Beating the heat

Despite the complexity of drought tolerance, researchers are making progress in the search for crops that can produce seed with limited water. Emily Waltz reports.

A revolution is quietly underway in the mid-West and Great Plains of the US. Following water shortages that have ravaged corn yields, the first of a new generation of drought-tolerant crops are being put to the test in the field. In March, Johnston, Iowa-based DuPont Pioneer announced that its newly developed transgenic corn, which downregulates production of the phytohormone ethylene, enhances grain yield after exposure to drought stress[1]. It could join DroughtGard maize, a variety expressing a *Bacillus subtilis* cold-shock protein made by Monsanto of St. Louis, that has already been planted on more than 200,000 ha by thousands of farmers.

With registrations elsewhere in the world—last year, Indonesia approved a sugarcane expressing choline dehydrogenase with enhanced resistance to water deprivation—and a half-dozen other transgenic approaches to drought tolerance and water use efficiency (WUE) in testing (**Table 1**), biotech is making strides in bolstering crop resistance to drought. But it may not be happening fast enough. Global population increases are putting greater demand on food, feed and water, and climate change models are predicting prolonged periods of drought and heat.

What it means to be drought tolerant

Drought-tolerant plants can withstand a finite period without water, be grown with less water or use water more efficiently. (Some make a distinction between drought tolerance and WUE, and although the two differ slightly, here 'drought tolerance' is used broadly to include both.) But for the drought-tolerant crop to be commercially useful, it has to do these things without sacrificing yield.

However, being drought tolerant does not mean the plants can grow without any water. Researchers have been careful not to suggest that genetic engineering may someday confer this miracle capability in plants, yet it has become a point of misunderstanding when communicating the benefits of biotech to the general public. "If a soybean is planted in sand with 100-degree temperatures and no water for a month, nothing is going to save it," says Eric Rey, CEO of Arcadia Biosciences in Davis, California, which has drought-tolerant rice and canola in development.

Interest in drought tolerance as a trait has been on the rise over the past decade, both in industry and academia. At least 117 field trials for drought tolerance were given the green light in 2013 by US regulatory authorities alone, up from just 29 in 2004, according to data from Information Systems for Biotechnology (ISB) in Blacksburg, Virginia, a group that tracks regulatory activity. And these numbers may not include trials of drought-tolerant plants that are categorized under a more general description or as an undisclosed phenotype.


Drought tolerant crops are making an appearance in the US.

Monsanto is currently conducting far more field trials of drought-tolerant crops than anyone else in the US, according to data from ISB (**Fig. 1**). Over the past 15 years, 64% of field trial permits or notifications have gone to Monsanto, DuPont Pioneer follows with 12% and Paris-based Biogemma, Basel-based Syngenta and Rutgers University in New Brunswick, New Jersey, have been granted 3% of field trial authorizations. More than two-thirds of these studies have been in corn, and a smaller percentage have been in cotton and soybean.

Leading the way

Most efforts to confer drought tolerance involve keeping a plant's natural responses to drought stress in check so that yields are not penalized. "A plant's natural tendency during drought is to focus less on producing a lot of grain and more on making sure a few grains survive," says David Lobell, an agricultural ecologist at Stanford University in Stanford, California. This reaction probably evolved in the plant as an adaptive mechanism: it goes into survival mode, rather than productive mode.

Monsanto's DroughtGard, the first drought-tolerant variety worldwide to be approved for market, works by quelling stress responses. The plant borrows a gene from the bacteria *B. subtilis* that encodes cold-shock protein CspB, which binds and thereby stabilizes RNA, and unfolds RNA secondary structures, which often fold in response to environmental stress. This chaperoning of RNA is thought to minimize the effects of drought on photosynthesis, stomatal conductance and carbon fixation—cellular functions that affect grain yield. "The plant acclimates to the stress more quickly and utilizes water more efficiently, leaving it with more water to help it through critical periods of growth," says John Fietsam, a technology development manager at Monsanto. "It allows the plant to put more resources toward the ear over vegetative growth."

DroughtGard (MON87460), was deregulated by the US Department of Agriculture (USDA) in December 2011. Monsanto in 2012 and 2013 made MON87460 hybrids available commercially on a limited basis to farmers in the western Great Plains states, where the climate is drier than in the Corn Belt states of the US Midwest. About 250 farmers participated during the first year, each planting no more than 40 acres along with varieties of corn that did not contain the new transgene. More than 2,000 farmers participated in the second year, planting MON87460 in a broader range of environments. In both years, the growers saw an average yield increase of five bushels per acre during drought, or about 280 pounds of grain, compared with competitive drought-tolerant commercial hybrids that do not contain the transgene encoding CspB, says Fietsam. A dry land acre in this region will typically yield 50–125 bushels of corn.

This year Monsanto will continue to focus on the western Plains states, and is expanding commercialization further east to involve about 200 farmers in Missouri, southern Iowa, southern Illinois, Kentucky and southern Indiana. MON87460 hybrids in 2013 were approved for import in China, a key export market. MON87460 is available as a stacked trait with insect resistance and herbicide tolerance. Monsanto has an ongoing collaboration with Ludwigshafen, Germany–based BASF to develop additional pathways to confer stress tolerance in corn and other crops. "The next generation is still very early in our pipeline," says Fietsam.

Table 1 Transgenic drought tolerant crops in commercial development and on the market

Developer	Crop	Mechanism	Implementation location and status	Field trial results
Monsanto	Corn	Expresses a cold-shock protein B from *B. subtilis*, which stabilizes RNA	Deregulated in US in December 2011; stewarded commercialization in US western Great Plains and Midwest	Average increase of five bushels of corn per acre during drought
PT Perkebunan Nusantara XI; University of Jember (East Java, Indonesia); Ajinomoto	Sugarcane	Expresses glycine betaine from *Rhizobium meliloti*	Approved in Indonesia by the National Genetically Modified Product Biosafety Commission in May 2013	20–30% higher sugar production than conventional counterparts during drought
Performance Plants (Kingston, Ontario)	Canola, corn, petunia and rice	Uses RNAi driven by conditional promoters to suppress farnesyltransferase; shuts down stomata	Licensed to Scotts (Marysville, Ohio), Syngenta (Basel), Bayer CropScience (Monheim, Germany), DuPont Pioneer, Mahyco (Jalna, India), RiceTec (Houston) and DBN (Beijing)	Canola, 26% higher yield; petunia, double the number of flowers
DuPont Pioneer	Corn	Expresses an ACS6 RNA construct to downregulate ACC synthase and decrease biosynthesis of ethylene	Field trials in the US and Chile	2.7–9.3 bushel per acre advantage over nontransgenic varieties in drought conditions
Arcadia Biosciences	Rice and canola	Expresses isopentenyltransferase from Agrobacterium, which catalyzes the rate-limiting step in cytokinin synthesis; accompanied by SARK promoter from bean	Two years of US field trials in rice with combined water use efficiency, nitrogen use efficiency and salt tolerance; technology licensed to developers who have put the gene into their own varieties of soybean, wheat, rice, cotton, sugar beets, sugarcane and tree crops	13–18% under various nitrogen application rates; 12–17% under water stress conditions; 15% under combined stress
Verdeca, a joint venture of Arcadia Biosciences and Bioceres	Soybean	Overexpresses Hahb-4, from sunflower thought to inhibit ethylene-induced senescence	Field trials in Argentina and the US	7–15% yield advantage over comparable varieties during drought and other stress
Japan International Research Center for Agricultural Sciences	Wheat, soybean and sugarcane	Expresses DREB1A transcription factor under the control of the rd29A promoter	Field trials via collaborations with International Maize and Wheat Improvement Center, International Rice Research Institute, International Center for Tropical Agriculture, Brazilian Enterprise for Agricultural Research	Varies
University of Tokyo and Japan International Research Center for Agricultural Sciences	Rice and peanut	Expresses DREB1A transcription factor under the control of the rd29A promoter	Field trials via collaborations with University of Calcutta (India, rice) and International Crops Research Institute for the Semi-Arid-Tropics (India, peanut)	Varies
Agricultural Genetic Engineering Research Institute (Giza, Egypt)	Wheat	Expresses HVA1 gene from barley, which confers osmotolerance	Conducting field trials and generating biosafety data required for approval by Egypt's regulatory authorities	Not disclosed
Indian Agricultural Research Institute (New Delhi)	Tomato	Overexpressing osmotin-encoding genes under the control of the 35S CMV promoter	Greenhouse studies in India	Better survival and growth: yield data not yet available

The other transgenic drought-tolerant crop approved for commercial cultivation is Indonesia's sugarcane. In May 2013, the country's regulatory authority, the National Genetically Modified Product Biosafety Commission, approved a variety with the *betA* gene isolated from the bacterium *Rhizobium meliloti*. *betA* encodes choline dehydrogenase, which converts choline into betaine aldehyde, which is then converted to glycine betaine by the enzyme betaine aldehyde dehydrogenase. Glycine betaine is a compatible solute thought to act as an osmoprotectant, helping plants acclimate to drought conditions. When a plant cell is exposed to water or salt stress, glycine betaine stabilizes the structure of macromolecules, helping maintain the integrity and proper function of the membranes. But the full details of the role of glycine betaine has not been firmly established.

Indonesia's sugarcane was developed by researchers at the state-run sugarcane producer PT Perkebunan Nusantara XI. The company, headquartered in Surabaya, produces 16% of the country's sugarcane on an 83,000-ha estate. Nearly 40% of that land is not irrigated and receives a limited amount of rain during a four-month wet season. The company developed the drought-tolerant variety to address the need for better yield on this dry land, says Bambang Sugiharto, a molecular plant physiologist at the University of Jember, who co-developed the sugarcane and is a consultant with the company. Sugar production during drought in the transgenic varieties is 20–30% higher than in conventional parental lines, he says. The company developed the sugarcane in collaboration with Tokyo-based Ajinomoto Company and does not plan to sell the technology, according to Sugiharto.

PT Perkebunan Nusantara XI began commercial planting of the crop this year, says Sugiharto. The sugarcane is the first of 14 biotech crops being assessed for deregulation by Indonesia's biosafety commission.

Many paths

Among drought-tolerant crops still in the testing phase, a number of pathways are under investigation. "You do not have one drought-tolerance pathway," says Jeff Habben, a crop molecular biologist at DuPont Pioneer. One approach that DuPont Pioneer has had success with is modulating ethylene, which regulates plant growth under abiotic stress. Through the expression of an RNA-interference (RNAi) construct that targets the enzyme ACC synthase (ACS) that catalyzes the rate-limiting step in ethylene biosynthesis, Habben's group downregulated ACS, which decreased biosynthesis of ethylene, leading to increased grain yield in corn. The authors report, however, that it is "too simplistic to directly relate ethylene suppression to a positive yield response." Other related factors are likely to be involved, including ethylene perception and subsequent downstream signaling and the interaction of ethylene with other hormones.

DuPont Pioneer has field tested the technology on controlled irrigation plots in California, Texas and parts of the US Midwest. This year the company will conduct field trials in Chile as well. The company already has a nontransgenic drought-tolerant line on the market called Optimum AQUAmax, which it hopes to combine with the gene-silencing approach, says Habben.

Arcadia Biosciences is targeting ethylene-related pathways as well, through a joint venture with Bioceres of Rosario, Argentina, called Verdeca. Verdeca is developing drought-tolerant soybean varieties for South and North America. The soybean has been transformed to overexpress Hahb-4, a homeodomain–leucine zipper transcription factor from sunflower that is thought to inhibit or delay ethylene-induced senescence[2], while allowing ethylene to properly regulate leaf stomatal opening, according to Arcadia. The delay in senescence may enable active photosynthesis for longer periods during drought, allowing plants to synthesize osmoprotectants and other metabolites that improve drought tolerance.

Verdeca's field trials have shown an increase in yield of 7–15% over comparable varieties during drought and other stress conditions, according to Rey at Arcadia. He expects initial soybean varieties with this technology to be commercially available beginning in 2017 or 2018.

Separately, Arcadia is testing a drought-tolerance technology aimed initially at rice and canola that targets production of cytokinin, another stress-response hormone that regulates stomatal behavior. During drought stress, cytokinin decreases, leading to stomatal closure, decline in leaf health and a smaller canopy. Arcadia's plants are transformed to express isopentenyltransferase (IPT), an enzyme that catalyzes the rate-limiting step in cytokinin synthesis. The *ipt* gene from *Agrobacterium* is accompanied by a senescence-associated receptor kinase (SARK) promoter from *Phaseolus vulgaris* that is turned on at the earliest signs of senescence, which are triggered by drought and other stresses[3]. "We're trying to buffer out the stress signal so the plant doesn't go into freak-out mode," says Rey. The technology was developed by Eduardo Blumwald, at the University of California, Davis, and licensed to Arcadia.

Rey says the company considers its product more of a WUE trait. Because the gene is designed to kick in at the earliest sign of stress, it should help plants during episodic drought, such as the middle of a hot, dry day, as well as more extended periods of drought, he says. The company hopes to bring this line of products to market beginning sometime between 2018 and 2020, Rey says.

One of the best understood mechanisms of a plant's adaptation to drought stress is the way in which the plant hormone abscisic acid (ABA) controls the opening and closing of the stomata.

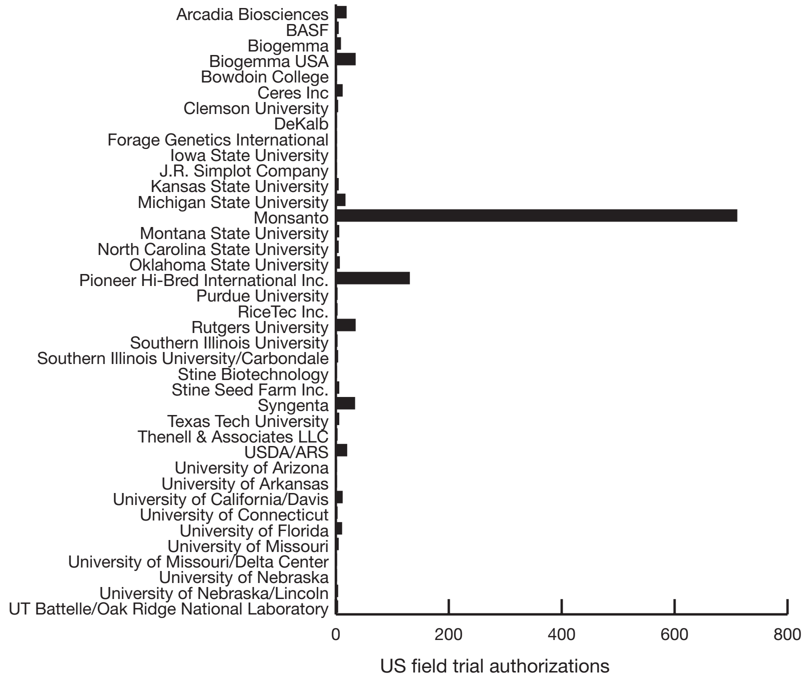

Figure 1 Total US drought-tolerance field trial authorizations by institute, 1998–2013. USDA/ARS, United States Department of Agriculture/Agricultural Research Service. (Source: ISB)

That is the mechanism on which Performance Plants in Kingston, Ontario, is focusing. Plants avoid potential damage from water shortage by the rapid biosynthesis of ABA, which triggers the guard cells surrounding the plant's stomata to close and hold on to moisture. Plants do this naturally, but "you can sensitize those guard cells even more," says Yafan Huang, chief scientific officer at Performance Plants.

Huang's approach involves suppressing the protein farnesyltransferase, which is thought to regulate the sensitivity of ABA in guard cells. The company has used RNAi driven by a variety of conditional promoters, such as the drought-inducible rd29A promoter from *Arabidopsis thaliana* to downregulate farnesyltransferase in canola, corn, petunia and rice. This approach shuts down stomata earlier and gives the plant a chance to survive the drought by reducing transpirational water loss, according to the company. "Then hopefully the rain comes and the plant can go back to regular metabolism," says Huang. "We like this approach because it's a reversible process," he says. Because the promoters are conditional, they are induced only during drought conditions, and when the drought is over, stomatal functions revert to normal.

The complexities

Drought tolerance may not be the easiest trait to confer, with hundreds, and sometimes thousands, of genes up- or downregulated under drought stress in the laboratory[4]. The genes may be associated with a large number of metabolites and proteins, and gene expression can vary by plant part and growth stage. Plus, the interaction between the plant's genotype and the environment can vary. "Drought tolerance is not one particular pathway in one particular plant," says Blumwald. In addition to drought-tolerant crops, Blumwald is also working on drought tolerance in fruit.

Most genes that are known to be involved in the regulation of stress responses are also involved in the regulation of plant growth and development. That makes it hard to fiddle with drought response pathways without affecting yield. Genes that confer first-generation traits such as insect resistance and herbicide tolerance often do not depend on these intricate connections to function.

And droughts are unpredictable. They vary in severity and timing in relation to crop growth. This not only wreaks havoc on field trials, but can also leave crops useless if they have been tailored to withstand only certain types of drought stress, as some stages of plant development are quite sensitive to drought. DroughtGard's yield advantage, for example, will vary widely from field to field and year to year, depending on the environmental conditions and the severity and timing of the drought, Monsanto has said. If a bad drought occurs during flowering or grain filling, yields can be reduced to zero, the company has warned. Indeed in 2012 such a drought occurred in the US. "We did lose some trials because the drought was so severe," says

Fietsam at Monsanto. “You have to have water to grow corn.”

Crop sensitivity to drought, at least in corn, may be increasing. In a report published in May, Lobell reported that yields of corn have steadily increased in the central US since 1995, but so has the crop’s sensitivity to drought[5]. That means that when there is a drought, absolute yields are probably better than they were 15 years ago, but the percentage of corn lost relative to the current trend under good conditions is increasing.

The benefits of drought-tolerance traits on yields is further confounded when heat comes into play. In a 2013 study of nontransgenic crops across multiple growing sites, researchers at the International Maize and Wheat Improvement Center (CIMMYT) in Harare, Zimbabwe, found that corn that is tolerant to drought might not function that way when exposed to heat stress[6]. “Tolerance to combined drought and heat stress in maize was genetically distinct from tolerance to individual stresses,” the authors concluded.

Some scientists have expressed doubt that there will ever be a gene that confers drought tolerance in a significant way. “If there was an allele that improved yield under drought with no penalty under optimal conditions, it would have been selected [by now],” says Seth Murray, a corn breeder and crop scientist at Texas A&M in College Station, Texas. “Drought tolerance is not a trait, it’s a fantasy word.”

Pamela Ronald, a plant pathologist from UC Davis, disagrees. “People said the same thing about submergence tolerance. Breeders had tried for almost 50 years to bring in a trait from an ancient variety through conventional breeding. But with modern genetics, breeders were able to show[7] the trait was not so complicated after all. In the end it was a single gene that had a huge effect on submergence tolerance,” she says.

Some researchers have overcome obstacles in drought tolerance by trying different promoters, particularly inducible promoters that kick in only when triggered by stress. Stress-inducible promoters allow the plant to go back to normal when the stress is over—a strategy that may help avoid negative effects on crop growth. Husband-and-wife team Kazuo Shinozaki and Kazuko Yamaguchi-Shinozaki at the Japan International Research Center for Agricultural Sciences and the RIKEN Institute, both in Tsukuba, learned this after engineering the model plant *Arabidopsis thaliana* to constitutively overexpress the gene encoding *DREB1A* (dehydration response element-binding protein 1A) under the control of the 35S cauliflower mosaic virus (CMV) promoter. The transgenic plants showed tolerance to stress but were severely dwarfed in size. But when Shinozaki and his colleagues used the stress-inducible rd29A promoter to drive expression of *DREB1A*, the resulting plants were even more stress tolerant, and the effects on plant growth were minimized[8].

Going global

The work of Shinozaki and his team has inspired a host of worldwide research and field trials of crops that feature their promoter-transcription factor combination. The University of Tokyo, where Yamaguchi-Shinozaki is now stationed, is developing rd29A-*DREB1A* rice and peanut in collaboration with researchers at the University of Calcutta and the International Crops Research Institute for the Semi-Arid-Tropics in Patancheru, India, respectively.

Arcadia’s technology went through a similar evolution. When transgenic plants expressing IPT were under the control of a constitutive promoter or promoters inducible by light, copper, heat or senescence, they did not grow well. But Blumwald discovered that in plants expressing IPT under the control of the SARK promoter, yield loss was minimized.

But the best way to approach drought tolerance, most researchers agree, is in sheer numbers of field trials: “The only way we know how to tackle this thing is to do a lot of testing. Lots and lots of testing. There’s no surrogate. And that’s not cheap,” says Habben at DuPont Pioneer.

Researchers working with the public-private partnership Water Efficient Maize for Africa (WEMA) know this well. Monsanto licensed MON87460 to the WEMA project, but the organization still has years of field trials ahead of it in an effort to incorporate the event into varieties suited to Africa and to satisfy regulatory requirements. It does not help that some of WEMA project’s drought tolerance field trials have been wiped out by—ironically—rain, including a 2010 trial on the west cape of South Africa, which is “more or less a desert,” says Sylvester Oikeh, WEMA project manager.

So far, researchers working for the WEMA project have conducted up to six years of field trials in five sites across three countries: Kenya, Uganda and South Africa. Yields have been 8–14% higher, on average, than those of nontransgenic varieties, he says. The group expects to commercialize the crop in 2016 or 2017 in South Africa, says Oikeh. But in Kenya and Uganda, the regulatory environments are not as conducive to commercialization (although field trials are allowed). For example, in Kenya the regulatory framework is in place for the biosafety review of transgenic plants, but in 2012 the government banned genetically modified organisms from entering the country, leaving its regulators and scientists in limbo.

WEMA researchers are not alone in facing regulatory obstacles in Africa. Scientists in Egypt are also pushing ahead with a drought-tolerant crop without confidence that their regulatory authorities will allow commercial cultivation. Researchers at the Agricultural Genetic Engineering Research Institute in Giza are testing a transgenic wheat variety that borrows the *HVA1* gene from barley. The gene encodes a member of the group 3 late embryogenesis abundant (LEA) proteins, and has been reported to confer osmotolerance for seed embryos as an adaptive response during cellular dehydration. The *HVA1* gene exists in wheat and is expressed during seed development, but the researchers reintroduced it to be expressed in the root to enable the entire plant to withstand drought stress, says Ahmed Bahieldin, a plant biologist at Ain Shams University in Cairo and King Abdulaziz University in Jeddah, Saudi Arabia, who has led the collaboration.

Bahieldin has been working since the mid-1990s on stress-tolerant wheat for Egypt, where the only arable land is found along the banks of the Nile River. His group conducted field trials between 2000 and 2004 in the Nile delta region and reported that the transgenic wheat needed less than 25% of the water that nontransgenic wheat required[9].

A breeding program and field trials have continued since Bahieldin’s latest report was published, but results have not been disclosed, he says. His team is working on generating the data required for approval by the Egyptian National Biosafety Committee. The committee has approved just one transgenic event—*Bt* corn from Monsanto—for cultivation, and getting the green light for drought tolerance will not be easy. “Frankly speaking, I am not optimistic that we can commercialize our transgenic lines at their present status and at the present time,” says Bahieldin.

Drought tolerance is a tough field to be in, but there is a bright side: other traits are as complex or even more complex than drought tolerance. Consider virus resistance: “the added complication to that is that your enemy [the virus] is evolving all the time,” says Rey at Arcadia. At least the composition of water is not a moving target. But, unfortunately, its availability is.

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