THE PLANT ENGINEER

Dan Voytas has worked tirelessly to make targeted genome editing of plants a reality

By Elizabeth Pennisi

At age 5, with some gentle guidance from his parents, Dan Voytas grew more tomato seedlings than could fit in the household garden. So his dad suggested the young gardener put the extras in his wagon and market them to neighbors. The small boy was hooked—the next year, he grew even more plants. A few years later, his dad built a greenhouse off the family’s laundry room so the dryer vent would warm the plants, giving his son a head start on the growing season. Voytas added petunias and other bedding plants to his product mix; by high school, he netted $1000 a summer, impressive for a teenager in the late 1970s.

Now, Voytas’s green thumb and entrepreneurial spirit are poised to reshape 21st century agriculture. Over the past 20 years, he has pioneered novel ways of precisely editing a crop’s DNA to give it new traits or delete undesirable ones. It’s an approach that is potentially more powerful than the traditional way of making genetically modified (GM) crops: using bacteria to smuggle in genes from other species. And because it can leave no foreign DNA behind, it could free these products from the stigma and regulatory burden of being labeled as GM organisms (see sidebar, p. 1222).

Voytas, now 54 and director of the Center for Genome Engineering at the University of Minnesota (UM), Twin Cities, has had to overcome recalcitrant technologies, navigate intellectual property fights, and endure commercial failures. But he has emerged from it all as a recognized world leader in plant engineering. “When you think of gene editing in plants, he’s one of the first names that comes to mind,” says Michelle Christian, a molecular biologist at the Seattle Children’s Research Institute in Washington. Voytas helped invent a key genome-editing platform, transcription activator-like effector nucleases (TALENs), and a company he’s partnered with already has new modified soybean, potato, and wheat varieties in the works, including a reduced-gluten wheat.

Voytas has also made a name for himself by rallying the field, forging collaborations, and disseminating new tools including CRISPR, the genome-editing system that is taking biology by storm (Science, 18 December 2015, p. 1456). Voytas is excited by CRISPR’s ease of use and versatility, but he is not abandoning TALENs. It’s like cellphone technology, he quips: “The iPhone 6 comes out and everyone wants it, but the iPhone 5 can still do everything you need.”

“What makes Dan stand out is he’s not wed to one tool or approach,” says Adam Bogdanove of Cornell University, a co-inventor of TALENs. “It’s about adopting whatever tools you need to gain control of [editing] crops.” Indeed, Voytas is committed to sparking a revolution in plant science by any means necessary. In a world with a burgeoning population and limited land and resources, he says, it’s a matter of survival. And one of the few things that can shake his habitually mild manner is what he believes is misguided suspicion of GM organisms: “At some point, it will come down to: Are we going to use genome-edited plants or are people going to starve?” he says.

VOYTAS’S LOVE OF PLANTS was a constant in a childhood that took him from rural Minnesota, where his dad was a forester for the U.S. Department of Agriculture (USDA), to northern New Hampshire, and finally to Harvard University. When he arrived at the Cambridge, Massachusetts, campus to start his freshman year, he immediately looked for the horticulture department—he planned to major in plant science—but dis-
covered to his chagrin there was none. The best he could do was take a course in plant taxonomy. But he loved Cambridge, despite his mom’s concerns about there not being enough green grass and too many cars, and after he found a plant lab at Harvard that would take him, he stayed on to get a Ph.D.

Working for Harvard geneticist Frederick Ausubel, “I immediately felt at home,” Voytas recalls. The lab was pioneering the use of a then–little-known mustard plant, Arabidopsis thaliana, as a model for plant biology. Voytas was willing to look into whether it had so-called transposable elements: pieces of mobile DNA that can jump from one chromosome spot to another. Watching what happened to maize and insects when transposable elements disrupted a DNA sequence had proven useful in determining the function of many genes, but it was unclear whether they even existed in Arabidopsis.

“He was willing to take somewhat of a risk and start working at the dawn of a new field,” Ausubel says. Papers in Nature, the Proceedings of the National Academy of Sciences, and Genetics attested to the lab’s success. “Serious and earnest but also a lot of fun,” is how Ausubel remembers Voytas, who once ordered baseball hats with an Arabidopsis plant on the front and the logo “Today’s weed, tomorrow’s paradigm.”

Ph.D. in hand, Voytas postponed a move to Iowa State University (ISU) in Ames as an assistant professor because he was under the spell of transposable elements and their potential for genome manipulation. Instead, he made a detour to Johns Hopkins University in Baltimore, Maryland, joining yeast biologist Jef Boeke for a brief postdoc to study transposable elements in yeast, which is even easier to work with than Arabidopsis. With bioinformatics savvy unusual for biologists in the early 1990s, Voytas scoured the newly sequenced chromosome 3 of baker’s yeast, Saccharomyces cerevisiae, for unknown transposable elements.

Even at that early stage of his career, when he and Henry Levin, a fellow postdoc in Boeke’s lab at the time, regularly swam laps at a local Baltimore pool, Voytas would talk with enormous enthusiasm about his dream of precisely engineering plant genomes as a better way to improve
When is a GM plant not a GM plant?

By Elizabeth Pennisi

The recently developed genome-editing methods, from zinc finger nucleases to transcription activator-like effector nucleases (TALENs) to CRISPR, are shaking up the debate over how to regulate genetically modified (GM) plants. Canada, for example, stuck to its rule that a plant should be regulated as GM if a novel trait has been introduced to it, regardless of the technology used. But the U.S. Department of Agriculture (USDA) has so far exempted plants altered by TALENs and CRISPR from its GM regulations.

Developed decades ago, when “gene guns” and bacteria were used to insert new genes, USDA’s rules focus on whether a plant contains “foreign” DNA from a plant pest or pathogen. Advocates of the latest technologies say they leave no such DNA behind, so the plants that result should be indistinguishable from ones that could be naturally bred with enough time. In accepting this reasoning, “USDA has been very progressive [even though] they are working with statutes that were put in place before anyone could imagine you could edit a genome,” says Dan Voytas of the University of Minnesota, Twin Cities, a genome-editing pioneer who is also chief scientific officer at the New Brighton, Minnesota, company Calyxt (see main story, p. 1220).

Developing, testing, and getting regulatory approval for a plant modified with earlier techniques could take decades and cost more than $100 million, limiting commercial use of GM plants primarily to large companies trying to improve commodity crops. But USDA exemptions for CRISPR and TALENs-edited crops favor smaller firms such as Calyxt, allowing them to work with food companies and respond quickly to consumer preferences, Calyxt’s CEO, Federico Tripodi, argues. The development of Calyxt’s first TALENs-modified soybean strain, which produces oil with fewer polyunsaturated fats, has taken just 6 years and cost $6 million, he notes.

USDA, however, has only ruled so far on crops in which the new genome-editing methods knocked out DNA bases, not those in which they’ve introduced, replaced, or added genes. And the United States may still alter its existing policy on GM organisms—the National Academies of Sciences, Engineering, and Medicine and a White House group examining the issue and will release recommendations soon (see Policy Forum, p. 1211).

Many other countries haven’t taken a clear stance yet. China has encouraged research into genome-edited crops, but public opposition to GM organisms in general has kept the nation from clarifying its approval process. The European Union has postponed its own decision on CRISPR- and TALENs-edited crops for almost a year now, and some suspect it will leave the issue to individual members—a compromise it has made in the past for GM plants.

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crops than the then-current methods. Levin would shake his head. “It was a shout out into the desert,” says Levin, now a molecular biologist at the Eunice Kennedy Shriver National Institute of Child Health and Human Development in Bethesda, Maryland.

The DNA-editing techniques of 20 years earlier techniques could take decades enough time. In accepting this reasoning, “It was the best tool out there.”

Bogdanove says. “Before CRISPR came out, it worked as good as we had hoped,” Voytas and a colleague filed for a patent on using Ty5 to manipulate genomes, and in 1999 he left ISU to found a company called Phytodyne to develop targeted plant gene-editing technologies.

Voytas and Phytodyne never got very far with transposable elements, but around the same time a few researchers were playing around with another way to target distinct places in a genome: harnessing pieces of transcription factors, the proteins that home in on specific DNA sequences to regulate a gene’s activity. In a few cases, molecular biologists had linked the targeting portions of the proteins, called zinc fingers, to DNA-cutting enzymes called nucleases. At Phytodyne, Voytas and colleagues began to make these molecular scissors for plants, but they struggled to make progress. “The science was too hard and it was at too early a stage,” Voytas says.

“It was frustrating,” recalls co-founder David Wright, an agronomist at ISU. “We ran out of cash and we ran out of time.” Voytas took the failure hard: “I vowed I would never do a company again.”

WHEN PHYTODYNE CLOSED its doors in 2004, Voytas went back to ISU. One day, he got a call from Keith Joung, then a new pathologist at Massachusetts General Hospital in Boston, who was eager to develop zinc finger technology so that he could try it in mammalian cells. Their collaboration produced a 2009 Nature paper reporting that zinc finger nucleases (ZFNs) efficiently modified a tobacco gene to make the plant resistant to herbicides. They also founded a consortium that developed a set of ZFNs everyone could use, a tricky experience because a company was vigorously protecting its patent rights to the hard-to-make molecules and because of the inherent difficulties of teamwork. “I didn’t quite realize it at the time, but he was a good mentor about how to handle the complex politics,” Joung says. “I always felt that I could completely trust Dan.”

Voytas’s collaborative nature and willingness to try new things is legendary. Despite running a plant lab, he once took in an undergraduate who wanted to explore using ZFNs to cure AIDS—and later helped the student get a technician’s job in Boston. Others liken him to a big brother “that knows everything and knows what help you need,” says Caixia Gao, a plant biologist at the Chinese Academy of Sciences’s Institute of Genetics and Developmental Biology in Beijing.

A new chapter began in 2009 when Bogdanove, then at ISU, approached Voytas with a proposal that could render ZFNs obsolete. Bogdanove and, independently, a team in Germany had figured out that plant pathogen proteins nicknamed TALEs home in on specific DNA sequences based on the arrangement of their amino acids (Science, 14 December 2012, p. 1408). Re-arranging the amino acids in TALEs to target different stretches of DNA was much easier than re-engineering zinc fingers, Bogdanove argued.

Voytas hesitated for a while, but after moving from ISU to UM, he teamed up with Bogdanove to link specific TALEs to nucleases—voilà, TALENs were born. “It worked as good as we had hoped,” Bogdanove says. “Before CRISPR came out, it was the best tool out there.”

And as he had done for ZFNs, Voytas became an evangelist for the new techno-
A bumper crop of genome-edited plants

Challenging older forms of genetic modification, four recently developed genome-editing methods—meganucleases, zinc finger nucleases (ZFNs), TALENs, and CRISPR—have produced many plant varieties with useful new traits, some highlighted below. A few are in field trials already.

### Genome-editing methods

- CRISPR
- TALENs
- ZFNs
- Meganucleases

### Altered growth

- Corn
  - *Zea mays*
  - DuPont Pioneer plans a CRISPR-altered version of its “waxy corn” variety; Dow AgroSciences is using ZFN technology to make corn simultaneously healthier and herbicide resistant.

- Soybean
  - *Glycine max*
  - Calyx’s TALEN®-edited soybeans may help food companies reduce trans fats in their products by 2018, as required by the United States for health reasons.

- Wheat
  - *Triticum aestivum*
  - Chinese plant geneticists used TALENs to knock out all six copies of a gene that confers susceptibility to powdery mildew. Calyx is readying field trials of the disease-resistant strain.

### Disease resistant

- Rice
  - *Oryza sativa*
  - USDA gave a green light to Iowa State University’s bacterial blight-resistant rice made by TALENs, but there are no plans for commercialization yet.

- Tomato
  - *Solanum lycopersicum*
  - Genetic engineers have been trying to improve the shelf life of tomatoes since the 1990s, and now new genome editors are making the fruit resistant to bacterial and viral infections.

### Herbicide resistant

- Poplar
  - *Populus sp*
  - At least two groups have used CRISPR to alter the lignin and tannin content of poplar seedlings, paving the way for trees better suited for biofuel and other uses.

- Potato
  - *Solanum tuberosum*
  - Calyx’s TALEN®-altered potatoes store longer in the cold, and yield less of a potential carcinogen when cooked; CRISPR’ed spuds are resistant to herbicide.

### Yield, storage, or processing improvement

- Lettuce
  - *Lactuca sativa*
  - Using a version of CRISPR that involved no introduced DNA—just RNA and proteins—a South Korean group altered lettuce to be more stress resistant.

- Grapefruit
  - *Citrus paradisi*
  - After demonstrating that CRISPR works in oranges, a team knocked out a grapefruit gene that confers susceptibility to citrus canker, a widespread bacterial disease.

- Tomato
  - *Solanum lycopersicum*
  - Genetic engineers have been trying to improve the shelf life of tomatoes since the 1990s, and now new genome editors are making the fruit resistant to bacterial and viral infections.
Three other products have the green light of trans fats in the food supply by 2018. Administration requirement of getting rid of trans fats in the food supply by 2018. USDA has already said it won’t treat the soybean or the potato as GM, which has sped their development.

Recently, the new CEO of Calyxt came to Voytas and asked whether TALENs were dead given the emergence of CRISPR. In response, Voytas showed him data on TALENs activity in diverse crop species. The company can make 1000 novel TALENs a week, more than enough to develop new products at a competitive rate, he argues. And whereas CRISPR technology is mired in intellectual property disputes, ownership of TALENs is clearly defined. TALENs can also be better than CRISPR at hitting an exact location in the genome and making clean cuts in DNA, Bogdanove says.

Voytas acknowledges his group was slow to embrace CRISPR. The first CRISPR reports “sort of seemed too good to be true,” says Colby Starker, Voytas’s longtime lab manager at UM. “We got burned a little bit by not jumping on that bandwagon.” But by now, 80% of their work is CRISPR-based, and, true to form, Voytas is developing tools for all to use. “Dan has a very strong sense that the technology we develop should be shared,” Starker says.

One challenge for CRISPR and other editing platforms is the difficulty of getting the DNA-editing molecules past plants’ rigid cell walls. To solve that problem, Nicholas Baltes, now at Calyxt, and others in Voytas’s group have pioneered the use of plant viruses called geminiviruses, which naturally deliver genetic material into plant cells. The researchers have engineered the viruses to carry the genes encoding CRISPR’s components—a nuclease and a so-called guide RNA that directs the nuclease to a target DNA sequence—into plant cells and then get the cell to churn out the gene editor. So far, these modified viruses have done the job in tobacco and tomato, increasing CRISPR’s gene-editing activity 10- to 100-fold, Voytas says.

Already requests are pouring in for an extensive set of CRISPR tools that Voytas’s lab soon plans to release. They’ve crafted 150 different CRISPR constructs—some encoding nuclease proteins that make double- or single-stranded breaks; others that simply ferry molecules, such as fluorescent tags, to specific spots in the genome; still others that can go after up to 12 targets at once.

Now that genome engineering is such a hot field, Voytas is almost in as much demand as the inventors of CRISPR—in June he was traveling so much for talks and conferences that he spent only 6 nights in his own bed. That’s particularly tough on him because his house is one of his few nonwork passions. The midcentury modern building, designed by one of Minnesota’s most famous architects, had fallen into disrepair before Voytas restored it to almost its original state, adding an orchid garden that he tended for several years in the basement. He is also torn between his work and his dedication to his mother and handicapped brother, whom he visits weekly, bringing flowers from his community garden plot. Lately he has been so distracted by his many projects that his mom sometimes sends him home. “I’m talking and he’s not hearing,” she says.

It’s all worth it to Voytas, who believes crops created with TALENs or CRISPR will be vital to the world’s food security. Giving a talk about Calyxt’s soybean project one day, he recalls, “I got all choked up and had to stop and take a long, hard breath.” And last year, when he visited a big field of the company’s soybeans, he snuck a few pods home, and kept them on the kitchen counter, picking them up every so often. A proud thought went through his mind: “My TALENs did that.”
The plant engineer
Elizabeth Pennisi (September 15, 2016)