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Plant Science Special Issue 'Synthetic Biology Meets Plant Metabolism'

Title:

Where are the drought tolerant crops? An assessment of more than two decades of plant biotechnology effort in crop improvement

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Highlights
- The significant global industry investment in the application of genetic engineering technology to improve plant response to water deficit has so far produced few products.
- Significant progress has been made in the identification of genes that have a positive effect on plant response to water deficit.
- There remains a significant disconnect between simulated water deficit in pots and the impact of water deficit on crop productivity.
- Field research confines drought tolerance trait evaluation to one season per hemisphere per year, which slows progress.
- The cost of bringing a GM product to market limits the development of drought tolerance GM traits to large acreage crops like corn which has a high likelihood of generating a positive return on investment.

Abstract
Since the dawn of modern biotechnology public and private enterprise have pursued the development of a new breed of drought tolerant crop products. After more than 20 years of research and investment only a few such products have

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reached the market. This is due to several technical and market constraints. The technical challenges include the difficulty in defining tractable single-gene trait development strategies, the logistics of moving traits from initial to commercial genetic backgrounds, and the disconnect between conditions in farmer's fields and controlled environments. Market constraints include the significant difficulty, and associated costs, in obtaining access to markets around the world. Advances in the biology of plant water management, including response to water deficit 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. As biotechnology looks to improve crop productivity under drought conditions, the environmental and food security advantages will influence public perception and shift the debate toward benefits rather than risks.

Keywords
Drought tolerance, GMO, crop genetic engineering, agricultural biotechnology

1. Introduction

Drought and water use efficiency are important factors that contribute to agricultural productivity worldwide [1]. Most cropland is rain fed leaving overall productivity to less predictable weather patterns. Furthermore, increasing global temperatures introduce additional uncertainty [2]. In addition, there is a push to maintain or increase productivity in an environmentally sustainable way [3]. Finally, the rate of productivity improvement for many crops is also in decline [4]. All this is happening in a world that is projecting nearly 2 billion more mouths to feed in the next 30 years [5].

Researchers and growers around the world recognize that water is the single most important abiotic factor limiting crop productivity [6]. Biotechnology is considered one of the most promising ways to develop new cultivars with a substantially improved tolerance to water deficit [7,8]. In this context, biotechnology encompasses the introduction of transgenes that directly affect plant water use, much in the way transgenes were used to enable herbicide tolerance or insect resistance. This is also known as genetic engineering, genetic modification (GM) and the creation of genetically modified organisms (GMOs), one of many gene-based technologies being applied to crop improvement [9].

Industry research is not often published in peer-reviewed literature, but the few examples highlight research to develop drought tolerance traits for corn [10–15]. Despite significant effort, Monsanto's DroughtGard® is the only drought tolerant corn biotechnology product on the market [10,16]. DroughtGard® has not had a significant impact in the marketplace and does not appear to exhibit an advantage over non-GM efforts to improve drought tolerance [17] that would justify the cost of registration and research. Additional drought tolerance biotechnology products include Verdeca's HB4 soybean [18] which is in the regulatory approval process in the U.S. (https://www.aphis.usda.gov/brs/aphisdocs/17_22301p.pdf) and Argentina, and PT Perkebunan Nusantara XI's NXI-4T sugarcane which is approved

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for cultivation in Indonesia [19]. Other drought tolerance products developed using molecular marker assisted breeding include Dow-DuPont 's AQUAmax® [20] and Syngenta's Artesian® [21] product lines.

The lack of products does little to illustrate what has been accomplished. It is no small feat to develop a drought tolerant GM product with measurable performance in the field. The above results demonstrate that GM technology can contribute to improving crop response to water deficit [22]. It also indicates that metabolic processes that contribute to crop drought tolerance are not fully understood. New screens will be necessary to identify genes that significantly contribute to these important plant mechanisms.

The lack of marketable products despite significant investment is not lost on the scientific community [23,24]. Many have openly questioned the way these products are developed [23], and recent presentations to investors suggests that some companies have shifted their research and development investments to other areas. This review examines the pursuit of drought tolerant or water use efficient crops through application of biotechnology. While there are significant technical hurdles, the global regulatory environment also imposes constraints that affect the way research is conducted. The presence of GMO products in the food supply chain remains a flash point in many countries [25–28]. This is well-understood by industry research teams. New plant breeding innovations, such as genome editing, offer promising opportunity to advance the development of drought tolerant products [29]. While some government agencies such as the United States Department of Agriculture have issued responses to letters of inquiry from developers seeking clarity on the regulated status of their products, the global regulatory landscape remains uncertain about genome-edited plants.

2.1 Research to improve crop drought tolerance

Both public and private investment in drought tolerant crops saw a steady increase through the 1990's and early 2000's. Figure 1 is an examination of PUBMED for articles with the terms 'drought' and 'plant' in the Abstract/Title since 1990. There is a rapid increase in publications starting in the early 2000's which continues to this day. This broadly captures the field of drought research. The term 'gene' was added to the search to narrow focus toward biotechnology research which is often gene centric. These publications rose from less than 1% in the early 1990s to 46% in 2005-2010. The results indicate that while drought research continues to increase, the share of published research attributed to biotechnology has fallen to just over 36%.

The level of commercial drought research can be estimated in a similar way. A search of U.S. Patent Applications, in Figure 2, with the same terms in the Claims section indicates a sharp rise in applications from 2001 to 2003 which remains steady then rises in 2009 and again in 2013. The share attributed to biotechnology rose to nearly 45% in 2009 and has since declined to 25% today. Granted U.S. Patents, in Figure 3, follow a similar trend. This rather crude assessment of drought

research is meant to reveal trends. A fair question is why hasn't biotechnology research resulted in more drought tolerant products? To address this, we examined the research produced by industry and academic groups and discuss the similarities and differences.

2.2 Defining Drought

Drought or water deficit means many things to growers [30]. It is a consequence of the environment which varies with place and time. No two environments are exactly alike and weather conditions on a farm change throughout and across 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/intensity. Genetics that provide durable resistance to periods of water deficit are likely already fixed in commercial germplasm or are the subject of on-going breeding programs [31–33]. Molecular biologists and biotechnologists initially sought to apply the basic strategy that drove the development of commercial insect control and herbicide tolerance traits. This often began with proxy or surrogate assays, such as withholding water from potted plants, often conducted in controlled environments [22,34].

Many approaches were developed to simulate water deficit. They ranged from imposing osmotic stress with chemicals like sodium chloride or polyethylene glycol (PEG) or withholding water for a period of time or until differences between experimental and control groups where obvious. The latter evolved into the common practice of applying a lethal drought, where most control plants die and most traited or transgenic plants survive the treatment. Many studies identified genes with activity in these assays and several papers discuss the relevance of this research to water deficit in a production environment (reviewed in [23]). But the discovery process begins with 100's to 1000's of genes to test and surrogate assays are the most efficient way to conduct an initial evaluation [22]. This is of particular importance if drought tolerance is one of many traits in a biotechnology program [35]. Industry groups often employ research strategies to control costs and maximize throughput/efficiency which are often referred to as platforms. The investment in platforms typically makes it necessary for the research to fit the platform. The limitations of a discovery pipeline approach do not only apply to agriculture, similar issues affected success in pharmaceutical discovery [36], particularly in translation from early discovery to clinical trials (the so-called Valley of Death) [37].

The challenge is defining a practical water deficit problem that is compelling enough to initiate a product development project. This informs all the downstream work, including the crop to focus on, what genes to work on, how to express candidate genes and how to evaluate their effect. The initial hypothesis needs to be granular enough to connect metabolism to the desired phenotype, and many drought researchers know that this is non-trivial. One approach hypothesizes that there is a class of genes that confer drought tolerance when expressed using the CaMV 35 promoter [38] in plants exposed to water deficit but, do not impact productivity in well-watered environments. The bacterial cold shock protein [10] in Monsanto's

DroughtGard® trait is an example, and given the breadth and depth of their drought research program the evidence suggests these genes are extremely rare. Another approach only considers the impact of water deficit during early reproductive development [14]. Most crop production incorporates a package of technologies to make it as efficient and profitable as possible. Many modern traits are based on single genes, but it might be possible to extend this to a few genes per trait. Ideally a drought tolerance trait complements other technologies and focuses on problems not easily addressed by other approaches. The obvious advantage of biotechnology is its ability to introduce novel genetic information. Another advantage is its modularity, for example the ability to create novel combinations of regulatory sequence and protein coding sequence.

2.3 Crop Choice for Drought Tolerance Traits

Biotechnology trait development is an expensive enterprise [35]. It is not cheap or easy to produce transgenic crops, and many crops cannot be easily transformed. A recent assessment estimates, based on successful trait development, that more than 19 cumulative years are required from Early Discovery to the marketplace, including 37% of the total timeline for regulatory science and registration activities [39]. Furthermore, the total cost to bring a GM trait to the marketplace is greater than $136 million [35,39], this limits the choice to commodity crops like corn, soy, rice, wheat and canola. The potential market size must be large enough to justify this investment. Not all consumers want transgenic products directly in their food, which further limits options to crops that are primarily used to feed livestock.

It is difficult to determine how successful a drought tolerance trait might be. It's very easy to argue that crop production requires an enormous amount of humanity's fresh water supply [40]. Most authorities suggest that agriculture requires around 70% of the fresh water supply [41,42]. This is primarily due to the physiological demands of crop growth and development. Several models have been developed to help growers understand the relationship between the water supply and crop productivity [43,44]. Most water is stored in the soil and augmented by seasonal rainfall. In dry regions irrigation provides water needed to grow a crop. Water deficit impacts crop productivity when demand outstrips supply. A confounding factor is that most crop breeding programs aim to maximize productivity when inputs like water are not limiting. This can result in germplasm that is highly productive when water is plentiful but does poorly when water is limiting [45]. It's important to know if growers will pay a premium for drought tolerance traits, and this varies with crop and region.

Most companies cannot justify the development of drought tolerance traits on crops with only small or local markets. In addition, the route to market depends on the crop. Commodity crops, developed using biotechnology, typically require government approval not only in the country where the crop will be grown but also in all major import countries with functional regulatory systems for agricultural biotechnology. This is because commodity crop products enter global distribution networks, often starting at local grain elevators and many importers have strict

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guidelines for biotechnology-based grain. Larger seed developers have programs to secure import permits for new biotechnology traits. The expense associated with global market access, limits biotechnology traits like drought tolerance to crops like corn. It is the crop that many companies consider an entry point for drought tolerance traits and is the major focus of this review.

3.1 Trait Gene Identification

A basic outline of the drought tolerance trait development process is in Figure 4. Much public and private research focused on the discovery of genes that might form the basis for drought trait development [7,8,46]. Liang [5] provides a comprehensive summary of recent reviews. Early genes were identified in forward/reverse genetic screens in model organisms like Arabidopsis and rice. Genomics opened entire genomes to evaluation with respect to drought.

Large-scale differential expression analysis using microarrays and DNA sequencing technology identified 1000's 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 microarray technology [47–49]. The draft rice genome extended the ability to probe for drought tolerance genes on a genome scale [50]. This has been extended to more than 100 plant species in the last 15 years [51]. Several approaches to interrogate microarray data were developed [52,53]. The role of microRNAs in drought response is also being studied on a genome scale [54]. The accumulated data enable assembly and interrogation of drought responsive gene regulatory networks [55,56]. This body of work led to the identification of candidate genes for the development of drought tolerance traits. Many of these studies were conducted on plants subjected to artificial water deficits. 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 signaling 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, independently its ability to bind C-repeats in cold-responsive promoters. It was an early candidate for drought tolerance trait development, however over expression using the CaMV 35S promoter caused pleotropic growth defects [8,57]. SnRK1 was shown to be a key regulator of the feast/famine response in Arabidopsis, affecting at least 1000 genes [58]. 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 [59,60]. 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 [14]. The ABA signaling pathway is central to plant water status, including response to water deficit [61]. ABA sensitivity can be tuned by over-expressing 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 [62]. Signaling 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 subgroup 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 signaling [61]. 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 [63,64]. 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 [65]. This is by no means comprehensive, rather it illustrates how some drought tolerance genes investigated by industry were identified.

Many focus on model plants grown in controlled environments and subject to an artificial form of water deficit. While this is a logical approach to the problem it's applicability to real world crop production has been questioned [23]. 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 [66]. Interestingly, high transpiration in the field - not lower as would be seen in engineered - plants is used as a selection criterion to identify high-yielding wheat genotypes and as an important predictor of yield performance under drought [67]. Survival or resilience as a crop trait is not 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 terminater in event MON87460 (http://www.isaaa.org/gmapprovaldatabase/event/default.asp?EventID=98). In bacteria, cspB is an RNA chaperone that regulates translation in response to cold stress, an adaptation to cold shock [68]. The cspB trait gene confers tolerance to high light plus cold in Arabidopsis and, heat, cold and drought in rice [10]. It also confers drought tolerance in maize, and contributes positively to chlorophyll content and photoassimilation [10]. This required an intact RNA binding domain indicating the mode of action is at the RNA level [10]. At the physiological level the DroughtGard® trait reduces leaf growth which decreases water use and makes more water available during the critical flowering period [16]. This increases ear growth and improves productivity, particularly during water deficit. This is a rare example of a stress tolerance gene discovered in a model plant using controlled conditions, that also confers drought tolerance in maize in a field environment.

3.2 Trait Gene Expression

It is widely accepted that genetic programs that respond to abiotic stress like water deficit redirect metabolic energy. This usually reduces productivity. Drought response programs are therefore under tight control, and typically express only transiently. Many papers describing transgenic plants expressing a drought tolerance gene typically express that gene using strong constitutive promoters such as the CaMV 35S [38], maize ubiquitin [69] or rice actin [70]. It's likely that only a few drought tolerance trait genes work well in this context. Most drought tolerance transgenes slow growth as described above when over expressed this way. Drought-responsive promoters have also been described [71,72]. The lack of promoters is likely one of the most important constraints on drought tolerance trait development [73].

This is not limited to drought research. There has been comparatively little work on the development and characterization of promoters that respond to abiotic stress, relative to candidate gene identification. A review of 77 rice promoters lists just a few that respond to water deficit [74]. Researchers are also exploring construction of synthetic promoters from well-characterized component parts [75–77]. Detailed analysis of genes that respond to water deficit provide opportunity to develop synthetic promoters to drive drought tolerance genes [78]. Other research demonstrates how modern genomic information can be leveraged to generate promoters from almost any plant gene [79]. The lack of diversity with respect to trait gene expression tools may explain why more effective trait genes have not been discovered. In trait development, promoters are at least equal to coding sequence with respect to their role in expressing the trait, and each trait gene may require unique expression control for optimal activity. One explanation may be that companies consider trait gene discovery to be the most important aspect of the drought trait development problem, at the expense of their regulation, and prefer well-characterized promoters such as the constitutive regulatory elements listed above to the challenge of developing novel promoters. Also, it may prove difficult to identify targeted promoters that are active in all the necessary environments.

3.3 Gene Evaluation

Effective trait gene discovery programs aim to cover as much ground as possible, as efficiently as possible. Like most transgenic trait development programs, transgene evaluation begins in controlled environments [22,35]. For drought tolerance trait candidates this translates to a primary evaluation step in pots. Common transgenic traits like insect control and herbicide tolerance traits afford the opportunity to evaluate trait gene activity/efficacy on primary transformants. This is not possible for drought tolerance traits, where significant replication and stringent trial design are required to measure the effect of a gene in a drought tolerance experiment. Also, some drought assays require entries to be homozygous for the trait gene, so it may take up to two generations before candidate transgenic events are assayed. During this period candidate events are selected based on molecular attributes such as the quality and copy number of the transgene, and transgene expression which is usually carried out at the RNA transcript level. Generally, multiple events per transgene construct are produced because molecular characterization and fertility

issues can eliminate up to half the events before the first drought evaluation is conducted [10,12,14].

The controlled environment drought tolerance assays used by industry reflect those reported elsewhere in the literature. The general principle is to determine if a meaningful difference in a drought tolerance attribute, such as a change in transpiration or growth when water is limited, can be assigned to the transgene. These assays are conducted in dedicated facilities that are usually located near transformation labs, for logistical purposes and to minimize the paperwork required for transport. In the U.S., GM events are subject to interstate movement and environmental release guidelines set forth by the USDA. For this reason, Syngenta evaluates 10-15 distinct events per construct in controlled environments and advancement requires that most of them express the attribute. Those that don't are set aside, and this is where work stops on most constructs/drought gene candidates. It is unfortunate that most data produced in these pipelines are not accessible to the scientific community. It's likely that many of the drought tolerance candidate genes described in the scientific literature have been evaluated for their ability to confer drought tolerance in one or more crops. But industry research groups have no compelling incentive to publish pipeline data.

Product candidate events, no matter the trait, are regulated as transgenic organisms, which places strict guidelines on how these plants and their seed are managed. In particular when and where they can be planted. Regulated crops cannot contaminate commercial crops and there are two primary ways to reduce this risk, spatial and/or temporal isolation. Guidelines for spatial isolation dictate that there must be anywhere from several hundred meters to several kilometers distance from the nearest sexually compatible crop, depending on the jurisdiction. Temporal isolation requires that the regulated crop be planted at a time that insures it reaches sexual maturity substantially before or after sexually compatible crops in the vicinity. This means it must be planted a few weeks before or after non-regulated crops. Since modern cultivars are bred to take full advantage of the growing season this usually means the regulated crop cannot be taken to full maturity to measure trait gene effects on production attributes like yield. Spatial isolation is more practical but still presents challenges. An ideal environment for evaluating corn is typically in regions where corn is widely grown, which can make it difficult to achieve the necessary isolation distances.

The first step in field evaluation is to produce the seed that will be planted. For corn this is usually done in continuous nurseries just before the field trial season. The hybrid tester contributes genetic attributes that are appropriate to the region where the trial will be conducted, and perhaps other useful traits such as insect control and herbicide tolerance. It's important that trial seed are produced in the same nursery, at the same time. This ensures that the environment where the seed are produced is not a variable in the analysis. Also, most seed are produced by manual pollination which can be very costly depending on the crop, the size of each trial and the number of trials.

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Drought research requires that the environment be one in which water the crop receives can be controlled. Rainout shelters are useful but they are expensive and significantly constrain the planting area. More often companies select regions that receive little rainfall during the growing season and, where the local climate and soils reflect conditions found in production regions. One such region is the Central Valley of California. Ideally these farms are outfitted with state of the art drip irrigation systems to simulate a variety of water regimes throughout the crop cycle.

Regulated crops also require dedicated equipment to handle all aspects of cultivation including seed preparation, planting and harvesting. Most of these trials follow a crop destruct protocol, enabling measurement of yield attributes. This means that all the transgenic plants and their seed must be devitalized at the end of the trial. The fields must also be monitored into the next season to ensure volunteers, plants from seed that escaped the harvest, do not germinate. But if they do, the plants must be destroyed prior to seed set. This type of management system is very expensive, and is typically reserved for the top tier drought trait candidates.

The data collected in typical drought tolerance field trials are limited to a few attributes that are recorded on a plot basis. For corn these include emergence, plant density, anthesis-silking interval, a stress rating at one or more time points and yield. Sometimes ear height, plant height, barrenness, stay-green are recorded but these depend on how many times researchers are deployed to the field to collect data. Harvest data decide which traits advance, and these are usually measured by research combines, custom harvesters that process plants on a 1, 2 or 4 row basis. While these data are very meaningful to breeders and growers, they are subject to environmental variability that must be taken into account. Researchers can calculate the number of plots necessary to detect a given trait effect with high confidence but generally makeup of these trials is a compromise between the trait performance target and the available resources. The goal is to identify traits that meet predetermined performance criteria, so trials are not designed to study trait biology. That information becomes necessary when a trait gene shows promise in the field, which is rare.

Ideally, each candidate transgenic event is evaluated over 2-3 seasons in multiple locations. Often, results come from one season in the northern hemisphere and one in the southern. The large agricultural biotechnology companies operate several research farms throughout the world. Smaller companies and the public sector typically lack the resources to conduct production-based field trials on drought tolerance traits, which constrains progress toward understanding how drought tolerance traits behave in real grower environments.

If a candidate gene is selected for product development, this triggers a new round of transformations in which the transformation construct is remade to commercial standards and thousands of events are typically produced to identify lead and backup events [35]. These have a molecular profile that generally consists of a

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single-intact copy of the transgene with high activity in a non-disruptive region of the genome. These events are introgressed into commercial lines and evaluated for activity at locations where the trait will be marketed. This multi-year effort includes the preparation and submission of regulatory dossiers to the appropriate authorities to ensure that the necessary permissions are in place when the trait is ready for market.

Due to cost and complexity, this evaluation is often reserved for commercial candidates only. Trait genes do fail during this phase of the product development cycle. A common cause is the lack of commercial levels of efficacy in the appropriate germplasm. In corn it is nearly impossible to predict how a drought tolerance trait will interact with the underlying genetics in commercial germplasm. This is a 4-7 year process if everything goes well.

4.1 Trait Genes Verses Germplasm Improvement

An important consideration for drought tolerance trait development is germplasm improvement. Most commercial crops, and in particular crops like corn, soybean and rice are products of breeding programs. Although the rate of genetic gain is slowing most agree that it is close to 1% per year. Breeding and plant biotechnology programs take more than a decade to complete, and they often pursue similar objectives. If the goal for a drought tolerance trait is to increase yield by 10% when water deficit occurs, which is a reasonable target, it's quite possible that a trait candidate's efficacy will diminish during the product development process. The dependence of plant transformation on very specific genetic backgrounds in most species, which generally lack commercial drought tolerance adds to this problem. A recent discovery found that specific combinations of the BABYBOOM and WUSCHEL genes vastly improves plant regeneration and goes a long way toward addressing this problem [80]. Finally, drought tolerance traits may not function across all commercial germplasm. These important considerations inform how drought tolerance traits are developed, and may further explain why there are not more drought tolerance traits on the market.

It's very easy to see breeding and biotechnology programs focused on drought tolerance as competing. But, they are largely complementary. The former screens available genetic diversity and breeding materials for genetic loci that confer robust tolerance to drought. The latter introduces genetic diversity, often but not necessarily from outside the species. Drought tolerance is often one of many attributes being targeted in a breeding program [31]. Germplasm development is an ongoing process to continuously improve cultivars and it's rare for a particular line to stay on the market for more than 7 years. Finally, the genetic backgrounds that a trait might be deployed in are probably not yet in production at the discovery stage.

4.2 Government Regulation of Drought Tolerance Traits

There are two important aspects to consider when preparing to introduce a plant biotechnology product to the market: generating data demonstrating the product functions properly and establishing that the product is safe for people, livestock and

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the environment. Developers must also show how the new GMO product they wish to market will benefit society. These affect how a GM drought tolerant product is evaluated.

Regulatory systems in different jurisdictions around the world employ varying degrees of scientific evidence to make decisions about approval of biotechnology products. The typical food safety requirements for biotechnology traits, including drought tolerance, have been reviewed elsewhere [81,82]. In the United States the Coordinated Framework for the Regulation of Biotechnology brings together the United States Department of Agriculture (USDA), the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) to evaluate new products prior to market introduction. Each agency addresses different areas within their statutory authority. Other jurisdictions are driven more by politics than science. For example, the European Union (EU) have implemented a rigid interpretation of the precautionary principal into their regulation of GM products [83]. Depending on the crop and product concept, a variety of cultivation and import approvals are required to bring a new GM product to market, each with their own set of requirements. Research to address regulatory concerns adds significant time and cost to the development of new drought tolerant GM products.

4.3 Prospects for the next wave of biotechnology products
Despite the slow appearance of biotechnology-based drought tolerance products, knowledge and capability continues to grow. The advent of new technologies based on genome science promises to expand the biotechnology tool kit for drought tolerance research [51,84]. In addition, knowledge of the mechanisms that contribute to crop drought tolerance have led to the development of chemical applications [85,86]. Microbes that facilitate soil interactions which improve crop response to water deficit are being discovered [87]. Genome editing technology enables very precise gene manipulation to introduce new allelic diversity or alter endogenous gene activity [88–91]. Collectively, these are improving the ability to identify important genes involved in drought tolerance and produce meaningful advancement in product development.

The continued advancement of DNA sequencing and plant phenotyping technology increases the power to detect genetic components associated with crop response to water deficit. Significant evidence suggests that variation associated with gene regulation underlies many drought tolerance QTL [92]. Long-read DNA sequencing technology is addressing the repetitive nature of many crop genomes, enabling quick and inexpensive assembly of ever larger DNA contigs. Analytical tools to take advantage of this information continue to advance, and enable rapid identification of the genetic components that contribute to drought tolerance [93,94].

Genome editing is a relatively new and exciting technology to directly manipulate important crop genes. Tool development is principally driven by the human therapeutic sector and advances are quickly translated to plant/crop applications [95–97]. Most work continues to rely on traditional transgene technology to

introduce gene editing reagents but work to circumvent this dependency is underway [98–100]. Many examples demonstrate the ability of gene editing technology to knockout gene activity [101–103], create new diversity [104,105], introduce novel traits [100,106,107] and replace genes [108]. Techniques to introduce base changes or new genetic information are less efficient but many groups are working on improvements [109,110]. By directly manipulating critical genes in crop response to water deficit, gene editing may prove a far more effective approach relative to traditional GM technology. It also has the potential to lower the regulatory hurdle of traditional GM technology and encourage more groups to create and test genome edited plants.

Knowledge of the biological mechanisms involved in crop response to water deficit also reveal opportunity to manage crop response to water deficit using chemistry. ABA receptors were discovered in a chemical genetic screen using pyrabactin, a selective ABA agonist [111]. ABA has potential to be 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 [64]. The quinabactin structure was further explored for molecules with improved properties identifying promising new variants [112,113]. Modification of four amino acids in AtPYR1 enabled high-affinity binding to an agrochemical without losing its signal transduction activity [65]. In addition, novel caged derivatives of trehalose-6-phosphate were shown to improve grain size in wheat [114]. This exciting new area is just beginning to be explored and is complementary to genetic strategies as the target sites manipulated by these new molecules are highly conserved across angiosperms.

5. Conclusions

The road to commercializing drought tolerance biotechnology products goes far beyond the identification of genes that might be central to plant response to water deficit. It begins with careful consideration of the problem to be solved. Drought tolerance is an over simplification of the challenges that water deficit imposes on crop production. Each crop and crop production system is unique with respect to the impact water deficit has on productivity and profitability. The more accurately the water deficit problem can be defined, the more likely a tractable drought tolerance trait can be developed. Researchers must also be mindful of competing technologies, particularly breeding that will change the germplasm background for any trait during the course of its development. Crop management technology will also influence the environment the trait is meant to perform in.

Drought tolerance trait technology will likely be one part of an integrated crop management strategy that includes other traits like insect control and herbicide tolerance. Modern growers are technology agnostic when it comes to management tools. They will likely use any chemical, software, mechanical or genetics tool that that effectively and economically enables control of crop response to water deficit.

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Crop management is a multifaceted discipline that a drought tolerance trait must seamlessly integrate into. Biotechnology trait developers must be aware of the many possible solutions available to growers to effectively position drought tolerance traits.

Genomics and the development of genome editing has the potential to greatly expand the plant breeding tool kit. But it remains to be seen what regulatory hurdles genome edited products will face. It is clear that modern genomic tools have the potential to identify key regulators of plant water management that earlier tools missed. Taken together these capabilities promise to advance our ability to define and develop effective drought tolerance trait technology.

An unfortunate reality with respect to industry research is that much of it, particularly discovery research, remains unpublished. This is mainly because companies do not want to inform their competition of ongoing R&D activities and they need to protect intellectual property. Some industry research is published as a result of public/private partnerships, a few of which are supported through government funding schemes. Perhaps governments could encourage publication of R&D results through creative tax incentive programs similar to those that encourage R&D investment. Another possibility is for industry to consider early discovery research pre-competitive to encourage more open discourse. The challenge with either is insuring participation by a broad range of companies.

To answer our original question, a few drought tolerant products have reached the market, and more are in development. It is interesting to note that the only commercial drought tolerant GM product is based on an RNA chaperone [10] and that trait candidates developed by commercial researchers are based on trehalose metabolism [14], ethylene signaling [12], transcriptional regulation [11] and amino acid biosynthesis [15]. This certainly supports the complex nature of crop response to water deficit and suggests there is more than one way to engineer drought tolerance in crops. When work began, researchers did not know how to confer drought tolerance in crops. Current achievements and technology advancement provide significant reason and opportunity to further improve crop drought tolerance. Continued effort by public and private research groups will no doubt lead to exciting new products.

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Figure Legends

Figure 1. Growth of peer-reviewed literature in PubMed focused on drought tolerance. PubMed was surveyed for articles with 'drought' and 'plant' in the title/abstract. 'Gene' was added to identify articles informing biotechnology applications.

Figure 2. Growth of United States Patent applications focused on drought tolerance. The U.S. Patent Applications database was surveyed for articles with 'drought' and 'plant' in the Claims each year since 2001, the earliest year for which data are available. 'Gene' was added to identify applications focused biotechnology.

Figure 3. Issued United States Patents on drought tolerance. The U.S. Patent Issued Patents database was surveyed for patents with the terms 'drought' and 'plant' in the Claims section. 'Gene' was added to identify Patents focused on biotechnology applications.

Figure 4. General outline of a pipeline to develop drought tolerance GM trait technology. Each stage operates independent of the others. The timeline represents how an average trait gene progresses to product development. The discovery stage can benefit from automation and standardization. The proof of concept stage generally includes at least one field evaluation. Product development includes the production of 1000's of new events and more in-depth event characterization. It also includes biological studies to determine mode of action which supports governmental regulatory requirements and health/safety studies. Per sample costs

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break down as low ($1000's to 10,000's) moderate ($10,000's to 100,000's) high ($1,000,000's to 10,000,000's). Moderate success rates are around 1-5% and low success rates are below 1%.
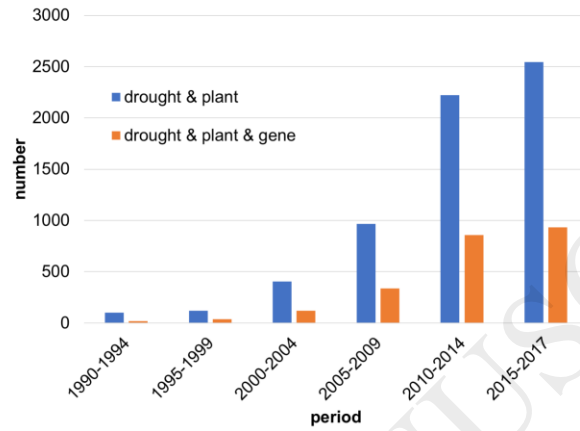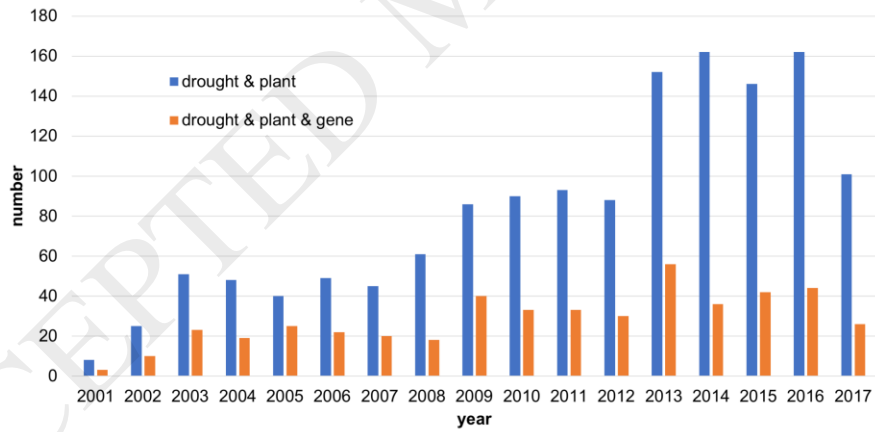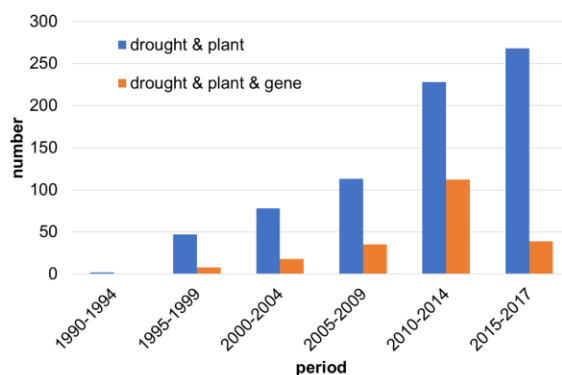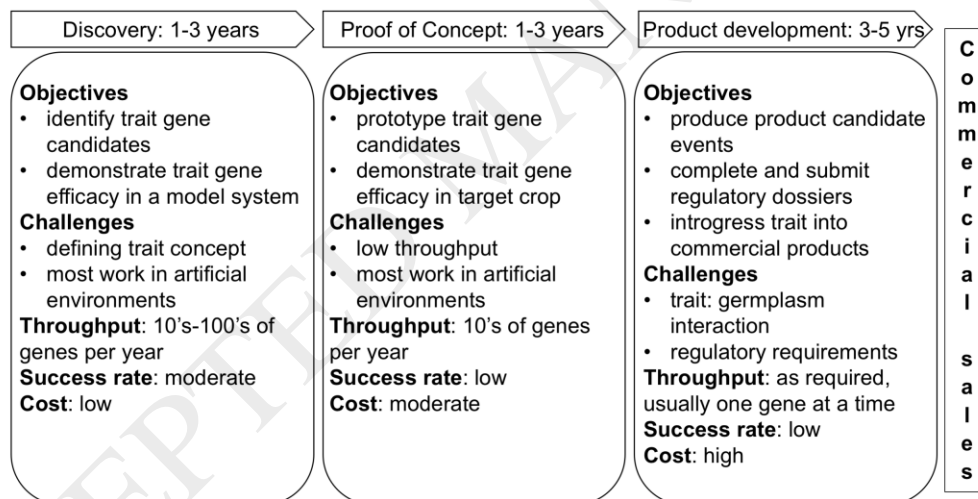

Figure 1

Figure 2

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Figure 3

Discovery: 1-3 years	Proof of Concept: 1-3 years	Product development: 3-5 yrs	Commercial sales
Objectives • identify trait gene candidates • demonstrate trait gene efficacy in a model system **Challenges** • defining trait concept • most work in artificial environments **Throughput**: 10's-100's of genes per year **Success rate**: moderate **Cost**: low	**Objectives** • prototype trait gene candidates • demonstrate trait gene efficacy in target crop **Challenges** • low throughput • most work in artificial environments **Throughput**: 10's of genes per year **Success rate**: low **Cost**: moderate	**Objectives** • produce product candidate events • complete and submit regulatory dossiers • introgress trait into commercial products **Challenges** • trait: germplasm interaction • regulatory requirements **Throughput**: as required, usually one gene at a time **Success rate**: low **Cost**: high	

Figure 4

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