexpression using the CaMV 35S promoter caused pleotropic growth defects.

SnRK1 was shown to be a key regulator of the feast/famine response in *Arabidopsis*, affecting at least 1 000 genes. This activity extends to wheat, and it was later found that, in certain circumstances, the sugar metabolite trehalose-6-phosphate is an allosteric effector of SnRK1 activity directly linking the trehalose pathway to stress response. This may explain the mechanism by which expressing a trehalose-6-phosphatase in very young maize ears improves productivity when drought is imposed during reproductive development.

The ABA signalling pathway is central to plant water status, including response to water deficit. ABA sensitivity can be tuned by overexpressing ABA receptors, which can be leveraged to increase water productivity in *Arabidopsis*. However, there appears to be a threshold after which increased ABA sensitivity reduces yield by restricting growth.

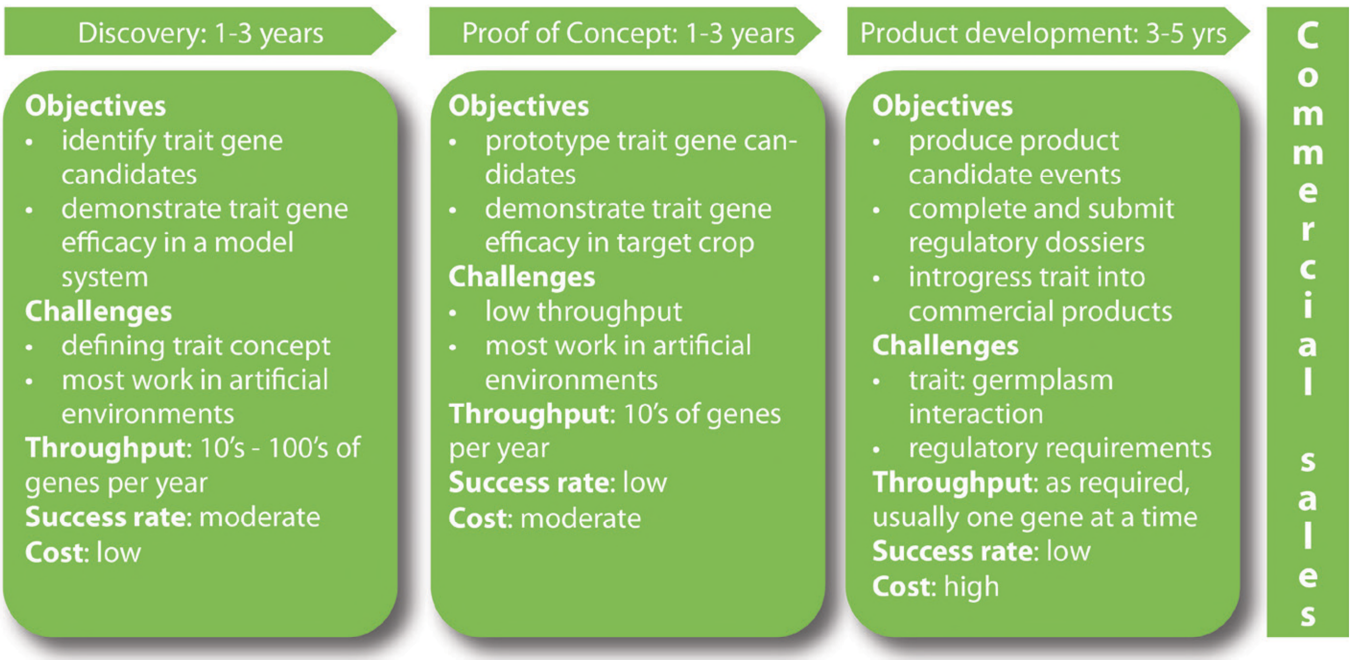
Signalling is initiated when ABA receptors bind ABA and undergo a conformational change that enables them to bind and inhibit the activity of clade A PP2Cs, which dephosphorylate a sub-group of stress activated SnRK2 kinases under basal conditions.

The ABA-mediated inhibition of PP2C activity leads to SnRK2 activation and subsequent phosphorylation of downstream effectors and activation of ABA signalling. A mutagenesis screen identified conserved amino acids in ABA receptors that alter their sensitivity to ABA and these mutants provide new tools for rationally modulating crop ABA sensitivity and water productivity by genome engineering approaches.

Directed mutagenesis even reprogrammed receptor ligand specificity so that ABA receptors could be controlled by a crop protection chemical, which opens the door to chemically modulating transpiration using existing agrochemistry.

Many attempts to improve drought tolerance in transgenic plants result in plants that survive an often-extreme drought. However, this survival is usually achieved because the engineered plant is growing more slowly than controls with less leaf area and lower stomatal conductance. Survival or resilience as a crop trait is not

Figure 1: General outline of a pipeline to develop drought tolerant GM trait technology.



acceptable if it means that productivity under good growing conditions is lower.

Monsanto's DroughtGard® trait consists of the *Bacillus subtilis* cold shock protein B (cspB) fused to a rice actin promoter, and an *Agrobacterium tumefaciens* transcript 7 terminator in event MON87460.

In bacteria, cspB is an RNA chaperone that regulates translation in response to cold stress, an adaptation to cold shock. The cspB trait gene confers tolerance to high light plus cold in *Arabidopsis* and heat, cold, and drought in rice. It also confers drought tolerance in maize and contributes positively to chlorophyll content and photo-assimilation.

This required an intact RNA binding domain indicating that the mode of action is at the RNA level. At the physiological level, the DroughtGard® trait reduces leaf growth which decreases water use and makes more water available during the critical flowering period.

Trait gene expression

It is widely accepted that genetic programmes that respond to abiotic stress such as water deficit redirect metabolic energy. This usually reduces productivity. Drought response programmes are therefore under tight control and typically express only transiently.

Most drought tolerant transgenes slow growth as described above when overexpressed this way. Drought-responsive promoters have also been described. This is not limited to drought research. There has been comparatively little work on the development and characterisation of promoters that respond to abiotic stress, relative to candidate gene identification. Detailed analyses of genes that respond to water deficit provide an opportunity to develop synthetic promoters to drive drought tolerant genes.

Next wave biotechnology products

The advent of new technologies based on genome science promises to expand the biotechnology toolkit for drought tolerant research. Additionally, knowledge of the mechanisms that contribute to crop drought tolerance have led to the development of chemical applications.

The continued advancement of DNA sequencing and plant phenotyping technology increases the capacity to detect genetic components associated with crop response to water deficit. Significant evidence suggests that variation associated with gene regulation underlies many drought-tolerant quantitative trait loci. Long-read DNA sequencing technology is addressing the repetitive nature of many crop genomes, enabling quick and inexpensive assembly of ever-larger DNA contigs.

Modern genomic tools have the potential to identify key regulators of plant water management which earlier tools have missed. Taken together, these capabilities promise to advance our ability to define and develop effective drought tolerant trait technology.

Genome editing is a relatively new and exciting technology to directly manipulate important crop genes. Most work continues to rely on traditional transgene technology to introduce gene editing reagents, but work to circumvent this dependency is underway. By directly manipulating critical genes in crop response to water deficit, gene editing may prove a far more effective approach than traditional GM technology.

Knowledge of the biological mechanisms involved in crop response to water deficit also reveals opportunities to manage crop response to water deficit using chemistry.

ABA receptors were discovered in a chemical genetic screen using pyrabactin, a selective ABA agonist. ABA has potential as an excellent tool to manage crop response to water deficit, but it is costly to produce and metabolically and environmentally unstable. Chemical library screens identified quinabactin, a novel ABA agonist that addresses ABA's liabilities as an agrochemical and provides new avenues for dynamically tuning crop water consumption throughout a growing season.

The quinabactin structure was further explored for molecules with improved properties identifying promising new variants. Modification of four amino acids in AtPYR1 enabled high-affinity binding to an agrochemical without losing its signal transduction activity.

Furthermore, novel caged derivatives of trehalose-6-phosphate were shown to improve grain size in wheat. This is complementary to genetic strategies as the target sites manipulated by these new molecules are highly conserved across angiosperms.

Solution of combined capabilities

The road to commercialising drought tolerant biotechnology products goes far beyond the identification of genes. It begins with careful consideration of the problem to be solved.

Drought tolerance is an oversimplification of the challenges that water deficit imposes on crop production. Each crop and crop production system is unique with regard to the impact water deficit has on productivity and profitability. The more accurately the water deficit problem can be defined, the likelier a tractable drought tolerant trait can be developed.

Biotechnology trait developers must be aware of the many possible solutions available to producers to effectively position drought tolerant traits. Genomics and the development of genome editing have the potential to greatly expand the plant breeding toolkit. Modern genomic tools have the potential to identify key regulators of plant water management which earlier tools have missed. Taken together, these capabilities promise to advance our ability to define and develop effective drought tolerant trait technology.

When work began, researchers did not know how to confer drought tolerance in crops. Current achievements and technology advancement provide significant opportunities to further improve crop drought tolerance. Continued efforts by public and private research groups will no doubt lead to exciting new products.

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