

Drying out. Australian farmers know all too well the devastating effects of drought, which has greatly reduced harvests.



PLANT GENETICS

The Blue Revolution, Drop By Drop, Gene by Gene

Researchers probe the secrets of how plants cope with water stress to improve crop yields

Eight years ago, then-U.N. Secretary-General Kofi Annan called for a “Blue Revolution” in agriculture. With Earth’s water resources under strain, population growth booming, and desertification increasing, the need to wring more crops out of dry land is becoming urgent, Annan said in his April 2000 Millennium Address.

It was a call to arms for plant genomicists. But they are fighting a battle on many fronts. “Drought stress is as complicated and difficult to plant biology as cancer is to mammalian biology,” says Jian-Kang Zhu, a molecular geneticist at the University of California, Riverside. Plants have evolved complex mechanisms to deal with water shortages, which vary in timing and severity from place to place, season to season.

Researchers armed with the latest sequencing and gene-expression technologies are making progress in rounding up the genes that can help plants stand up to dry conditions, both in the greenhouse and in the field. “We do know a bit more about what the effects [of stress] are in biochemical detail,” says Hans Bohnert, a biochemist at the University of Illinois, Urbana-Champaign. But some skeptics doubt that it will be possible to manipulate one or a few genes to get tougher breeds. “There isn’t a single, magical drought-tolerance trait,” says Mark Tester, a plant physiologist at the Australian Centre for Plant Functional Genomics in Glen Osmond.

Others counsel patience. Companies and governments are eval-

uating promising new strains of corn, rice, and other crops—some genetically modified (GM) and some the products of conventional breeding—in the field. Australian farmers, for example, are eagerly awaiting results of a field trial of GM, drought-tolerant wheat that has just been harvested. There’s been “a constant increase in interest, particularly from the private sector,” says Roberto Tuberosa of the University of Bologna in Italy. “Drought and tolerance to water stress are very hot topics at this moment.”

Drop by drop

During the green revolution, researchers and breeders focused on improving productivity by providing optimal environments—fertilizing poor soils, irrigating dry lands, destroying weeds and pest insects—and developing high-yielding crops that thrive under those conditions. But looming water

shortages are changing the equation. Agriculture consumes 70% of the water people use, and its needs will increase 17% by 2025, according to the International Water Management Institute (IWMI), based in Sri Lanka. But competition from urbanization and development means that “less water is becoming available for agriculture,” says Henry Nguyen, a plant geneticist at the University of Missouri, Columbia.

Given this anticipated shortfall, IWMI’s *Comprehensive Assessment of Water Management in Agriculture*, released in 2003, called for a 40% improvement by 2025 in yields of crops where water is the limiting factor. Unfortunately, historically, improvements have inched along by only about 10% per decade, says John Passioura of CSIRO Plant Industry in Canberra, Australia, and it’s not clear that even that pace can be sustained.

Climate change also has agronomists worried. Rainfall in some areas is declining and becoming more erratic, wreaking havoc on markets. A case in point: A drought in Australia has cut world supplies of wheat and barley, leading to a spectacular price hike in those crops. And rice farmers in northern Italy have been forced to shift from growing rice in paddies to flooding their fields for very short periods.

Plants have mechanisms to cope with drought. In some places, local crop varieties have evolved to survive, if not thrive, as long as water shortages are not too severe. How efficiently a plant draws water from the soil, how



Rain or shine. In China, movable shelters shield drought-tolerant rice from rain during field trials.

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well cells retain water, how much water is released through leaf openings called stomata, the timing of flowering relative to the seasonal onset of drought—all factor in drought tolerance. Roots produce molecules that allow them to suck water out of ever-drier soils; leaves respond by closing stomata. Cells mop up free radicals produced during dehydration or produce molecules that preserve their ability to hold on to what water they have.

Researchers are employing two strategies to decipher and harness these responses to drought. Some take a relatively traditional approach, growing and crossing varieties and evaluating how the progeny vary in their ability to deal with stress. They then select and grow the best-adapted plants. For these breeders, genome studies have identified DNA markers that speed the identification and selection of plants worthy of further study.

Some researchers head straight for the genes. In 1998 at the University of Arizona, Tucson, Bohnert and his colleagues began teasing apart the genetics of stress response using the model plant *Arabidopsis*, whose genome was sequenced in 2000. They evaluated thousands of mutant *Arabidopsis* strains, each of which contained a fluorescence protein attached to a stretch of regulatory DNA known to be involved in the plant's responses to stress. Lack of fluorescence would indicate that the responses were switched off, perhaps because a gene in the pathway that triggers them had been mutated.

The genetic technology they used enabled them to home in on the mutated gene or DNA region. They then manipulated the activity of these genes in transgenic plants to better understand the gene's role in weathering tough conditions. Other groups have done broad-scale surveys of genes and proteins active when plants are under stress, coming up with many promising candidates.

But precious few of these leads have panned out, possibly because drought responses are extremely complex. "We still do not have all the pieces of the puzzle, not even the key pieces," says Zhu, who worked with Bohnert. His work, for example, is showing that beyond genes, small RNAs help regulate stress responses in ways he does not yet fully understand.

"There are now several hundred papers published, tweaking individual genes," says Bohnert. "Under controlled conditions, one can see an effect but not in the field."

Gene by gene

A few genes have helped in the field, however. After looking at expression patterns of 1500 genes in *Arabidopsis* plants grown under drought conditions, Donald Nelson, now at Monsanto in Mystic, Connecticut, and his colleagues found 40 that appear to be involved in drought adaptation. They looked at effects of the most promising ones by causing each to be permanently turned on in GM plants. *Arabidopsis* with a constantly active *NF-YB1* gene didn't wilt as much as wild-type *Arabidopsis* and maintained higher photosynthetic rates.

Nelson tracked down the equivalent gene in maize and switched it on permanently. Sim-

ulated drought conditions typically reduced maize yield by more than 50%. Under those conditions, the GM maize produced as much as 50% more than unmodified plants, they reported online 8 October 2007 in the *Proceedings of the National Academy of Sciences*.

Subsequent field studies show that the transcription factors produced by these genes increase yields by an average of 10% to 15% under a variety of stress conditions, Paul Chomet, also at Monsanto in Mystic, Connecticut, reported in Washington, D.C., in February at the 50th Annual Maize Genetics Conference. This work was "a proof of concept," Chomet said. Monsanto now has a large-scale program screening for genetic enhancements that can improve yield despite water shortages, with one new variety about to enter the regulatory pipeline. (The company has also just agreed to develop this and other drought-tolerant technologies with the African Agricultural Technol-

ogy Foundation through a royalty-free agreement that will make these crops available to small farmers.) This effort is much more challenging, than, say, developing GM Bt maize, in which "you flip a switch and Bt works," he pointed out. "With drought, we are trying to dial into the physiology of the plant."

Yafan Huang has also tried dialing into the physiology of the plant. In 1996, Peter McCourt of the University of Toronto in Canada and his colleagues discovered a mutant *Arabidopsis* that was overly sensitive to the plant hormone abscisic acid, which influences plant development and activates stress responses, including closing stomata to inhibit water loss. Two years later, McCourt's group demonstrated that the mutated gene, *ERAI*, typically countered abscisic acid's propensity to close stomata. Mutants lacking a functional *ERAI* were more sensitive to the hormone and their stomata more prone to closure—a change that helped the mutants withstand drought stress (*Science*, 30 August 1996, p. 1239; 9 October 1998, p. 287).

Encouraged by this discovery, McCourt and Huang, of Performance Plants Inc. in Kingston, Canada, fiddled with this same gene in canola. They created a line of canola that carried anti-sense DNA that disables the



More crop per drop. Tests of genetically modified rice (*top right*), canola (*middle right*), and corn (*bottom right*) show that they withstand dry spells better than unmodified controls (*left*).

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Getting to the Root of Drought Responses

Plants are bit like giant straws. Water in the ground gets “sucked” up the corn stalk or tree trunk and leaks out of microscopic pores, primarily in the leaves. The pores, called stomata, must open to let in carbon dioxide for photosynthesis, but the water loss—up to several cubic meters a day for a tree—can be severe, especially in drought-ridden areas. Not surprisingly, researchers seeking to develop drought-resistant crops have homed in on ways to keep stomata shut down (see main text). But Henry Nguyen, a plant geneticist at the University of Missouri, Columbia, thinks they should also be looking at the bottom end of the straw. He and Missouri colleague Robert Sharp are establishing a drought research center at Missouri that will concentrate on root biology. “From a drought research [perspective], this will be a new frontier,” says Nguyen.

It’s been difficult to unearth how roots cope with arid conditions. Unlike leaves, their workings are hard to observe. Nguyen is peering deep inside the roots, at the genes themselves. His group has surveyed gene-expression patterns in different parts of maize roots under various stresses and plans to compare them with expression patterns in soy roots. Early analyses suggest that different biochemical pathways underlie drought adaptations in the two species, he says.

Lewis Lukens, a plant geneticist at the University of Guelph in Canada, and his graduate student Tina Wambach are finding different patterns of gene expression in the roots of plants from different inbred lines of maize, some of which grow more than others when water is in short supply. Lukens and Wambach looked at gene activity in a half-dozen of the more diverse responders. They grew seedlings suspended in water solution to see what was happening, get relatively pure samples of RNA, and precisely control water availability. They started the seeds in normal conditions, then subjected them to “drought” stress for 24 hours, sampling root tissue during prestress, “drought,” and recovery periods.

To Lukens’s surprise, the number of activated genes varied threefold across the different inbred lines. In one, 4500 genes out of 40,000 changed their expression; whereas in another, only 2000 did so. Furthermore, “there were very few changed genes shared across all the genotypes,” says Lukens. Even when the same gene was activated, it was



In full view. Maize seedlings grown hydroponically varied in their roots’ resistance to water stress.

revved up to varying degrees, he reported in February in Washington, D.C., at the 50th Annual Maize Genetics Conference.

Although the diversity of responses was initially confusing, Lukens later discovered that many of the genes were active in the same enzymatic pathways. “We saw much more conservation,” with about 40% of the pathways shared across all the lines, he notes. It seems that many lines had adapted in a similar way, but the activity of different genes in particular pathways changed. Lukens hopes that by crossbreeding lines that show different patterns of gene expression in the same pathway, breeders may come up with hybrids with even better drought tolerance than either of the parent lines. Meanwhile, “the next step is to do the genetics and identify the regions of the genome that explain that diversity,” he says.

—E.P.

ERAI gene in drought conditions. In both lab and field studies, the plants produced significantly more seeds than wild-type canola, they reported in 2005. The work “for the first time showed it was possible to engineer a single gene and have an effect,” says Nguyen. Moreover, when water supplies were adequate, the GM canola yields were on par with the non-GM canola, which is key to any drought-tolerant crop’s commercial success. Both results have held up through three more years of field studies.

Ultimately, “whether the Performance Plant work will advance drought hardiness is hard to say,” says Lewis Lukens, a plant geneticist at the University of Guelph in Canada. But, Huang notes, tests are under way for similarly modified corn and other plants. Several companies are evaluating whether to use Performance Plant technology in their drought-resistant lines.

Control over stomata is also key to a drought-tolerant rice being developed in China. Instead of shutting down a gene, researchers at the National Center for Plant Gene Research in Wuhan cause a different one to be overactive. This gene, called *SNAC1*, enhances the ability of abscisic acid to shut the stomata. Under severe drought conditions, the GM rice produced 22% to 34% more seeds than non-GM rice and produced about the same number of seeds under normal conditions, Lizhong Xiong and colleagues reported in 2006. The rice tested is not one widely grown commercially, and thus the work is a “proof of idea,” says Xiong. His team is now generating new transgenic plants from commercial rice varieties.

Meanwhile, in Australia, Molecular Plant Breeding CRC just finished a small field trial in a dry part of Victoria of several transgenic wheat lines designed to withstand drought.

Each line contains one of six different genes for drought tolerance derived from maize, *Arabidopsis*, a moss, or a yeast. The company will not reveal the nature of the genes, and the test is just to see if the transgenic lines outperform the control lines under field conditions, says Michael McLean, a spokesperson for Molecular Plant Breeding CRC in Bundoora.

“Clearly, recently we have seen some very promising advances in terms of drought tolerances in crop plants,” says Nguyen. “Now it’s a question of how to optimize the system.” And Tester is also optimistic: Ultimately, “I think there will be a palette of genes from which breeders and crop scientists will select for putting together the drought tolerance for a particular region.”

The revolution Kofi Annan called for in 2000 hasn’t yet occurred, but the seeds have at least been planted.

—ELIZABETH PENNISI