

Tiptoeing around transgenics

New techniques for manipulating plant genomes are yielding plants touted as nontransgenic. Will that relieve regulatory burden? Emily Waltz investigates.

Last August, it came to light that the US Department of Agriculture (USDA, Washington, DC) has been quietly informing crop trait companies that plants made with certain novel approaches to genetic modification would not require regulatory oversight. In a letter dated 26 May 2010, the USDA informed Indianapolis-based Dow AgroSciences that genetically modified (GM) corn developed using a zinc-finger nuclease (ZFN) technique would fall outside of the agency's authority. Six years earlier, in correspondence dated 24 March 2004, the USDA informed Cibus Genetics in Annapolis, Maryland, that plants made with the company's chimeric DNA-RNA oligonucleotide-directed repair technology would also not warrant review. These letters effectively give a green light for the two companies to begin field trials and commercialize GM plants without further review, much as for new varieties created by mutagenesis or conventional breeding. The letters were retrieved through Freedom of Information Act (FOIA) requests submitted by industry experts and reviewed by *Nature Biotechnology*.

The two techniques exemplify a host of new approaches to creating GM plants that, although not always developed with this intent, may allow companies to avoid burdensome regulations designed for the technology of a previous era. As opportunities to, in effect, tiptoe around regulations have appeared, industry players have begun capitalizing on them. "Every time we get together with companies—particularly small companies—the question is posed: How else can we circumvent these regulations?" says Alan McHughen, a biotechnologist and Jefferson Science Fellow in Washington, DC. "It has always been a question," he says, and now companies are putting their theories into practice.

Clear sailing for targeted mutagenesis

In the US, the USDA's regulatory domain over GM plants arises from decades-old statutes that give it authority to regulate 'plant pests' (as defined in the Coordinated Framework for the Regulation of Biotechnology of 1986, available here: <http://usbiotechreg.nbio.gov/>). Genes taken from plant pests are commonly used in the construction of transgenic plants. For example, the use of the cauliflower mosaic

virus 35S promoter has been commonplace as a means to constitutively activate transgenes, and the plant pest *Agrobacterium tumefaciens* has been the workhorse delivery system for shuttling foreign genes into plant genomes. What's more, non-plant-derived genes have been transferred into plants to confer desired traits (the *Agrobacterium* sp. 5-enoylpyruvylshikimate-3-phosphate synthase gene to confer glyphosate resistance) or for selection purposes (antibiotic-resistance genes). Because the creation of nearly all GM plants thus far has involved these tools, the USDA has maintained its authority to regulate essentially all GM plants (see p. 211).

But advanced technologies are quickly supplanting the old methodologies¹. The technologies used by Dow and Cibus, for example, fall outside the USDA's authority because neither involves genetic material originating from plant pests. Instead of adding foreign DNA, the companies edit or alter plant genes through site-specific mutagenesis techniques.

In the Cibus approach, chemically synthesized chimeric single-stranded DNA oligonucleotides direct the modification of an existing gene—similar to work pioneered by Eric Kmiec at the University of Delaware with chimeric DNA-RNA oligonucleotides. The oligonucleotides complement plant genes except for a single base pair. When introduced into a plant, the oligo hybridizes with the plant gene, creating a single mismatch; this is recognized as an error and repaired by the plant cell's DNA-repair enzymes using the oligonucleotide as a template. The chimeric oligonucleotide itself is digested by nucleases in the cell within hours, and the plant is left with a gene that codes for a desired trait. Cibus distinguishes its products from those made through more traditional genetic modification. According to Peter Beetham, senior vice president of research at Cibus, "They're not genetically modified and they're not transgenic," a message repeated in videos on the company website. Of course, this all depends on how one defines 'genetically modified'.

The USDA's 2004 letter to Cibus says that the agency has no authority to regulate Cibus' technology, a position that followed an extensive review of the technology by the agency, according to Beetham. The company hopes

to market its first product, herbicide-tolerant canola, in the US in 2012, he says. However, the product is pending approval by the Canadian Food Inspection Agency, which regulates the environmental release of plants with novel traits, including those created through biotech, mutagenesis or conventional breeding techniques. It is unclear how the technique would be treated under European regulations; some independent researchers argue that it would be excluded from European regulation because mediating genome changes by means of oligonucleotides is equivalent to mutagenesis, which is not regulated in the EU².

Dow's product relies on ZFN technology, another site-specific approach. ZFNs are engineered proteins that can be designed to make DNA double-stranded breaks at specific genomic locations. Using a cell's own repair machinery to repair the break, specific gene modifications can be made. Dow used the technology to delete sections of the gene for inositol-1,3,4,5,6-pentakisphosphate 2-kinase, which catalyzes the final step in phytate biosynthesis in corn seeds. The method reduces the corn's level of phytate, an antinutritional component of feed grain.

The USDA's letter to Dow effectively gives the company a green light to begin experimental field trials and commercialize reduced-phytate corn without further review by the agency. Dow has not yet initiated field trials of the corn and has no plans to do so, according to Brad Shurdut, global lead for regulatory and government affairs at Dow. The company consulted with the USDA provisionally in case it should decide to move forward with development, Shurdut says.

Dow will continue to consult with the USDA on new ZFN products in its pipeline, Shurdut says. The company has many applications for the technology, including some that involve foreign DNA, so some products would be likely



Fast track to market. Rather than the three to ten years normally required for a seedling plum to produce fruit, FasTrack plum lines carrying the early-flowering gene produce fruit less than a year after being planted from seed. (Source: Agricultural Research Service, Washington, DC.)

to fall within the agency's authority. "I don't see zinc fingers being categorically exempt" from regulatory oversight, Shurdut says. "USDA has been very explicit that they will make decisions on a case-by-case basis." Over time, if a particular ZFN application can be shown to make specific, repeatable changes in a plant, Shurdut hopes to see the regulatory burden reduced. "We think that the extent of regulation should depend on the nature of the final product, not on the process employed in its development," adds Garry Hamlin, a spokesperson for Dow.

The USDA's decisions in the Dow and Cibus cases are of great interest to industry players developing these as well as other targeted GM technologies, such as transcription activator-like effector nucleases and meganucleases. "Targeted mutagenesis companies are seeking clarification on whether their products are or are not going to be regulated," says Scott Thenell, a regulatory consultant at Thenell & Associates in Walnut Creek, California, who filed one of the FOIA requests on behalf of a client. "These letters are some of the first examples to help clarify that question," he says. The topic "is of great interest to me and my clients and we have particular views on how products of the technology should be regulated."

Now that the letters have become public, more companies will likely seek similar passes from the USDA for products created with targeted mutagenesis techniques, says Drew Kershen, a law professor at University of Oklahoma in Norman. The increased interest may force the USDA, and other authorities globally, to come up with formal regulatory plans for these techniques. "It's going to force regulators to rethink the rules," he says. "All the regulatory agencies are going to have to face the reality that science is moving on."

Jennifer Kuzma, an associate professor in the Science, Technology and Environmental Policy program at the University of Minnesota in Minneapolis, had also filed a FOIA request for the letters. She says the USDA should be consulting with experts in a more public way before granting exemptions to specific companies. "These decisions are really under the radar," she says. "They're not being debated openly, and that is a concern." She suggests that the agency convene an advisory committee composed of academics and stakeholders to openly discuss the new techniques and how they should be regulated.

The USDA has had to give regulatory passes to some old techniques as well. In July 2011, the agency found that herbicide-tolerant bluegrass, made by Scotts Miracle-Gro of Marysville, Ohio, fell outside of its authority because no plant pests were used. The gene for herbicide resistance, *EPSPS* (5-enolpyruvylshikimate-

3-phosphate synthase), came from thale cress (*Arabidopsis thaliana*), and other genetic elements came from corn and rice. And instead of using *Agrobacterium* to deliver genes, the company used a gene gun, which blasts DNA into plant cells on pellets made of gold. None of these technologies is new, but Scotts' calculated effort to combine them with the intent of bypassing federal oversight exposed a critical weakness in USDA's regulations.

Stealth genetic engineering

Another USDA decision spurring surprisingly little academic debate is its announcement in June that it would deem the progeny of a new transgenic corn line as "nontransgenic." The line, a type of corn used to increase the volume of female parent seed for hybrid seed corn production, belongs to Johnston, Iowa-based Pioneer Hi-Bred.

In common hybrid seed production systems, inbred male lines of corn pollinate inbred female lines. To prevent the females from pollinating themselves, seed companies physically remove the pollen-producing tassel on the female plants—a labor-intensive part of hybrid seed corn production. Pioneer has created, through conventional breeding, a female parent that cannot self-pollinate. This ensures that the female plant will be pollinated by its male counterpart in the field and eliminates the need for detasseling. But it also puts a wrench into the production of inbred female parent seed. So Pioneer developed a transgenic helper line of seed corn, called a maintainer, to increase female parent seed production. The maintainer line contains a cassette of genes that restores fertility and prevents functional transgenic pollen from being produced. The cassette also includes a color marker gene that makes the seed fluoresce and appear pink under ultraviolet light.

In designated production fields, the maintainer line is planted alongside sterile female parents for pollination. The progeny of the two do not contain the cassette of transgenes, and those progeny go on to be used in hybrid seed corn production. To ensure that none of the progeny going into hybrid production contain the transgenes, the seeds are scanned under ultraviolet light. The company deemed the process 'seed production technology', or SPT.

In June, the USDA approved, or deregulated, the maintainer line, and upon Pioneer's request, deemed the commercial progeny of the corn "nontransgenic." "We are very excited about the way USDA worked with us," says Tracy Linbo, global biotech affairs and regulatory lead at Pioneer. The designation of "nontransgenic" from the USDA is important to Pioneer for international commerce pur-

poses, says Bridget Anderson, a spokesperson for Pioneer. The company would like commercial grain from hybrids produced with the SPT process to be exported without additional regulatory review by importing countries. The company has spoken with regulators in Canada, Japan, Mexico, Taiwan and South Korea, and all have verbally agreed that grain produced with the SPT process is nontransgenic, Linbo says. "We described the science to them," she says. "We want to make sure they know it's not transgenic."

Transgenic or not, the product is still the progeny of genetic engineering, and consumers should be made aware of this, says Michael Hansen, a senior scientist at the Consumers Union in Yonkers, New York. "They are trying to play with terminology," he says. "All these new technologies are ways to weasel around a very narrow definition of transgenic," he says. "I would consider that misleading to the public." Hansen says he reviewed Pioneer's analysis of the efficiency of its SPT process and is concerned that transgenic material could find its way into the seeds. "It's not a foolproof system," he says. His organization plans to alert the public, organic growers and concerned groups in importing countries that SPT seeds are offspring of genetically engineered plants.

Whether or not transgenic material pops up in Pioneer's seeds, consuming the progeny of a GM plant will likely still conflict with the values of those opposed to GM organisms, says Kuzma. "The people who are concerned or more wary of these technologies—my sense is that those people are not going to care that the gene is not in the progeny," she says.

Manipulating flowering

The USDA's ruling on SPT corn may be of particular interest to developers of transgenic early-flowering breeding systems, which yield nontransgenic progeny. In this breeding scheme, plants are genetically engineered to flower early, and once they do, the transgene is outcrossed through conventional breeding.

Scientists at the Agricultural Research Service (ARS) in Kearneysville, West Virginia, a research arm of the USDA, have applied the breeding scheme to plums. The plums are transformed with an early-flowering gene from poplar (*Populus trichocarpa* flowering locus T1, *ptFT1*). The gene shortens the tree's juvenile period to less than a year—a useful trait for tree breeders who would normally have to wait up to six years for the tree to flower and reach the reproductive stage.

Once the plant flowers, it is crossed with nontransgenic varieties with desirable traits, such as disease resistance and fruit quality. Markers are used to pick out those that have

the right traits. “We keep going until we have a population with the qualities that we want,” says Ralph Scorza, a research horticulturist at the ARS who is developing the plums. At the end of the breeding process, Scorza and his team select those that do not contain the ptFT1 transgene. Outcrossing ptFT1 is necessary for growing robust trees: if the gene is left in the trees, “they’re bushy, and the branches are weepy,” Scorza says. “But when the gene is out of there the plants grow fine.” Developing a tree that is nontransgenic wasn’t Scorza’s goal, but rather a consequence of the entire system, he says. “It’s a side aspect.”

Scorza calls the system “FasTrack,” and he sees it as both a means to speed up breeding of new varieties and a research tool to study gene function. Regulators at the USDA on 27 October informed Scorza that plum cultivars resulting from his FasTrack breeding system will fall outside of the agency’s regulatory authority, as long as those cultivars do not contain transgenes or pieces of transgenes.

German researchers developing a similar early-flowering breeding program in apple are unsure of how European regulators will view their product³. “We were discussing exactly this point at our last project meeting,” says Matthias Fladung, deputy director of the Institute of Forest Genetics at Johann Heinrich von Thuenen Institute in Grosshansdorf, Germany. Fladung says his group has asked German and European regulators in an official way whether the apples would be considered GM organisms, and is waiting for a response.

Keeping transgenes down

In a different kind of creative tree-development scheme, scientists are studying chimeric grafting, in which transgenic rootstocks are joined with nontransgenic scions: the branches and upper portions of trees. Through traditional recombinant DNA methods, genes for disease resistance or other useful traits are introduced into a rootstock, and the rootstock is grafted to a nontransgenic scion. The junction is like a skin graft: it is wrapped in tape and kept moist, until the vascular systems of the two ends grow together.

This approach raises the question, Does the transgenic material in the rootstock make its way past the graft junction and up to the branches where edible fruit is produced? Guido Schnabel, a plant scientist at Clemson University in Clemson, South Carolina, is investigating this question in plum trees. In a 2010 study, Schnabel found that *Gastrodia* anti-fungal protein-1 (*GAFP-1*), a gene from an

orchid that confers pest and disease resistance in plum tree rootstocks, was not migrating into the grafted shoot or leaves⁴. The resulting plums, then, would not contain the *GAFP-1* transgene. “It would be good to know if the consumer would accept something like this,” Schnabel says. But he cautions: “Although our studies have shown no recombinant gene transfer to the canopy, we cannot exclude the possibility that over time that might happen.” The transgene may also be transferring at a rate lower than the detection threshold, he says.

All in the family

One way to get around foreign genetic material altogether is to source material for a putative genetic modification from a sexually compatible species, a category called cisgenics or intragenics. J.R. Simplot in Boise, Idaho, has developed a cisgenic potato low in acrylamide, a compound that has been linked to health issues and to bruising, a cosmetic defect. All the genetic material to confer this trait came from potato. “We didn’t use transgenic approaches because there was no need to do so,” says Caius Rommens, director of R&D at J.R. Simplot. “The most important potato issues, both in terms of food quality and sustainable agriculture, can be solved through cisgenics,” he says.

The company has petitioned the USDA to approve the potato, and a decision is pending. Although all the genetic material came from potato, the USDA has regulatory authority over the product because the plant pest *Agrobacterium* was used as a transformation method.

Companies developing cisgenics may also soon get a regulatory break from the US Environmental Protection Agency (EPA). Normally, GM plants with an insect-resistance trait must be reviewed by the EPA as well as the USDA. (Plants containing material harmful to pests, so-called plant-incorporated protectants, are regulated like pesticides.) But EPA is considering exempting cisgenic plants from its review process⁵. In March 2011, the EPA shared a draft of the proposed rule change with two other federal agencies. “The initial steps in rulemaking require vetting with other agencies and costing it out,” says Doug Gurian-Sherman, a senior scientist at the Union of Concerned Scientists and a former risk assessor at the EPA. “That is a substantial commitment and is not done unless the agency is very serious about moving forward.” If the EPA exempts cisgenics, the USDA is likely to follow suit, he says.

Going the cisgenics route might pay off in the marketplace as well. Educated consumers are more likely to choose cisgenic food

over transgenic food, Simplot researchers have found through their own studies and a review of the literature. “The closer we can stay to breeding, the easier,” says Rommens. “The consumer prefers genes from inside the species.”

Consumers beware?

But foreign DNA isn’t the only thing that concerns consumers, say some researchers. “The concern over GM organisms is not restricted to the inserted gene or its presence in the plant,” says Hansen at Consumers Union. “There is also concern about the unintended effects that could occur as a result of insertional mutagenesis.” As in plant mutagenesis, which is not covered by regulation, many GM techniques can result in random insertions of the transgenic DNA into the host plant’s genome, which can cause unintended mutations that often can’t be detected. Some of these changes can hang around for generations of plant breeding. “Where the gene is inserted into the genome makes a big difference,” adds Gurian-Sherman.

Targeted approaches address that issue to some degree. In their ZFN research, Dow scientists have not, to date, identified unintended changes from the use of the technology in their assessments in their assessments carried out by deep sequencing genes related to the target sequence, which are the most likely to be hit, says Hamlin at Dow. However, “any form of cell reproduction, whether uncontrolled, in-nature or assisted by man, has the inherent ability to introduce random genetic changes,” he says. “Biology does not offer any gold standard for flawless reproduction of cells.”

But the differences between the targeted approaches and the older, less precise methods of genetic modification may be lost on the masses. “I don’t think the public is going to make that distinction,” Kuzma says. “People are concerned about choice and access to information and having trust in the people who oversee the regulation of the technology.” She notes that the USDA’s letters to Dow and Cibus were forced out of the agency by FOIA requests—not the most public-friendly way to go about regulation.

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