Agbiotech 2.0

As parts of the developing world embrace biotech, the focus is shifting from food production to fuels, industrial chemicals and even drugs. Daniel Grushkin investigates.

As Europe increasingly becomes a genetically modified (GM)-free zone, countries in Asia and South America are embracing the technology. Even African states are beginning to come around. Only five days before last year's vote in the European Parliament to give individual member countries the right to ban GM crops on the grounds of environmental and health concerns, Kenya became the fourth African country to approve the import and production of GM crops.

The thawing environment for transgenic products outside of Europe partly reflects a realization that grain commodity prices are threatening food security and that, according to the United Nations, agricultural production will need to rise by 70% by 2050 to meet the needs of the world's growing population[1]. To stave off a hunger pandemic and dire projections about the wilting effects of climate change on agriculture, new agbiotech tools and applications will be a key part of the solution. As a result, multinational companies are quickening the pace and widening the variety of innovation they are undertaking, not only to compete with each other, but also to outpace low-cost competitors in emerging economies that are producing innovations of their own. Thus, begins Agbiotech 2.0.

The end of hegemony?

Despite controversy about efficacy and safety, the adoption of the limited variety of first-generation GM crops has been remarkably widespread. Since their commercialization in 1996, crops transgenic for *Bacillus thuringiensis* (*Bt*) toxin or herbicide resistance now cover 160 million hectares and are used by 15.4 million farmers in 29 countries, according to the International Service for the Acquisition of Agri-biotech Applications, based in Ithaca, New York (**Fig. 1**).

And although the majority of research and seed production comes out of agrochemical corporations in the developed world, these multinational operations are no longer the only game in town. The country with the second-highest acreage of GM crops is now Brazil. And it is projected that developing nations, which grow half of the world's GM crops, will be growing the majority in 2012. Although most of those crops are now patented by seed giants such as Monsanto of St. Louis and Pioneer Hi-Bred (a DuPont business in Johnston, Iowa). local seed companies popping up in developing countries are bound to bite into the multinational market share.

For example, China's largest GM seed company is Shenzhen-based Biocentury Transgene, a state-supported company whose cotton seed incorporating the *Bt* gene is grown on 90% of Chinese cotton plantations. In the Indian market, Biocentury now goes toe to toe with Monsanto, which cut its prices by nearly half to compete with the Chinese company.

By 2015, some 34 GM crops will have moved into advanced development in Asia, compared with only 26 in the US and Europe, according to a 2009 report by the European Union Joint Research Centre[2] (http://www.nature.com/nbt/journal/v28/n1/extref/nbt0110-23b-S2.xls). In the short term, these crops aren't a technological challenge. The technology the companies are adding to their crops has trailed the giants, and those crops are slated to be grown domestically. But according to the report, "in [the] future the adoption pattern may change fundamentally, with more new GM crops being adopted first in Asia (and then potentially spreading from there)."

Hello, generics

The seed hegemony will further be challenged in 2014, when the early patents on GM crops expire. As many as 29 seed patents could be on the chopping block, but the most important among them is the last of Monsanto's Roundup Ready soy seed, which contains a gene resistant to the company's herbicide Roundup. Herbicide-resistant soy occupies more acreage worldwide than any other GM crop. Just as generic versions of Roundup originating from China have wedged into the company's chemical herbicide business, cut-rate seed companies producing generics are also likely to rush in to challenge the giant for market share.

The expiration of these patents marks a turning point for GM crops. Whereas for the past 16 years, GM crops have exploited exclusively foreign genes to kill pests and tolerate herbicides, the next generation of biotech crops from the major seed companies goes much further. They have engineered seed that increases yield and addresses stressors from climate change: drought, heat stress and even the salinity in the soil. The giants have had no choice but to innovate.

Agro-genomics

A decade ago, Ceres, an agbiotech based in Thousand Oaks, California, adopted the tools of the Human Genome Project to study plants. It was the beginning of a revolution in agbiotech. In one project, the company upregulated 10,000 genes in *Arabidopsis thaliana* to test how the new GM plants would react under various conditions. It was act of random searching: "You look at the 20,000-odd genes that are in the plant and you say, 'I'll take this half,' " says CSO Richard Flavell. In the process, Ceres screened hundreds of thousands of plants

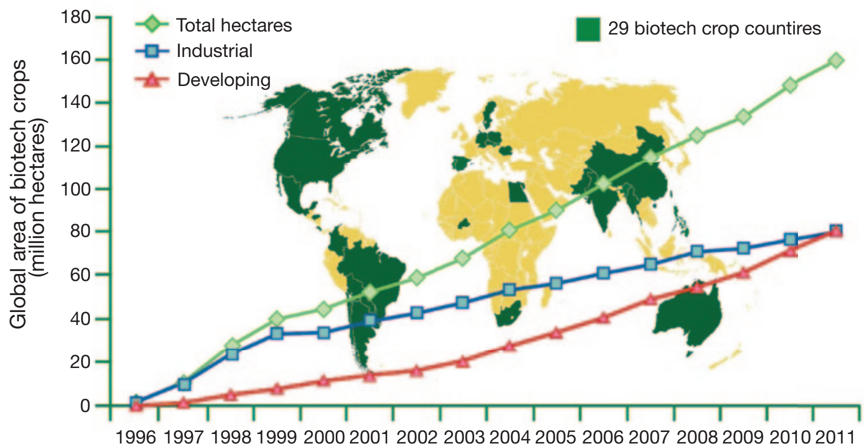

Figure 1 GM crops around the world. (Source: Clive James, ISAAA, 2012.)

for traits that might be useful. "That was our source of variation and we could do it at a scale that was essentially bigger than anyone else could do at the time," he says.

The project had the random quality of the experiments that plant breeders performed in the first half of the twentieth century. Leading up to the birth of genetic engineering, scientists zapped seeds with X-rays or dipped them in chemicals to induce mutations. If the mutant produced a useful trait, they'd breed the plant. The difference here was that Ceres could identify the gene and the mutation producing a particular effect. Instead of playing genetic potluck, they were slowly building a data set that could start to answer questions about an individual gene's relationships to plant physiology.

The trick to connecting genotype with phenotype was having sophisticated screening techniques and the tools of computational biology. The work gave birth to a five year $137-million product-discovery and development deal with Monsanto in 2002 and a stream of imitators. Although the technology under license has not been commercialized to date, through this process, agricultural-genetics research has begun to form a shadowy picture of the network of genes that determine certain plant traits. Last year, Ceres reported on two genes regulated by the *Arabidopsis* circadian clock that affect flowering. When upregulated, the genes (*At5g52250* and *At5g23730*) produced early flowering. The genetic alteration could one day be carried into food crops to increase yield[3] (see p. 215).

The widespread adoption of next-generation sequencing has now begun to fill in the blanks bit by bit. "DNA sequencing has evolved at a speed nobody could have predicted a few years ago. This is absolutely essential for going forward," says Michael Metzlaff, research manager at Bayer CropScience, headquartered in Monheim am Rhein, Germany.

Researchers have even begun to sequence multiple strains of crops to understand the relationships of various alleles to traits, something that would have been prohibitively expensive just a few years ago. One team in China, for example, has sequenced 14 domestic and 17 wild varieties of soybean to find specific genetic variants between wild-type and cultivated strains[4]. "Once you've got a conventional genome assembly, then to reassemble against an existing skeleton is very cheap and quick," says Jim Dunwell, a plant biologist at the University of Reading, UK.

In addition, marker-assisted breeding—in which short DNA sequences, associated with genes of interest can be used to determine whether a seed is likely to possess a desirable trait, without the need to grow a mature plant—has become a mainstay of major seed companies. For example, Pioneer Hi-Bred has developed a 120-watt laser to score a thin slice off of a seed in order to sample its genes without destroying it. According to Metzlaff, the use of molecular markers cuts breeding time in half.

Box 1 Pipeline to regulatory limbo?

Deregulation has always been a convoluted process in the US. A new transgenic crop might pass through three agencies—the US Food and Drug Administration, the US Environmental Protection Agency and US Department of Agriculture (USDA)—before being approved for commercialization.

Now, according to the Washington, DC–based Biotechnology Industry Organization (BIO), deregulation is more arduous than ever. In the USDA, the average processing time has risen from 140 days in 1996 to nearly 1,200 last year. Seed companies have paid on average $35 million in expenses associated with deregulating individual crops according to a September Crop Life International survey of seed companies.

The delay has coincided with a glut of new GM crop varieties. In 2008, there were 33 GM crops in worldwide commercial circulation. That number will reach 124 by 2015, according to a 2009 report from the Joint Research Centre (JRC) of the European Union[2]. Coupled with increasingly shrill debate over GM crops, the surge has created a regulatory logjam, both domestically and internationally. Alexander Stein, author of the 2009 JRC report, points to the controversy in India over brinjal (eggplant) engineered with a *Bt* gene. "When India posted a moratorium on eggplant, the whole Indian pipeline came to a stop. It's the politics involved," he says.

In the US, industry has blamed the backlog of 20 crops awaiting deregulation on a series of lawsuits made by public interest groups, particularly over alfalfa and the sugar beets modified with the Roundup Ready gene. The lawsuit over GM sugar beets, for example, hinged on whether the USDA had done a proper environmental impact study before approval. Last August, Jeffrey White, a district judge in San Francisco, declared that it had not.

"The whole system has been screwed up as a consequence of harassment of lawsuits filed by activists," says Val Giddings, a senior fellow at the Information Technology & Innovation Foundation in Washington, DC. For its part, the Washington, DC–based US Center for Food Safety, which filed the suit, complains that officials have never evaluated the crops in good faith. "Our experience is that the USDA fundamentally views all biotech products as a good thing, and their job is just to rubber-stamp approvals," says Bill Freese, Center for Food Safety science policy analyst.

To expedite the process, last April the USDA introduced a pilot program allowing seed companies to write their own environmental impact studies on new crops (or hire a third-party company). The announcement produced a new round of uproar over conflicts of interest.

If there's one thing that the both sides agree on, it's that regulation needs an overhaul. The USDA has been trying to update its regulations since 2004, with little headway. Until now, agencies have evaluated GM crops on the basis of the dangers associated with the genes' organism of origin or the vector by which they're inserted. "We have to start regulating by the properties of the crop, not the techniques by which it was modified, which is what we're doing now," says Nina Fedoroff, former science and technology advisor to the US Secretary of State.

Ironically, the next generation of gene-editing technologies may sidestep the regulatory process entirely. The USDA regulates transgenic crops through the Plant Protection Act, which gives it the power to rule on genetic parts that come specifically from plant pests. Zinc-finger nucleases (ZFNs), for example, don't originate from pests, and, therefore, appear to fall outside the regulatory framework. In 2009, Vipula Shukla at Dow AgroSciences used ZFNs to produce herbicide resistance in corn without adding any foreign genes. Presumably, the seeds will be treated like any conventional breed. "Because the changes you introduce by those techniques are exactly like those you can make by classical mutagenesis, it shouldn't be subject to this horrendous regulation," Fedoroff says. Similar constructs called transcription activator–like effector nucleases could make gene editing even easier, they originate from the plant pest *Xanthomonas*, and might be captured under the current framework.

The USDA's Animal and Plant Health Inspection Service (APHIS) has yet to decide on its role in the process. "APHIS is currently considering the regulatory status of zinc-finger nucleases and transcription activator–like effector nucleases," says spokesman Richard Bell. The decision has the potential to change the entire industry (see p. 215).

Genomics and markers, however, are only part of the equation. "You can sequence a genome in a week or outline a biochemical pathway in gory detail, but the question then becomes, how do you use that information to help the engineering or the modification of the plant?" says Vipula Shukla, scientist at Dow AgroSciences.

The latest technology in gene manipulation adopted by seed companies has been zinc-finger nucleases (ZFNs). Dow AgroSciences licensed the technology from Sangamo of Richmond, California, under the name Exzact Precision Technology. Using ZFNs, genetic engineers can target and manipulate precise sequences of DNA. These are useful not just for gene insertion, but for cutting specific locations on the genome to disable or edit specific genes.

In 2009, Shukla and a team from Dow and Sangamo used ZFNs to target and disable a gene encoding an inositol pentakisphosphate kinase responsible for the storage of 75% of the phosphorus that is found in corn kernels and is an unhealthy component of animal feed. The team not only reduced seed phytate but conferred herbicide resistance too.

Because the technique forgoes inserting foreign genes into crop genomes, the technology raises the regulatory question—are ZFN-manipulated seeds bred or GM (**Box 1**)? "This increasingly gray area might not come under the regulated definitions of GM," says Dunwell. If so, they may eliminate the regulatory lag between development and commercialization (see p. 215).

Old problems, new traits

Getting pests under control still occupies plant researchers: the emergence of herbicide-resistant weeds has seed companies exploring new genes for combating them. So far, 21weeds have shown resistance to glyphosate, the active ingredient in Roundup—many of them have appeared in the years since the release of herbicide-resistant GM crops[5]. To counteract these new weed strains, crops with resistance to multiple pests have been produced (**Box 2**) and crops resistant to other herbicides, such as Dicamba (Monsanto) and acetolactate synthase (ALS, DuPont), are in late stages of development. Meanwhile, researchers are taking new approaches to supplementing *Bt*, such as RNA interference, to enable crops to ward off pests[6].

Beyond new suites for pest management and herbicide tolerance, the goals for the GM crops coming down the major seed companies' pipelines are to both increase yield and address abiotic stressors "As we're looking toward this next wave of traits, what's different and required is a deep understanding of the fundamental biology of how the plant works. We need to understand the relevant biochemical pathways, the energetics, how they use nutrients and, most importantly, how those biological components interact in the environment," Shukla says.

In that direction, research is now underway to identify the genes underlying crop architecture, leaf area and leaf angle, with a view to using genetic technologies to create new varieties that maximize photosynthesis. Root structure can also be altered to increase crop density by maximizing nutrient uptake while occupying the smallest area, says Flavell. In one example of this work, scientists at the Chinese Academy of Sciences linked the *OsSPL14* gene to the number of tillers, or shoots, at the base of rice. A mutation in the gene decreased the number of tillers and increased yield by 10%[7].

And then there's water stress. One need only look at the American Southwest to understand the focus on drought. The last year has been the driest period Texas has seen in 115 years. In the coming years, with population growth and biofuel crops competing for irrigation, there will be increasing strain on water resources. "Investigating plants to tolerate heat and drought—that's the most important thing we could be doing for the generation after next," says John Bedbrook, DuPont's vice president for agbiotech.

One of the most promising developments comes from an insertion of a gene originating from *Bacillus subtilis* that encodes a cold-shock protein. When bacteria are exposed to sudden cold, protein synthesis slows. As a result, the cell begins producing cold-shock proteins, chaperones that rescue misfolded mRNA to restore translation. This produces a new state of equilibrium and allows the cell to adapt to the new temperature. When plants undergo stress—from heat, cold or dryness—their metabolism slows, too. "They tend to hunker down," says Bob Reiter, biotech lead at Monsanto. By having a single gene that continually produces cold-shock proteins, the plant is prevented from slowing down.

In one Monsanto study, a transgenic corn strain with a cold-shock protein gene yielded 30.8% more grain under drought conditions than plants without the gene, although the germplasm, timing and severity of drought can affect the outcome. The gene has also proven to be effective in stress conditions brought on by heat and cold; rice with the transgene grew 35% taller than rice without[8]. Monsanto is planning large-scale field trials this year.

Performance Plants of Kingston, Ontario, Canada, has also developed a drought-tolerance gene technology called Yield Protection Technology (YPT), which it has licensed to several seed companies, including Syngenta based in Basel, Bayer CropScience and Scotts Miracle-Gro based in Marysville, Ohio. Canola with a promoter that downregulates the production of farnesyltransferase have a 26% increase in yield; in petunias, the modification nearly doubles the number of flowers per plant.

Box 2 Bumps in the road

2010 was a bad year for Monsanto. The company's newest corn cultivar—SmartStax—failed to meet its hype. The seed had been billed as a marvel of biotech. It combined eight gene inserts, which had never before been accomplished in a product, and pooled Monsanto's suite of technologies with those of Dow AgroSciences. The crops possessed above- and below-ground pest resistance and two types of herbicide tolerance. Despite these bells and whistles, when the first corn harvests in the US were tallied, SmartStax ears yielded 2.5% less than the company's cheaper, less-sophisticated seed with only three gene inserts.

Farmers were angry. They had paid $24 more per acre for the product[9]. The company tried to appease them by offering free credits for the next season, but the damage was done. Commentators around the country decried the company's health. "This may be the worst stock of 2010," Jim Cramer shouted on CNBC's *Mad Money*.

The incident was telling. Even though the destiny of agbiotech seems cast—gene technology speeding ahead and more farmers adopting it every year—these are uncertain times for the seed giants. The SmartStax story raises an important question that has yet to be answered. Will farmers pay a premium for the next generation of crops?

In 2010, Monsanto priced SmartStax seed too high and US farmers didn't go for it. They bought seed for only 3 million acres of SmartStax, instead of the 4 million that Monsanto had hoped for. But things might be picking up. Monsanto is reporting that SmartStax corn outperformed its competitors' products in 2011, and saw a 10-million acre increase for all its GM corn products.

For farmers, the seed advances boil down to the bottom line: "I believe the only meaningful word for all these technologies is yield," says Yafan Huang, of Performance Plants.

According to the company, suppression of farnesyltransferase triggers stomata to shut earlier and tighter in the drought cycle, allowing plants to hold onto moisture and recover sooner when finally watered. Farnesyltransferase is thought to dull the effects of the phytohormone abscisic acid, which modulates the size of stomata. Performance Plants uses RNA interference to downregulate the gene encoding farnesyltransferase. Yafan Huang, CSO of Performance Plants, expects YPT, the first of its suite of gene technologies, to enter the market in 2013.

Fortifying plants

Customers can expect to see food on their grocery shelves that have had trans fats removed or omega-3 fatty oils added. Soybeans engineered by Monsanto to produce oil with stearidonic acid omega-3 are used in foods ranging from yogurt to granola. Next year, DuPont will release a soybean strain that is high in oleic acid. "It's a soy oil that has a fatty-acid composition of an olive oil," says Bedbrook. To produce the variety, DuPont scientists expressed a 600-base-pair fragment of Δ12-desaturase (*FAD2)* gene, which caused gene silencing in the seed. These soybeans have 75% oleic acid, which is a monounsaturated, healthy oil, in the seed oil. The gene silencing prevented the formation of a second double-carbon bond on the oleic acid, and stopped the production of linoleic acid, a polyunsaturated fat.

These oil traits are early examples of seed companies adding nutritional benefits to their crops. In the aftermath of opposition in the developing world to Golden Rice—a strain that had elevated levels of β-carotene (vitamin A precursor), which is often lacking in diets in the developing world—HarvestPlus, a non-profit in Washington, DC, has used conventional breeding techniques to fortify staple crops in South Asia and Africa with vitamin A, zinc and iron.

Beyond food

Even with all the headway made in food crops, agbiotech is moving well beyond food. For nearly a decade, many small companies that license their technologies to the seed giants have been building an industry of crop alternatives to petroleum-based fuel. "In the big picture, there will have been a slice of time when fossil fuels were the source of energy, but of course for the thousands of years before it, the sun and the land were the sources of energy. So unless there's going to be some extraordinary source of solar voltaics or other innovations, I think the land is going to be realized as a substitute for fossil fuel," says Flavell.

Nearly all the companies are scrambling to find the right mixture to meet the rising demand. Ceres, for example, has added a salinity-tolerance gene to switchgrass to allow it to be grown for biofuels in marginal land without competing for resources with food crops. Mendel Biotechnology based in Hayward, California has invested in *Miscanthus giganteus*, a fast-growing, tall grass that has been genetically engineered to increase yield, and is now in the third year of field trials. Syngenta, for its part, has created a corn strain specifically for ethanol production that contains the gene encoding α-amylase, so that the enzyme begins the process of converting starch into sugar inside the plant. Today, ethanol manufacturers pour amylase into the corn slurry as a first processing step.

Looking down the road, higher-value products are also likely to be engineered into feedstocks. Cambridge, Massachusetts–based Metabolix, for example, has inserted a pathway in switchgrass, camelina and sugarcane to grow beads of polyhydroxyalkanoate within seeds to be extracted for the production of biodegradable plastic.

Alongside industrial applications, there is still the possibility that medicines within crops will also find their niche. New York-based Pfizer is developing a treatment for Gaucher's disease that is produced in GM carrot and tobacco cells. The cultures produce recombinant glucocerebrosidase, an enzyme required for metabolizing fat. The treatment is now under review at the US Food and Drug Administration, with a 1 May 2012 Prescription Drug User Fee Act date. Elsewhere, Calgary, Alberta, Canada–based SemBioSys has engineered safflower plants to produce insulin for diabetics. Phase 1/2 trials were completed in the UK in 2009 (where an approval pathway for biosimilars exists), in which the safflower-produced product was indistinguishable from Humulin (insulin), produced by Eli Lilly of Indianapolis. The company has a joint venture with the Chinese pharmaceutical giant Tasly of Tianjin, and is positioning the product for eventual registration in the US, Europe and China, according to Rick Pierce, president for US and international operations.

Amber waves

Jüergen Logemann, director of research at BASF Plant Science based in Raleigh, North Carolina, was addressing the US Congress a few years ago about the future of agriculture when an old farmer stood before the hearing committee. Logemann remembers the farmer's words: "For the last 30 years nobody cared what I was doing. I was poor. Now, suddenly I feel honored and valued. I feel like I've become king."

The farmer voiced a sentiment that resonates with agbiotech insiders, who feel the sector is on the cusp of the next generation of crop technologies. "You begin to rethink the economy of the rural areas," says Roger Beachy, one of the first plant genetic engineers and former director of the National Institute of Food and Agriculture in the US Department of Agriculture. "The rural areas become the factories to make all these raw materials. You value them in a different way than, 'oh, that's just making more corn.' It's not. It's economy."

The slew of new applications flowing from agbiotech have those in the industry thinking about society's connection to farmland in a new way. As crops are engineered to produce more grain or products such as medicine or fuel, and to do so under increasingly variable conditions, every acre of land, even marginal land, gains value. Seed companies still bear the burden of proving this dream to the public, especially when climate indicators and food shortages imply otherwise. But, as Flavell says, "with a little bit of optimism, we can believe that the role of the land is going to come back to hold a new position in the way that perhaps cities did in the past hundred years."

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