THE BREEDING EDGE

Will the breeding evolution lead the next green revolution?


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Greetings,

Change can be difficult, especially when it comes to adopting new ways of farming and producing food. But there are big innovations underway in labs and universities that analysts describe as "revolutionary," enabling the creation of new plants and animals in months rather than decades. In this series of articles, Agri-Pulse explores “The Breeding Edge” – a deep dive into how these new precision methods for plant and animal breeding are set to transform global food production and the potential impact for agribusinesses, farmers and consumers around the world.

Best regards,

Sara Wyant
Founder and Editor of Agri-Pulse

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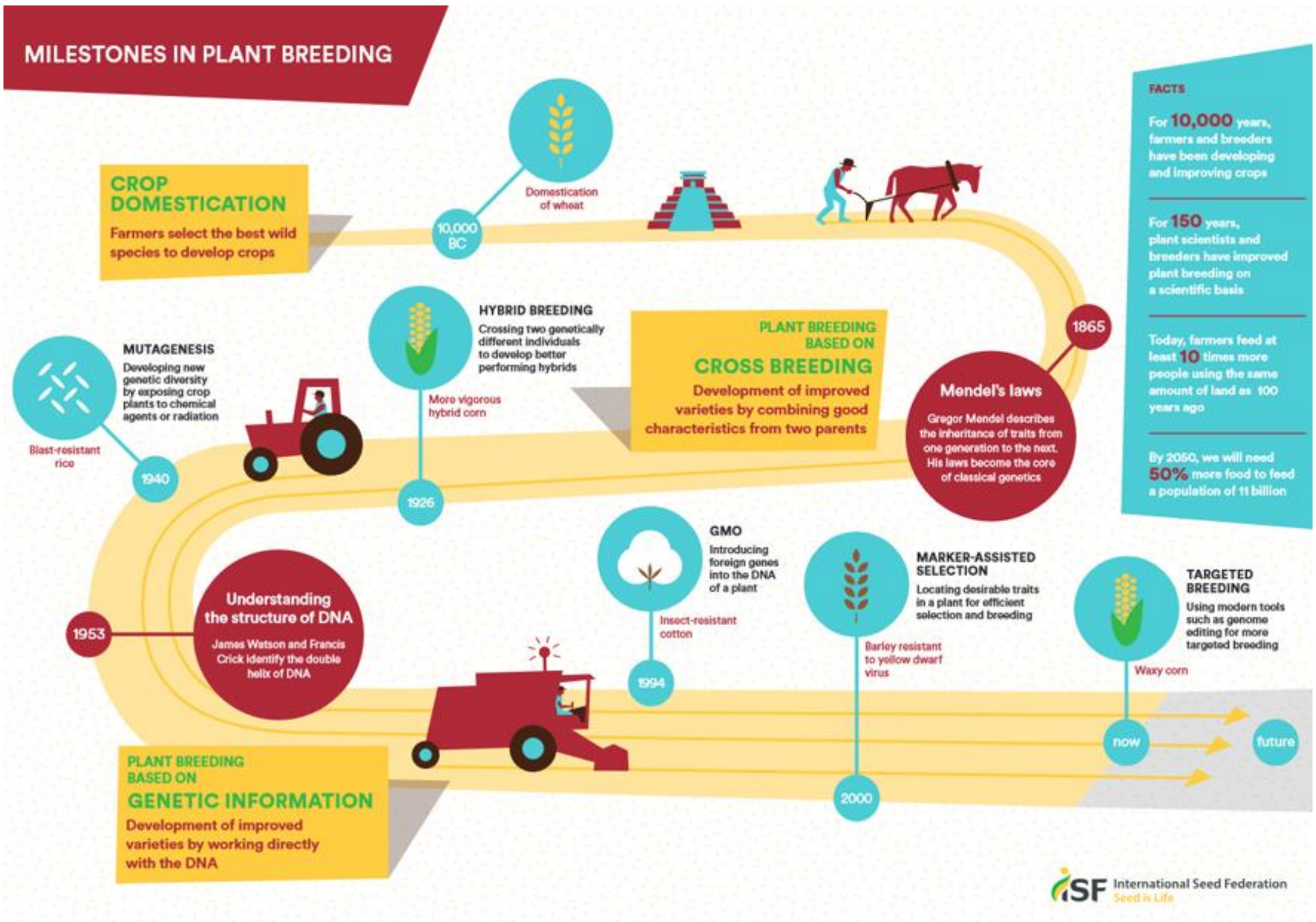
How gene editing can revolutionize feeding the world



By Ed Maixner

The process of producing food, protecting the environment, and improving animal health is advancing at a seemingly breakneck pace.

These advancements are driven in part by new scientific discoveries, genetic research, data science, enhanced computational power, and the availability of new systems for precision breeding like CRISPR—an acronym for Clustered Regularly Interspaced Short Palindromic Repeats.

The outcomes possible with different types of precision breeding today might have seemed impossible just a few decades ago and these new opportunities have strong implications for both producers and consumers. Consider just a few of the possibilities:

- A new cassava plant, engineered to be resistant to brown streak disease, could make the difference between small farmers in Africa having a crop to eat and having no crop at all.
- New breeds of livestock and poultry could be engineered to no longer be susceptible to widespread disease outbreaks, like pigs resistant to Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), which can cost hundreds of millions of dollars annually.
- Cover crops that naturally improve soil health can be developed to grow in more diverse climates, improving environmental sustainability, water quality and animal nutrition.
- Dairy cows can be bred without horns, removing the need for cows to endure the polling (horn removal) process.
- Fruits and vegetables could be engineered to resist browning, extending their consumer appeal and reducing food waste.

Indeed, the science is moving so rapidly that some are wondering if producers, as well as consumers, and regulators will ultimately be able to understand and embrace the changes.

As history demonstrates, new advancements in breeding have almost always been controversial – even though safety or environmental risks have not been proven.

"It is critically important that everyone in agriculture becomes rapidly conversant in this technology, as it already has been a game changer," notes Kevin Folta, who chairs the Horticultural Sciences Department at the University of Florida in Gainesville. "If these technologies are delayed because of misunderstanding, we will lose many opportunities to bring improved varieties to the field and better fruits and vegetables to consumers."

The long, long road to gene editing

Change has always occurred in plant and animal breeding, but by a long shot, agriculture's genetic advances haven't always been so brisk. Quite the opposite.

Even though the pace toward modern plant and animal breeding quickened remarkably in the 20th century, since the birth of farming about 8000 B.C., in what's now Iran and Iraq, and in Central America not much later, most improvements in strains of crop and animal species have gradually evolved over hundreds of years.

Farmers, gardeners and others learned a wide range of husbandry skills over the centuries, says E. Charles Brummer, plant breeder at the University of California, Davis, and president of the Crop Science Society of America. "Cloning of grapes goes back hundreds of years," for example, and manure has been used to enrich soils since the earliest agriculture; crop rotations have long been used, too, to improve soil and crop vigor, he points out.

K. Kris Hirst, of Iowa City, Iowa, an archaeologist who writes and speaks on early world agriculture, agrees that early farming R&D involved much more than selective breeding, and was "also a matter of the humans learning what the plants or animals need and finding a way to give it to them . . ."

Various water delivery systems, for example, were built thousands of years ago on several continents, she noted, "consisting of things like canal systems in Mesopotamia, rock terraces in Peru and Mexico, underground watering systems, such as the qanats that tapped groundwater in the Turpan Oasis of central Asia."

Plant breeder E. Charles Brummer is president of the Crop Science Society of America

But Hirst tells *Agri-Pulse* that selective breeding has been with agriculture from the beginning.

"Basically, you would collect the seeds from the best crops this year and replant them in the garden for next – picking the seeds from favored aspects and replanting them again, and not planting those without those preferred traits. Some early domestication changes had to do with moving the plant out of its normal habitat. So, the survivor plants were the ones that were best at adapting."

In her writings, Hirst describes the start of farms and agriculture in the Near East and what's called the New Stone Age: "The earliest structures made of stone were built in the Zagros Mountains, where people collected seeds from wild cereals and captured wild sheep."

That period "saw the gradual intensification of the collecting of wild cereals, and by 8000 B.C., fully domesticated versions of einkorn (wild) wheat, barley and chickpeas. And sheep, goats, cattle, and pigs were in use within the hilly flanks of the Zagros Mountains, and spread outward from there over the next thousand years."

While today's farmers plant about 25,000 different strains of wheat worldwide, Hirst says, the earliest evidence of domesticated einkorn and emmer wheats has been found at the Syrian site of Abu Hureyra," dated to 10,000-11,000 B.C. Evidence of the earliest rye is linked to "hunters and gatherers living in the Euphrates Valley of northern Syria" about 9000-10,000 B.C.

Maize cobs uncovered by archaeologists show the evolution of modern maize over thousands of years of selective breeding. Even the oldest archaeological samples bear an unmistakable resemblance to modern maize. Photo ©Robert S. Peabody Museum of Archaeology, Phillips Academy, Andover, Mass. All Rights Reserved.

Maize (corn) was domesticated as early as 7000 B.C. in Central America from a plant called teosinte, and cobs of domesticated maize identified in Guerrero, Mexico, were dated to before 4200 B.C. One theory has corn originating in Mexico's highlands as a hybrid of diploid perennial teosinte and early-stage domesticated maize. In Peru alone, she says, specimens of 35 strains of maize from before

Europeans' arrival have been identified, including popcorns, flint varieties, and others for uses such as making chicha beer and textile dyes.

Corn was used in what is now the U.S. Southwest by about 1200 B.C. and, by the first century, the crop joined eastern North American natives' other established foods such as pumpkin and sunflowers.

As with their crops, farmers around the world also selectively bred animals, adapting them to their needs for food and clothing and breeding them to flourish in new climates when, for example, they migrated to the Americas and Australia and brought cattle to those continents.

Hirst points out that farming migration in early agriculture was extremely slow. "People traveled much more slowly in the past – a person can travel about 12 miles in a day, and less than that if driving animals. So selective breeding was a very slow process" as groups "would move into drier climates or less ideal environments or respond to climate changes as they themselves ranged hither and yon. You would bring your goats with you and the ones that lived the longest or continued producing milk the longest would be, by definition, the ones that survived." Initially, today's hot- and cold-climate cattle breeds all began as aurochs (now extinct) in Europe and the Near East. Beginning about 10,000 years ago, they were domesticated and bred for mankind's purposes across Europe and parts of southern Asia and northern Africa.

Archeologist Kris Hirst

She said evidence for selective farm animal breeding in pre-history was apparently not, for example, focused on the biggest, toughest bulls or rams to protect the herd. Actually, she said, scholars believe most sought-after characteristics were those in animals that could adapt to living close to humans. "Domestication," Hirst said, "is always associated with getting smaller, calmer, sweeter-tempered animals, who didn't mind being milked and were disinclined to attack the humans or wander off."

Cross breeding catches on in the 20th century

Although Gregor Johann Mendel, a scientist and Augustinian abbot in Brno, Moravia (now in the Czech Republic), became hailed in the 20th century as the father of modern genetics, the long-delayed acceptance of his discoveries reflects the very slow pace of plant and animal breeding advances through most of human history.

He tested some 28,000 pea and other plants to learn about hybridization and demonstrate the dominant and recessive traits in evidence as a result of crossbreeding. He also experimented with breeding mice and bees. He was not alone. Other naturalists and farmers experimented with cross breeding plants in the late 19th Century as well, but the concept did not catch on.

So Mendel's meetings with the Natural History Society in Brno in 1865 and his now-famous paper, Experiments on Plant Hybridization in 1866 were soon after ignored until the start of the next century, many years after his death.

Plant breeding in perspective

Geneticist and plant breeder Donald F. Jones, who chaired the Connecticut Agricultural Experiment Station in the early 1900's, once described a banquet in Sir Walter Scott's 1820 novel, Ivanhoe, in which the meal did not include potatoes, turkey, cranberry sauce, pumpkin pie, coffee and cigars.

"Peas and cabbages were there, but no knight in armor ever ate a tomato salad. Queen Guinevere never tasted corn on the cob," he wrote in reference to plants contributed by the New World to the Old.

"History has much to say about generals and battles. Its pages are filled with the deeds of emperor and kings, too seldom glorious.

But the major factor in the growth of states and empires has been the origin and development of domesticated animals and cultivated plants," Jones wrote.

However, in the early 20th Century, other scientists, trying to better understand inherited traits, reproduced Mendel's plant experiments and bought into his theories. In 1909, Nils Heribert-Nilsson, a Swedish botanist, demonstrated how results between crosses, or hybrids, yielded plants that outperformed either parent. That result was labeled "hybrid vigor" and became the spark for broad use of hybrid crop production.

Also, in 1917, American agronomist Donald Forsha Jones showed the benefits of employing the double-cross pollination method of hybrid seed production and helped usher in the first American hybrid corn seed in the 1920s.

Meanwhile, English naturalist Charles Darwin, who researched and wrote concurrently with Mendel, posted his Origin of Species in 1859 and Natural Selection in 1875, broadly influencing scientific thought on genetics as well. The findings of Mendel and Darwin were at the center of genetics and evolutionary biology by the mid-20th century.

Through the decades since, the magic of hybridization, back-breeding to isolate and infuse a desired trait, and other cross breeding has enhanced farm production along with crop and livestock health. But of course, there were other factors.

Old fashioned selective breeding, along with fertilization, pest control, soil health enhancement, and other techniques have enhanced crop production, just as livestock breeding successes have been augmented by improved animal nutrition, disease prevention and husbandry skills.

Nonetheless, breeding has led to success across virtually all of the farm sector: veggies with enhanced flavors; apples, citrus and other fruit that are sweeter and with longer shelf lives; even popcorn that pops better.

Corn breeders plunged into hybrids in the 1930s, producing double-crosses to maximize advantageous traits, and seed companies began aggressive selling of their hybrid seed to Midwest farmers. The U.S. average corn yield soared from 20 bushels an acre in 1930 to 90-100 bushels in the 1970s and to more than 170 bushels in recent years. And even higher averages seem likely. The top yield in the 2017 National Corn Growers Association's annual yield contest topped 542 bushels per acre in the no-till/strip-till irrigated category.

Meanwhile, since 1950, the U.S. average yield for wheat has climbed from 16 bushels per acre to about 50 in recent years; soybeans, from 21 bushels to 50.

The accelerated growth rates of meat animals speak especially to selective breeding and rearing success. In the early days of the commercial poultry industry, each chicken required approximately 16 pounds of feed to achieve a four-pound weight. Today, that amount of feed has been reduced by more than half –

less than seven pounds of feed – to grow the same size bird, all without the use of growth hormones or steroids, according to the National Chicken Council.

Fryer-sized chickens used by restaurants reach 4-pound slaughter weight at 35 or fewer days of age, about twice as fast as in the 1960s. Meanwhile, tom turkeys for the holidays are now raised to the typical 14 to 16 pounds in nine to 10 weeks, versus the four months or more required, for example, in the 1980s.

America builds an R&D infrastructure

The road to modern science-infused farming in the United States wasn't built in a vacuum. America started laying the foundation for publicly-funded agricultural research and advancement with the Morrill Act of 1862, by which Congress granted tracts of land to states at 30,000 acres per member in Congress. The land was to be sold to start and maintain colleges "where the leading object shall be . . . to teach such branches of learning as are related to agriculture and the mechanic arts . . ." The act resulted in the birth of the national land grant universities. The Second Morrill Act of 1890 extended similar support to Southern states to open similar institutions to accommodate black students.

National support for agricultural R&D came with the Hatch Act of 1887, which provided money for the state land-grant colleges to set up agricultural experiment stations and disseminate information from the stations. Later, the Smith-Lever Act of 1914 established a system of cooperative extension services, linked to the land-grant colleges, to help people learn about and implement new farming practices and livestock discoveries, advances in home economics and to support 4-H clubs and other outreach to farms and rural communities.

The Green Revolution goes global

Cross-breeding of corn was not the only yield-building game in town. Especially with countries in Southeast Asia facing severe and chronic food shortages and starvation in the 1950s, governments and plant breeders joined in the 1960s in aggressive rice and wheat breeding of hybrid and selective strains, plus improving farming practices to boost crop yields and feed more people.

In 1960, the Philippines government joined with the Ford and Rockefeller foundations and established a rice breeding collaboration called the International Rice Research Institute (IRRI). Its first rice release, IR8, called "miracle rice," allowed the Philippines to more than double its average rice yield in about two decades.

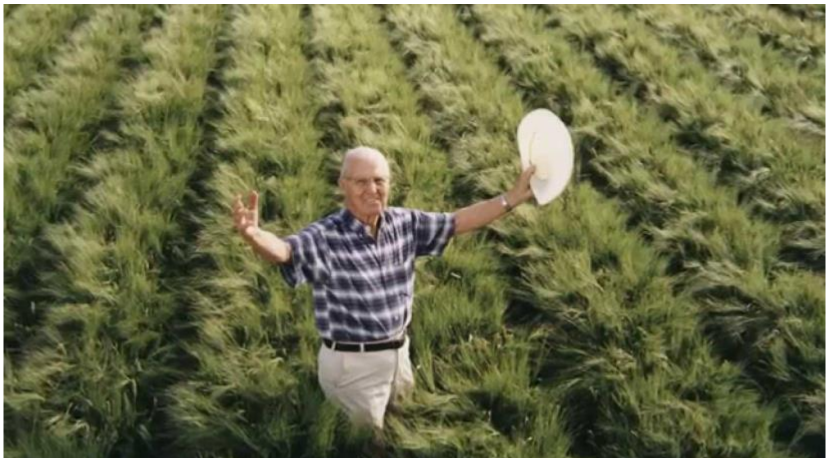
The IR8 variety led to other varieties of rice adapted to flourish in other countries in the region, including Indonesia, Cambodia, Malaysia, Vietnam and Myanmar (then, Burma). A new dwarf variety, IR36, was planted in India, and, when fertilized, it out-yielded other traditional rice by a factor of ten by 1968.

Norman Borlaug, the American agronomist who became known as the father of the Green Revolution, was working with the Rockefeller Foundation to improve wheat varieties in the 1940s in Mexico, which was succeeding in its own wheat improvement program. He went to India in 1961 at the government's request and began improving wheat to relieve hunger there, importing seed developed by breeders with the International Maize and Wheat Improvement Center.

Building on the global collaborative efforts to improve field crops in developing countries, the Ford Foundation, World Bank and others began in 1970 to set up a network of agricultural research centers under permanent direction. The result was the Consultative Group on International Agricultural Research (CGIAR), which is also supported by the United Nations Food and Agriculture Organization and others. CGIAR operates several research centers.

Breeders pry into the cell nucleus

The path toward breeders' genome manipulation required access to and knowledge of the millions of genes on chromosomes, which lie in the cell nucleus. That access began with British researchers like Rosalind Franklin, who, with an assistant, began producing the first high-resolution photos of deoxyribonucleic acid (DNA) fibers in 1951.


Norman Borlaug was a U.S. agronomist known as the father of the Green Revolution

Using such imagery, in 1953 molecular biologists James Watson and Francis Crick were able to describe chromosomes' double helix, the twisted-ladder structure of DNA. That discovery became the sort of first-grade graduation into modern molecular biology, which is focused on how genes control the chemical processes of an organism's growth and bodily functions.

Biochemist Frederick Sanger, another British scientist, and colleagues pioneered what became known as the "Sanger Method" of mapping the base pairs of genes, which are the letters of an organism's genetic code. His method was the original one for sequencing DNA and, in 1977, he published the sequence of a virus genome of over 5,000 base pairs.

The Sanger Method became the usual one for mapping an organism's genome. **In recent decades, with the expanding knowledge of DNA and massive capacity of computers to store and communicate such data, researchers have moved ahead, compiling entire genomes of plants and animals.** Sequencing of the 3 billion base pairs of the human genome, a 13-year international collaborative project, was completed in 2003.

Since 1995, scientists have sequenced the genomes of dozens of plants, including the major commercial crops. Sequencing the genomes of farm animals began with that for a chicken in 2004, and those for a cow (in 2009), and pig (2012) have since been published. Sequencing allows scientists to find specific genes on the chromosomes and learn how each gene works together with an organism's other genes to create its phenotype, which is its appearance and how it grows, functions, and responds to changes, and other characteristics.

At the same time, genetic engineering techniques were evolving to allow for the introduction of new traits as well as greater control over traits than previous methods such as selective breeding and mutation breeding, which is the process of exposing seeds to chemicals or radiation to generate mutants with desirable traits that can then be bred with other cultivars.

The first genetically modified plant was produced in 1983, using an antibiotic-resistant tobacco plant. In 1988, the Food and Drug Administration approved the first application of genetically modified organisms in food production. In the early 1990s, recombinant chymosin – an enzyme with a role in digestion in some animals -- was approved for use in several countries.

The first genetically modified food approved for release was the Flavr Savr tomato in 1994. This tomato was developed by Calgene to have a longer shelf life.

A year earlier, China had introduced virus-resistant tobacco, becoming the first country to commercialize a transgenic crop. Transgenics refers to processes that impose a gene or genes from an unrelated species into a plant or animal species nucleus.

The first pesticide-producing crop, Bacillus thuringiensis (Bt) potato, was approved in the U.S. in 1995. That same year saw the approval for marketing of other GM crops, including canola with modified oil composition, Bt maize, cotton resistant to the herbicide bromoxynil, Bt cotton, glyphosate-tolerant soybeans, virus-resistant squash, and another delayed ripening tomato. Golden rice was created in 2000, the first time scientists had genetically modified food to increase its nutrient value.

The full genomes of a rainbow trout and salmon were also sequenced a few years ago, and researchers used such access to tweak the genes of Atlantic salmon, inserting a gene from another fish, called the ocean pout. The change allows the salmon to grow and gain weight twice as fast as conventional varieties.

Two years ago, after more than 25 years of research, the U.S. Food and Drug Administration approved that fish, Aquadvantage, as the first genetically engineered food animal it has ruled as safe to eat. But Alaska lawmakers worked to stop the sale of the product in the U.S. and it's currently for sale only in Canada.

The new piece in the breeder's pipeline

Many genetics laboratories and crop and animal breeders are knee-deep in new gene editing processes.

Gene editing takes an approach to genetic alteration that is fundamentally new and different from the kinds of transgenic modifications – popularly known as GMOs -- that researchers have pursued with varying success for two decades. Instead, they've found quick, inexpensive ways to edit the proteins within plant and animal chromosomes and with precise and predictable results.

Biochemist and Professor Jennifer Doudna

Gene editing is akin to cutting and pasting text within a document, explained Jennifer Doudna, professor of molecular and cell biology and chemistry at the University of California, Berkeley, at a recent conference on gene editing on that campus.

In the 25 years of her cell biology and biochemistry career, she has "never seen science moving at the pace it is moving right now," and she sees GE as generating much of the stampede.

Brummer, the plant breeder from UC, Davis, says most breeders and agricultural professionals he knows, after decades of persistent popular suspicion and opposition to transgenic crops, see gene editing as potentially less objectionable.

He says they are "hopeful that regulatory concerns would be minimized and we could move forward. There are certainly a lot of positive things you could do with CRISPR."

He points out that, though CRISPR may spell less regulation, the breeder still must know the gene or genes to be removed or changed, and what the results should be, in order to succeed.

So, Brummer advises, "the availability of CRISPR doesn't so much speed up, but adds variability to genetics in the breeding program. You still have to go through the field testing, make sure that the yields are high, and produce the seed for farmers. So, there are still a number of years, in any event, you'll have to go through to get a new variety."

"Whether it's trans-genes, genetic markers, or other technology," he said, "these are all tools that are added to an existing plant breeding pipeline. And that pipeline is sort of our new cultivar delivery process. Maybe you can accelerate parts of it, or you can bypass parts of it, but, by and large, you still have that same pipeline . . . and all that stuff has to, in some sense, happen."

Advances in breeding almost always start out as controversial. Note that a century ago, some prominent scientists continued to condemn Mendel's 19th century findings as fraudulent, charging that his results were fudged to conform to the numbers he anticipated in his pea experiments.

And even now, some strains of transgenic corn and soybeans have still not been approved in some foreign markets – despite years of solid performance in the U.S. So, gaining acceptance by U.S and foreign governments for new gene-edited products is an ongoing challenge for all involved.

But failure to explore these new breeding techniques should not be an option, says Illinois pork producer Thomas Titus.

"It would be irresponsible not to continue to research and explore the possibilities with these new precision breeding tools" both for farm animals and humans, Titus said. "Think of the opportunities this holds for human health and the ability to eliminate certain diseases."

A rising tide of farm production challenges

Breeders don't rest. Their fight to keep farm plants and animals healthy and productive is nearly always a catch-up game or rear-guard action. So, crop and livestock breeders can never secure enough tools, and are always looking for new ones to counter the latest threat from pests or disease or to boost production to help producers remain efficient and keep up with competition.

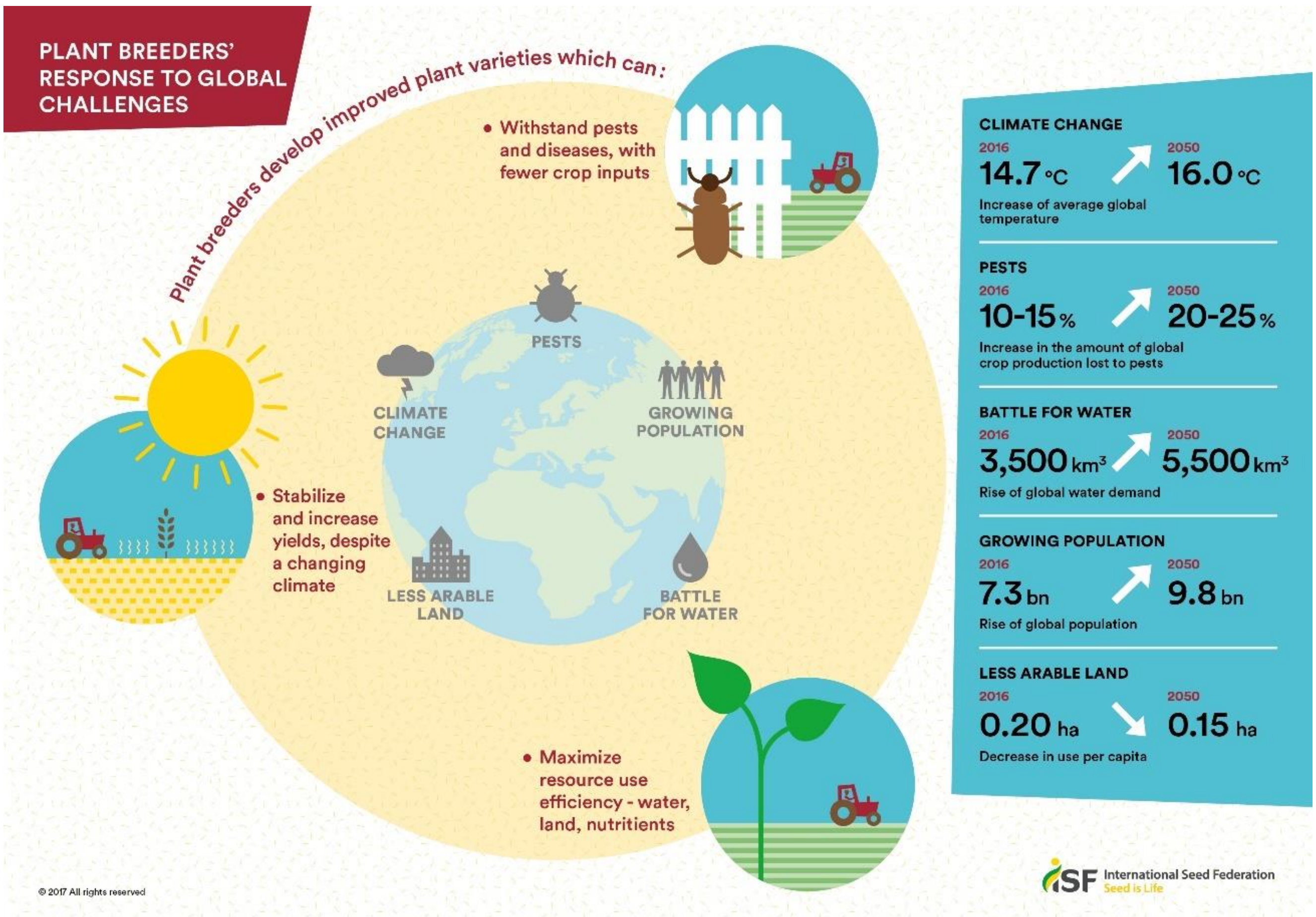
But while much of crop and livestock breeding means playing defense, the demand side of world agriculture is not sitting still either. Human population is climbing toward 9 billion by mid-century and folks have an increasing appetite for more diverse and nutritious foods.

Besides the prospect of more and more people to feed, the world's breeders are trying to find farmers new answers as they continue facing an array of ever-mounting production challenges:

- More crops and livestock on fewer acres: Available arable crop and pasture is declining as urban areas expand and industries take over farmland, so crop yields have to rise, and animals produce more meat, milk and such from available forage and feed.
- More crops with less later: Fresh water available to farms is declining as urban and industrial uses claim more water and aquifers are drawn down to keep food production going, and as climate

change leaves some farming regions with less water. The means selecting less-thirsty types of crops and breeding more drought resistant varieties.

- Limiting expansion of croplands to preserve forests and other wild areas.
- Improving plants' nutritional quality: More nutrition per calorie makes the best use of resources.
- Helping crops to adapt: Breeders need to help them adjust to rising temperatures and increasingly volatile weather.
- Conserving plant and animal genetic diversity: The broader our genetic diversity, the more resilient our crops can be against the next disease or natural disaster.



Hirst, meanwhile, isn't in the life sciences arena, but has a view about GE, the new laboratory magic entering the ancient plant and animal breeding pipeline.

"I'm all for developing new versions of our plants that may be better able to survive in the climate changes that are already in progress. We desperately need more plants that are adapted to drier and less stable climatic situations as soon as possible – shorter growing seasons, less dependency on irrigated water, stronger resistance to drought."

She adds a suggestion: "What about ways to clean extra nutrients out of the dead zones such as the one [in the Gulf of Mexico]? You could develop plants to do that."

But she advises caution, too.

"I am not for lowering regulatory bars" to the new GE products, she says, adding that she wants to see protection of biodiversity at the forefront when approval of gene editing products is considered.

"Biodiversity is a must have; not a nice to have, especially now, when we don't have a good grasp on what the future will bring," she says.

THE BREEDING EDGE

Will the breeding evolution lead the next green revolution?

Chapter 2

What is gene editing and why should you care?

By Ed Maixner

Animal and plant breeders are trying out a set of powerful new tools which have the potential to revolutionize agricultural practices and provide consumers with more healthy and safe food options.

Their new toolbox is called gene editing, and the instruments in it have strange-looking names: endonucleases, for example, which are enzymes that breeders can use to sever selected DNA proteins on an organism's chromosomes, allowing the breeder to make changes at the DNA break points, and thus alter the organism's genetic makeup.

"We use the term 'gene editing' rather loosely" in the world of science, said Bernice Slutsky, senior vice president for the American Seed Trade Association. At its core, gene editing is "plant breeding innovation," she said. Plant breeders have always used a range of tools – a toolbox of different

disciplines." With the new techniques, they are "doing the same things that breeders have always done, but very precisely," she said.

The precision Slutsky describes did not suddenly burst forth full-blown but is what researchers have built in recent decades. Some milestones include:

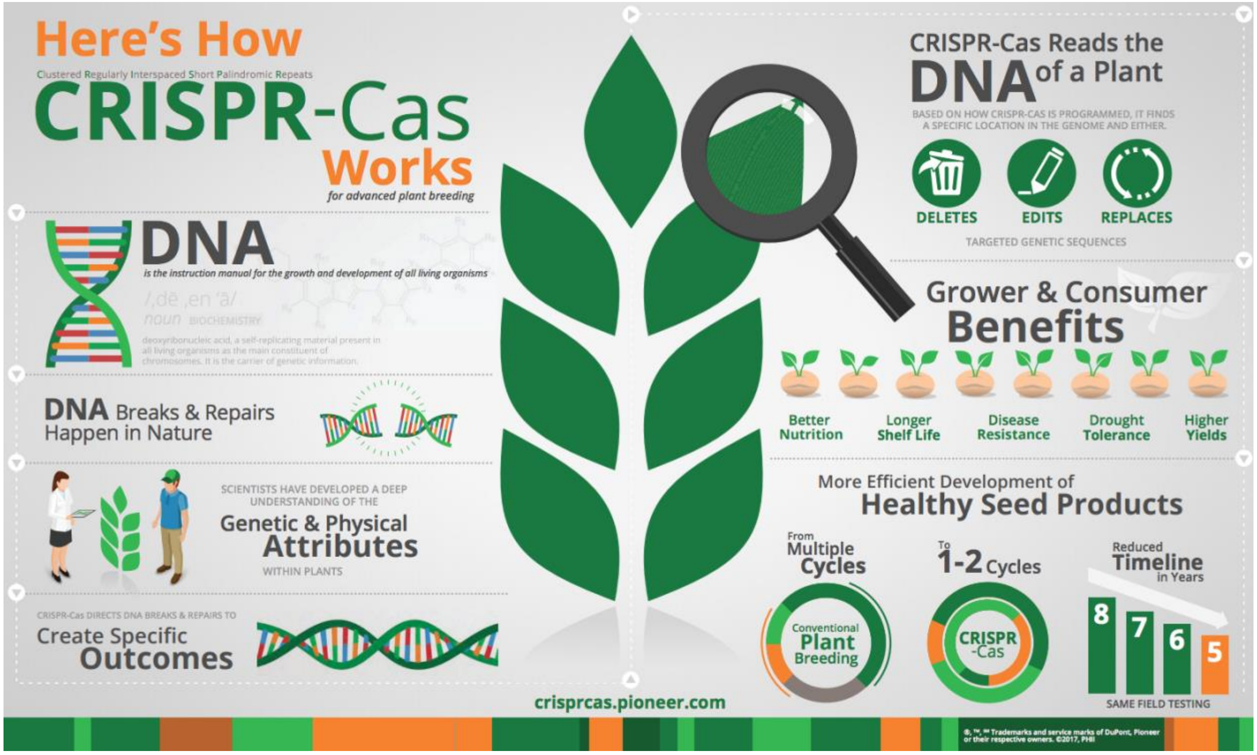
Bernice Slutsky, American Seed Trade Association

- Development of a technique called polymerase chain reaction in the 1980s. It allows researchers to duplicate a fragment of DNA proteins thousands or millions of times, providing a quick and cheap supply of specimens for their research.
- Another key gene editing tool, zinc-finger nucleases (ZFN), emerged more than a decade ago. ZFN are enzymes that target sequences of genes on chromosomes where genetic amendments are sought. This is similar to the newer gene editing processes but is considered more laborious and often less successful.
- What's more, in the past two decades or so, scientists have laid the table for gene editing by locating and cataloging – called sequencing – the entire genomes of a multitude of plants and animals. That makes virtually every gene potentially available for breeders to find and amend or delete. Full sequencing has been done for cattle, chickens and pigs. In addition, the sequenced genomes of almost 200 different plant species have been published, according to Todd Michael, who's been tracking plant sequencing as director of informatics for the J. Craig Venter Institute in California.

With such advances in place, two processes developed in recent years are accelerating breeders' ability to genetically alter crops and animals and apply the brakes to harmful organisms. Both can precisely improve a plant or animal without incorporating DNA from another species. One process is a mouthful called Clustered Regularly Interspaced Short Palindromic Repeats, or CRISPR, and the other is a similarly large swallow called Transcription Activator-Like Effector Nucleases (TALEN).

So, how do these genome-amending systems work?

Some have described CRISPR-Cas to be like editing text in a word processing application. With a specific goal in mind, the CRISPR-Cas system performs a specific search within DNA – an organism's complete set of instructions – to delete, edit or replace target genetic sequences.



Source: DuPont Pioneer. For more on CRISPR-Cas, go to https://crisprcas.pioneer.com)

Proteins, or genes, "in the nucleus of any organism provide all the information it uses to operate and grow," explained Jennifer Doudna, professor of molecular and cell biology and chemistry at the University of California, Berkeley, speaking at a conference on CRISPR in California. **"You can think of it like a text that includes instructions and a construction manual." Gene editing is "a way of modifying that document … you can cut and paste" the proteins in the nucleus, she said.**

Speaking more technically, both CRISPR and TALEN use enzymes that sever the double-strands of an organism's genes at targeted locations, making what is called double-strand breaks (DSBs), and often making several such cuts.

Then, DSBs are repaired by sending replacement genes to the severed site, using a sequence of genes as a template that includes the modification the breeder wants to make. Also, in both systems, other insertions or deletions of proteins are made at the break site as the broken ends are rejoined.

Jennifer Doudna, University of California - Berkley

In the CRISPR process (also called CRISPR-Cas9), the activating enzyme is a Cas9 nuclease, and the template of replacement genes is guided to its target in a single strand of ribonucleic acid (RNA). Similarly, TALEN uses a nuclease to make double breaks, but employs a pair of double-stranded DNA binding proteins rather than a single RNA strand. Luckily for plant and animal breeders, and perhaps all of agriculture, "there are lots of applications of gene editing," Slutsky points out. They are used to insert genes or knock them out, tag their location on a chromosome, correct genetic defects, etc. Thus, scientists hope to use them to benefit human health, first of all, but also to edit the genes of animals, plants, bacteria, fungi, and other organisms. They want to improve livestock breeds and crop varieties, but also eliminate diseases, wipe out pathogens, rein in harmful insects, and more.

Significantly, unlike other traditional gene-editing methods, employing CRISPR or TALEN is cheap, quick and relatively easy for breeders to use. That leads Doudna to call the new gene editing processes "a democratizing tool," because breeders in poor countries and in less-endowed labs worldwide – not just those in more lushly-funded corporate and university labs – will have broad access to gene editing.

What's more, in October, DuPont Pioneer, a leading global crop genetics company, and the Broad Institute of MIT and Harvard, which holds the initial CRISPR patent, granted agricultural researchers at all university and other nonprofit entities free access to use the CRISPR-Cas9 patent.

With such access and potential for quick results, not surprisingly, CRISPR has swept through labs around the world. **Slutsky says she travels a lot internationally to work on matters involving crop genetics, "and I probably spend 50 percent of my time in countries who are talking about (using CRISPR) … in South America, China, Japan, South Korea, Australia, Europe – countries that we would not necessarily consider biotech friendly, who ban GMO plantings. (But) they don't want these technologies to pass them by."**

The University of California-affiliated Innovative Genomics Institute has been collecting reports of successful genetic editing by scientists worldwide. Megan Hochstrasser, IGI science communications manager, is curator for the list and she says, "the tally is over 200 right now and only includes those organisms edited using CRISPR enzymes," rather than other gene editing processes.

IGI's list includes "all organisms … vertebrates, invertebrates, plants, and microbes," Hochstrasser reports, and "it seems that the microbes section is growing the most rapidly. Some of the microbes are pathogenic," and she thinks that "makes sense, since scientists need to understand how pathogens function in order to combat them."

Efforts to employ CRISPR to fight pathogens have perhaps most often been aimed at microbes that harm people: one that causes malaria, for example, and a parasite that causes Chagas disease. But researchers are also using CRISPR to build in defenses against rice blast fungus, corn smut, and the cotton bollworm, Hochstrasser noted.

Note, too, that the new processes aren't likely to be the world's last best answer in breeding techniques. A steam-powered car called the Stanley Rocket, for example, broke the world land speed record at 127.7 miles per hour more than a century ago (1906). Sure, that was jaw-dropping at the time, and even kind of impressive now. But, as with race cars, improvements will continue in gene-editing technique as well.

Here is one of perhaps many on the way. CRISPR actually often over performs in the cell nucleus, making an excessive number of cuts, including some in the wrong places, scientists report. But now, University of Wisconsin-Madison researchers have found a way to improve CRISPR-Cas9 technology, making genetic revisions much more likely to be exactly as desired. Their new method uses a molecular glue, keeping the Cas9 enzyme and RNA strand together as a complete repair kit at the DNA cut.

Meanwhile, a bioengineer at the Broad Institute has been working with a family of enzymes, named Cpf1, that work much like Cas9, and scientists in other labs around the world are looking for enzymes that can be most quickly and accurately aimed at the chromosomes of living things.

Although actual commercial use of gene-edited products awaits decisions by the U.S. and foreign governments about regulating them, a lively market has already emerged to sell the enzymes, engineered strands of RNA and other molecular bits for gene editing. Online, a market exists similar to what smart-phone users find in the Google Play Store when shopping for an app.

GeneCopoeia, for example, has been marketing biotech research tools and products for nearly 20 years. The Maryland-based company has partnered with a China-based FulenGen Co. and offers an array of CRISPR and TALEN tools "to help you every step of the way in your genome editing workflow." Meanwhile, Integrated DNA Technologies, with locations in Iowa and several sites abroad, has a similar display of products and services online.

Gregory Graff, Colorado State University

Considering the decline in public spending on agricultural research in recent decades, will enough funding be available to sponsor cutting-edge gene-editing agricultural research? Speakers at a Farm Foundation conference on ag research and innovation last fall said they expect it will.

First of all, the cost of using CRISPR and TALEN is low because of the speed of making the edits. Besides that, said Gregory Graff, an associate professor in agricultural economics at Colorado State University, with the "patent access granted to small companies and public agencies, it opens up potential for new products from small players," and "it renders those products viable in the marketplace."

What's more, said Graff, who tracks research startups in the U.S., America is not capital short: "There is more money out there than there are places to invest."

Bill Buckner, president of the Noble Research Institute in Oklahoma, agreed. "Venture capital folks don't know how or where to invest," leaving a lot of cash that could be available for genetic editing sorts of research. He noted, for example, a study by the Center for Rural Entrepreneurship that projects $29 trillion in wealth will be transferred between American generations from 2010 to 2040, and said "a lot of (the dollars) will be in the Midwest."

Some of the university research will likely be conducted in collaboration with commercial firms. For example, researchers at the University of Missouri, Kansas State University and Genus plc successfully bred pigs that are not harmed by the Porcine Reproductive and Respiratory Syndrome (PRRS) virus, a disease that costs North American farmers more than $660 million annually. **Click on the photo to watch the video from University of Missouri.**

"Once inside the pigs, PRRS needs some help to spread; it gets that help from a protein called CD163," said Randall Prather, distinguished professor of animal sciences in the

University of Missouri's College of Agriculture, Food and Natural Resources in a December 2015 release. “We were able to breed a litter of pigs that do not produce this protein, and as a result, the virus doesn’t spread. When we exposed the pigs to PRRS, they did not get sick and continued to gain weight normally.”

Researchers working in Prather’s laboratory also created the first miniature pigs that have the alpha 1,3 galactosyltransferase gene knocked out. This groundbreaking work has the potential to prove very useful for xenotransplantation: the transfer of pig organs into humans, Prather noted on his web site.

“We also created pigs with a mutation in the gene that is responsible for causing cystic fibrosis (CF). Now there is a pig model that mimics the symptom of CF so that physicians have something to invasively experiment on and develop treatments and therapies. This is especially important since the same mutation in mice does not result in a phenotype that is similar to humans.”

More recently, scientists at the University of Missouri worked with pigs to research stem cells and made a discovery that could significantly decrease the costs associated with in vitro fertilization in humans.

Nevertheless, gene editing will have to jump a huge policy hurdle before results of such plant and animal breeding show up on farms, in fields and in food stores.

A number of scientists, consumer and food safety advocates, and others fear the results of editing genes that are all naturally within a cell's nucleus the same way they do transgenic engineering, which alters plants and animals genetically by inserting genes from unrelated organisms. They want to see the U.S. and governments worldwide lump gene editing in with transgenic genetic alterations and regulate it as just another type of genetically modified organism, or GMO. That would almost surely ensure years of testing and approval for each product, as has been done for transgenic products, dramatically running up the costs to produce gene edited products commercially.

At the California CRISPR conference, where scientists were focused on the advantages and potential of gene editing, for example, Dana Perls, a spokesperson for Friends of the Earth, declared: “Let’s actually call this (CRISPR) genetic engineering. Why not name it what it is?”

THE BREEDING EDGE

Will the breeding evolution lead the next green revolution?

Chapter 3

The promise and potential for new plant varieties

By Sara Wyant

Most Americans have never experienced a famine or even chronic food shortages. We've grown accustomed to finding at least some types of food almost everywhere we look – the grocery, the gas station, the food truck, the corner store and of course, online.

So, when scientists talk about the need to improve productivity on the farm or create new plants and animals which can resist diseases that could potentially eliminate an entire crop or species, the words often fall on deaf ears to the consuming U.S. public. Why worry?

Perhaps there is no need to panic, but there is cause for concern. Economists and analysts agree that we need to improve productivity on farms and ranches just to keep up with feeding a global population that the **United Nations expects to grow from 7.6 billion to 9.8 billion by 2050.**

The Global Harvest Initiative's most recent Global Agricultural Productivity Report noted that, for the fourth straight year, agricultural productivity growth is not accelerating fast enough to sustainably feed the world in 2050.

"If agricultural productivity growth continues to stagnate, there will be significant ramifications for the economic vitality and environmental sustainability of food and agriculture systems. The availability of affordable, safe and nutritious food also will be undermined," the report noted.

But it's not only increased productivity that's important. **Consumers are increasingly concerned about the environmental impact of their food choices – how much water is consumed, how nutrients are utilized, and how much food is wasted. There are also concerns about nutritional benefits and the price and safety of what they eat.**

Farmers are ready to meet all these challenges, but they want access to new tools that will enable them to better cope with climate change, natural disasters and disease outbreaks. And that's where advanced, precision breeding shows so much potential.

"Different forms of breeding can address these challenges," emphasized Ian Jepson, Syngenta's Head of Trait Research and Development Biology, during a recent interview. He says the key challenges are the biotic stressors - the weeds, the insects, the fungal diseases and other pathogens, like nematodes, bacteria and viruses which all can significantly impact yields. However, the biggest losses are through abiotic stress, like heat, drought and cold.

"It's been estimated that $200 billion in losses a year are due to those biotic stressors," Jepson points out. "By 2050 we've got to increase productivity not only by protecting the crops from the bugs, but we've got to address fundamental yield and biotic stressors.

From 2011-2012, a severe drought caused a food crisis in East Africa. Photo by Oxfam East Africa

"Chemistry and biologicals do a great job on the biotic stressors. We have good chemical controls for weeds," Jepson adds. "We don't have good chemical controls for insects and fungal diseases and we're struggling to get new products to keep up with resistance pressures. So, on the biotic stressors, we need to supplement our chemistry and our biologicals with advanced breeding."

In order to increase a crop's yield, Jepson says "we can't keep throwing more nitrogen on it, because we've got runoff issues. We've got to work on the inherent productivity of crops. And we know that potential is there."

"If you take a crop like sugar cane and consider its photosynthetic capacity, you'll find that its ability to use the sun's energy and water to make sugars is way more efficient than most other crops. So, if we get the poor crops up to the level of the good crops, we can increase yields by 40 to 50 percent," he explained.

"But we've got to figure out how to do that. It's advanced breeding techniques that are going to make those breakthroughs."

Ian Jepson, Syngenta's Head of Trait Research and Development Biology. Photo courtesy of Syngenta

Jepson says there are a number of different efforts underway that can boost plant yields that now use only about 1 percent of the sunlight they receive in photosynthesis. For example, Realizing Increased Photosynthetic Efficiency (RIPE) is an international research project, headquartered at the University of Illinois, which is engineering plants to photosynthesize more efficiently to sustainably increase crop yields.

Formed in 2012, RIPE was originally funded by a five-year, $25 million grant from the Bill and Melinda Gates Foundation. In 2017, the project received a $45 million, five-year reinvestment to continue its work from the Gates Foundation, the Foundation for Food and Agriculture Research, and the U.K. Department for International Development.

"Photosynthesis is the process from which ultimately all our food and a lot of our fiber and many of our fuels actually come from," notes Stephen Long, a professor of plant biology and crop sciences at the University of Illinois in an interview with Illinois Public Media. "And the process really isn't that efficient."

Researchers found that by boosting levels of three proteins in tobacco plants, the crop grew 14 percent to 20 percent larger, according to a study published in Science in 2016. And they are confident that this process can be transferred to other crops, such as corn and soybeans which are widely planted in the U.S., or cowpeas, planted by small stakeholder farmers in Africa.

Meanwhile, researchers at the Donald Danforth Plant Science Center in Creve Coeur, Missouri**,** aim to identify new genes and pathways that contribute to photosynthesis and enhanced water-use efficiency - building on earlier research using the model grass, green foxtail (Setaria viridis).

"Understanding the network of genes involved in photosynthesis and drought tolerance will provide targets for plant breeders and genetic engineers to redesign sorghum specifically as a high value bioenergy feedstock to be grown on marginal soils and thus not compete with food crops," says lead principal investigator, Thomas Brutnell, director of the Enterprise Rent-A-Car Institute for Renewable Fuels at the Danforth Center.

Ultimately, they hope to deliver stress-tolerant sorghum lines, addressing the Department of Energy's (DOE's) mission in the generation of renewable energy resources. The development of a low input, environmentally safe and highly productive sorghum germplasm will help establish a lignocellulosic energy economy that can provide jobs to rural communities, ensure energy security and benefit the environment, the Center noted after receiving a five-year, $16 million grant from DOE in October 2017.

More recently, Andrea Eveland, an assistant member at the Danforth Center, and her team identified a genetic mechanism that controls developmental traits related to enhanced grain production in cereals. The work was also performed on Setaria viridis, which is related to economically important cereal crops and bioenergy feed stocks such as maize, sorghum, switchgrass and sugarcane.

"The genetics and genomics tools that are emerging for Setaria enable more rapid dissection of molecular pathways such as this one, and allow us to manipulate them directly in a system that is closely related to the food crops we aim to improve," says Eveland. "It means we are just that much closer to designing and deploying optimal architectures for cereal crops. The prospect of leveraging these findings for improvement of related grasses that are also orphan crop species, such as pearl and foxtail millets, is especially exciting."

Syngenta has been making advances in breeding, too. Their scientists solved the mystery behind an abnormal corn line responsible for revolutionizing corn breeding. The line produces haploid plants that contain just half the DNA of normal corn and was first discovered in 1959 by University of Missouri Professor Edward Coe.

"We (the seed industry) make millions of plants using this particular mutant line," Jepson explains. "But we had no clue how it worked – until recently."

They found their answer in 2013 and followed up with gene editing to verify the discovery in 2015. As a result, Syngenta hopes to make existing haploid-induction systems more efficient and potentially make breakthroughs in other crops.

Cassava Brown Streak disease is devastating a staple crop in parts of Africa, Source: Biosciences for Farming in Africa

All in all, researchers have made tremendous advances in plant breeding using a variety of different tools and relying on big advances in computational biology and computer storage that allow analysis of petabytes of data. But there's still a long way to go. And for some growers, help can't come fast enough. In some cases, entire farms, businesses and food supplies are being wiped out.

For example, Brown Streak Disease is devastating cassava plants in many African countries, especially in East Africa, where the root vegetable is a staple food for millions. In some cases, the disease, which has been dubbed the "Ebola of plants," exposes farmers to 100 percent loss, notes Biosciences for Farming in Africa.

Nigel Taylor, with the Danforth Center, is working with scientists in Uganda and Kenya to see if a relatively new-gene editing technology – CRISPR (stands for Clustered Regularly Interspaced Short Palindromic Repeats) - can be used to speed up the time it takes to grow cassava plants that are more resistant to the disease than conventional varieties.

In the United States, researchers are trying to find a cure for another particularly vexing problem - citrus greening disease, which was first confirmed in Florida in 2005. The Asian citrus psyllid (ACP) carries a bacterium infesting trees with huanglongbing (HLB or citrus greening). Infected trees produce fruits that are green, misshapen and bitter. Most infected trees die within a few years.

USDA reported in 2017 that HLB is currently the most devastating citrus disease worldwide and has affected all of Florida's citrus-producing areas leading to a 75 percent decline in the state's $9 billion citrus industry. Fifteen U.S. states or territories are under full or partial quarantine due to the presence of ACP.

Citrus tree leaves infected with citrus greening. Source: USDA-APHIS

Although there is no cure for the disease, growers have implemented several short-term solutions, including enhanced nutritional supplements, reflective mulch, bactericides and heat treatment to try to maintain fruit production. Longer term, researchers are looking at both traditional cross-breeding and new processes like gene editing and genetic modification to explore how to develop a disease-resistant breed of citrus tree which is not susceptible to greening and will not become diseased.

USDA's National Institute of Food and Agriculture (NIFA) recently announced new funding to combat the disease.

"The need to advance research and extension to develop management strategies for huanglongbing has reached a critical juncture," says NIFA Director Sonny Ramaswamy. "Severe damage to the Florida citrus crop from 2017 hurricanes further exacerbates the pressure on the industry and the need for new strategies to address the disease."

Florida's citrus industry has lost nearly half of its $1.5 billion on-tree fruit value in just 10 years due to citrus greening.

Harold Browning, the chief operating officer for the Florida-based Citrus Research and Development Foundation, reports that researchers in Florida, Texas and California continue to produce new citrus strains along with pursuing HLB resistance.

Yet, all types of citrus remain vulnerable to HLB to varying degrees, Browning says, and scientists haven't yet developed any commercial citrus varieties with strong resistance to the disease. "We really don't have a variety that is equivalent to a boat sitting on the pond with no holes in it," he notes.

CITRUS GREENING

The disease devastating the Florida Citrus industry

WHAT IS CITRUS GREENING?

Citrus Greening (also known as Huanglongbing or HLB) is a **disease spread by a small insect called the Asian Citrus Psyllid.**

The psyllid feeds on the stems and leaves of the trees, which infects the trees with bacteria that causes citrus fruit to turn green.

Greening impairs the tree's circulation and nutrition status, resulting in fewer and smaller fruit over time.

Once a tree is infected, there is no cure. The tree ultimately dies.

HISTORY OF CITRUS GREENING

ORIGIN: CHINA — Originated in China nearly a century ago

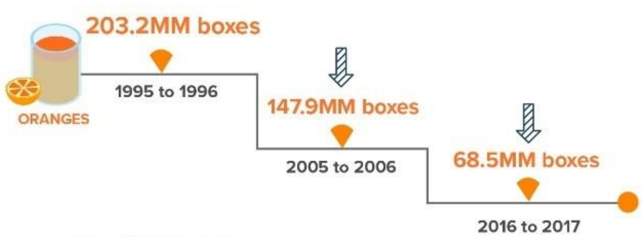
SIGHTED IN FLORIDA — Psyllid first appeared in Florida in 1998

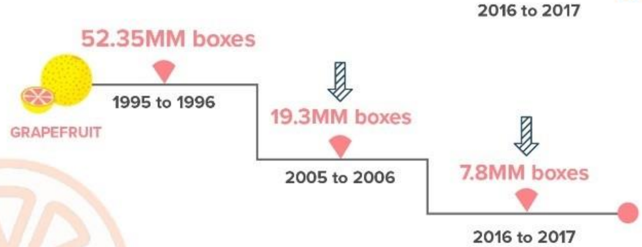
CONFIRMED IN FLORIDA — Disease first confirmed in Florida in 2005

CONFIRMED IN ALL CITRUS PRODUCING COUNTIES — In all counties by 2015

Movement of citrus greening across Florida.

SIGNIFICANT IMPACT TO THE STATE





AN IMPORTANT INDUSTRY

Florida provides MORE THAN HALF of the nation's **ORANGE JUICE** & is the **2nd largest producer in the world.**

(Florida Oranges: 90% processed for juice, 10% sold in fresh market)

FLORIDA IS THE ONLY U.S. PRODUCER OF **ORANGE JUICE**

FLORIDA IS THE LARGEST U.S. GROWER OF **GRAPEFRUIT**

The Florida Citrus Industry has an annual economic impact to the state of more than **$8.6 BILLION.** The Florida Citrus industry provides more than **45,000 JOBS.**

CITRUS IS THE STATE'S LARGEST AGRICULTURE CROP

https://www.floridacitrus.org/newsroom/citrus-411/citrus-greening/citrus-greening-infographic/

Pete Spyke, a veteran citrus grower who runs Arapaho Citrus Management in Fort Pierce, Florida, says there are no commercial citrus varieties that have been gene-edited or genetically-engineered because the work is still in research phases.

Researchers at USDA, the University of Florida, University of California, Texas A & M and other universities are working to find a source of tolerance, resistance or immunity, Spyke said.

Peter Spyke, Arapaho Citrus Management

"Once they do, they will have to go into every single variety and perform that edit. And then the tissue of that new edited variety has to be grown out and propagated material generated so that we can begin to propagate new commercial citrus trees.

"So, if someone arrived at my doorstep today with, for example, a perfectly-edited variety of navel orange, it would still probably be 10 years before there was an industry based on that new variety," he adds. "Every year we go, it's another 10 years from today."

The need for speed

For growers like Spyke, these advancements in genomics and precision plant breeding can't come fast enough.

But for the science to really take off, some big hurdles need to be overcome. Researchers are hungry for a federal and international regulatory system that clarifies how different plant varieties should be regulated. Plus, they'd like to see broad acceptance of these new breeding techniques by all parts of the food supply chain, including consumers.

NIFA Director Sonny Ramaswamy

"The EPA, USDA and FDA have to come up with an actual definitive regulatory framework," says NIFA's Ramaswamy. "Currently they don't have one in regards to gene editing."

For now, USDA has concluded that the new plants are not "regulated articles." But not everyone sees it that way.

There's already been some push-back on these new tools, driven primarily by people and organizations who either don't understand how the technology works or who aren't comfortable with anything they view as "messing with Mother Nature."

What most people forget is that Mother Nature has been changing plants for centuries, and that these new precision breeding tools can make the changes faster and more precisely.

"DNA is inherently stable, but breaks from time to time. And when it breaks, that break can be caused by UV light or chemicals or heat or mechanical damage," explained Syngenta's Jepson. "And when it breaks, it sticks itself back together. When it does that, it has a chance of making a

mistake and that creates variation. That's how editing works. But this is a random process and not very efficient."

Sweet corn is one example, he explained. "While regular corn in the field, you wouldn't want to eat it or put it on the grill. It's starchy. Sweet corn comes from one of the starch genes that has been broken and repaired and made a mistake. Instead of producing starch, it stays sweet and sugary. And that's how sweet corn was derived … where random mutations happened in nature and then were selected by plant breeders."

Starting in the 1920's, scientists discovered how to induce variation through mutagenesis breeding, sometimes called radiation breeding.

"They (researchers) would use a range of different techniques like x-rays or chemicals or they put plants through tissue culture and it would just increase the rate of mutation," Jepson said. "Using those processes, they would generate populations of thousands of plants and they would put them in the field. And most of them were mutations that were not beneficial. They would just disrupt pathways of interest so they would throw those ones away."

"The technique is still used today, sometimes called tilling," he added. "It's a bit more sophisticated, but it's still around."

Thousands of crop varieties have been developed using these mutagenesis approaches, including sweet potatoes, durum wheat for pasta, and the Ruby Red Grapefruit. Other varieties can be found on the joint Food and Agriculture Organization/International Atomic Energy Agency Mutant Variety Database.

Ironically, grocers and food companies have been selling crops produced through mutation breeding for decades without a label or any apparent consumer backlash about their genetic changes from chemicals or radiation. These varieties can even be labeled organic if they are grown according to other organic production requirements.

Yet, new precision breeding tools are creating such a buzz that some activists suggest techniques like CRISPR Cas9 - which involves "cutting and pasting" DNA within a plant at specific sequences – should be regulated the same as genetically-modified organisms (GMOs), which are created by the insertion of genetic material from a different species.

There is no science-based risk associated with either form of breeding, but GMOs have gotten a bad rap from several environmental groups which have pressured food companies to avoid them in food products.

Even the Non-GMO project, which attempts to verify and label food and beverage products that do not contain GMOs, wants to exclude gene-edited foods from consideration – even if they are not technically GMOs.

As a result, farmers and plant breeders are worried that much-needed research – aimed at solving some of the most pressing plant diseases - is at risk of being stymied in the commercial marketplace over unfounded fears about GMOs. Gene-editing could be one way around that barrier.

"I'm comfortable with GMOs, but big juice companies are skittish about utilizing them for brands like Tropicana or Minute-Maid orange juice," Spyke said. "So, at this point, the assumption is that for the main citrus industry (oranges for juice), the GMO route – where we introduce a foreign gene - is not the preferred solution.

“Using CRISPR for gene editing is the most hopeful at this point because it doesn’t change the fundamental citrus genome,” Spyke added.

Some firms are already marketing gene-edited plants as non-GMO or working to do so in the near future. And because the production and regulatory costs associated with gene editing are so much lower, a lot of smaller technology companies are jumping into the action alongside much bigger seed companies.

For example, San Diego-based Cibus developed sulfonylurea-tolerant canola using non-transgenic breeding technologies. Jim Radtke, Cibus’ senior vice president for product development, says, the company is “making changes in plants without incorporating foreign DNA and thus the plants are non-GM,” using a patented gene-editing tool called the rapid trait development system (RTDS). And companies like Cargill are paying a premium for the canola to make non-GMO oil.

Cibus expects to introduce non-transgenic glyphosate-tolerant flax in 2019, late blight resistant potato in 2020 and an herbicide tolerant rice after that.

Calyxt, is using a gene editing technique called TALEN, which is similar but not identical to the CRISPR Cas-9 gene-editing tool, to develop new crops. The Minnesota-based firm, which bills itself as a consumer-centric, food- and agriculture-focused company, is preparing for the commercial launch of its first product, high oleic soybeans, in 2018. Also in the Calyxt pipeline: a potato variety that doesn’t bruise and another that survives better in cold storage, high-fiber wheat, low-gluten wheat, herbicide tolerant wheat, and lower saturated fat canola.

Jim Radtke, Cibus' senior vice president, product development. Photo: Cibus

Building off technology developed at the University of Missouri, Yield10 Bioscience Inc. developed a gene-edited Camelina sativa plant line using CRISPR technology for increased oil content. The firm says it is “focused on translating initial encouraging yield improvement results in Camelina to canola, soybean, rice and corn.”

Syngenta, which has its U.S. headquarters in Greensboro, N.C., and its Advanced Crop Lab in the state's Research Triangle Park, recently obtained a non-exclusive license from the Broad Institute of MIT and Harvard to use CRISPR-Cas9 technology for agricultural applications. Syngenta said it will use CRISPR-Cas9 in various crops, including corn, soy, wheat, tomato, rice and sunflower.

Berkeley-based Caribou Biosciences, in partnership with DuPont Pioneer, is using CRISPR Cas-9 to produce a waxy corn. This next generation of elite waxy corn hybrids is expected to be available to U.S. growers within the next few years, pending field trials and regulatory reviews. DuPont Pioneer says it is establishing a CRISPR-Cas enabled advanced breeding platform to develop seed products for greater environmental resiliency with characteristics like disease resistance and drought tolerance, in addition to advancing the development of improved hybrid systems.

Monsanto has licensed two different CRISPR versions, CRISPR-Cas and CRISPR-Cpf1- which the firm describes as having the potential "to be a simpler and more precise tool" for making targeted improvements in a cell's DNA - as well as the Exzact technology and another gene-editing platform developed by TargetGene Biotechnologies Ltd. The company is focusing on potential improvements in corn, soybeans, cotton and vegetables in ways that will make farmers more profitable.

Germany-based Bayer, which is in the process of acquiring Monsanto, has its own joint venture centered on CRISPR gene editing and is expected to continue building on Monsanto's existing portfolio of tools.

Plenty of foundations and university researchers are also using new gene-editing techniques to improve vegetable crops, including tomatoes resistant to powdery mildew and virus-resistant cucumbers.

Scientists at the Noble Institute Research are using gene editing technology to improve the cover crop hairy vetch. Noble researchers are looking to improve the germination of seeds to make this legume more functional as a cover crop.

In 2016, Penn State University pathologist Yinong Yang used CRISPR-Cas9 to develop a button mushroom that resists browning and may have a longer shelf life and be better for automated mechanical harvesting. In approving the new mushroom, USDA wrote that because it "does not contain any introduced genetic material" it isn't subject to the agency's GMO regulations.

Regulatory uncertainty

But will new plant varieties produced using various forms of precision plant breeding continue to be regulated this way? That's potentially a multimillion dollar question. Farmers, researchers and investors would like some type of regulatory certainty in order to fully explore the potential to boost yields, protect plants from disease and provide added nutritional benefits.

"The challenge is this: You may get the regulatory authorities in the United States for some of those modified oils that don't need any regulatory permits to produce in the United States. But Europe and many other countries have not yet decided what is going to be required, if anything," notes Jepson. "That's the very limiting step here. How soon will the regulatory frame become established enough?"

Michal Bobek, advocate general of the European Court of Justice, recently delivered some relatively good news on that front. On Jan. 18, he ruled that new gene-editing technologies should be largely exempted from EU laws on GM food, although individual EU member states can regulate them if they choose.

That opinion drew a swift rebuke from Friends of the Earth Europe.

"Farmers and consumers across the EU expect that any new approach to producing food and crops should be fully tested to make sure they are safe for the public and the environment," said Mute Schimpf, food and farming campaigner at Friends of the Earth Europe. "They will be counting on the European Court of Justice (ECJ) to not uphold (Bobek's) opinion, and instead make sure that all new genetically modified foods and crops are properly regulated."

The ECJ is expected to make its final ruling in the coming months, taking into account the opinion. The European Commission is waiting for clarification from the courts before deciding whether new legislation – or an update of existing laws – could be needed for the new technology.

In Australia, regulators are proposing changes more in line with the current U.S. view.

Australia's gene technology regulator Raj Bhula recently proposed reducing regulations around CRISPR and other gene editing techniques, noting that they would not be considered "genetic modification".

"With gene editing you don't always have to use genetic material from another organism, it is just editing the [existing] material within the organism," Bhula told ABC Rural. "If there is no risk case to be made when using these new technologies, in terms of impact on human health and safety for the environment, then there is a case for deregulation."

Chapter 4

Protecting the herd: New opportunities through gene editing

by Ed Maixner and Sara Wyant

To pig farmer Thomas Titus, new scientific techniques could bring better disease resistance for his herd, saving baby pigs and potentially millions of dollars for the pork industry.

That is the foremost benefit "when I think about the possibilities of CRISPR (also called CRISPR-Cas9, the leading gene editing technique) and how it could help my farm," says Titus, who raises corn, soybeans and pigs near Elkhart, Ill.

The devastating PRRS virus causes disease in two ways: a respiratory form that weakens young pigs' ability to breathe and a more severe reproductive form that causes pigs to die during late pregnancy. In North America alone, PRRS is estimated to cost producers $600 million annually.

Thomas Titus farms near Elkhart, Illinois

"As a pig farmer, I see that one of the greatest diseases that impacts every pig farm across the United States is PRRS (porcine reproductive and respiratory syndrome). If there's an opportunity for us to eradicate diseases like that – or have resistance to diseases like that – it would be just astronomical!"

Indeed, at least two companies say they can deliver just what Titus says he wants. Both Genus, which owns a patent to its technique, and Acceligen, a division of Recombinetics, also involved in developing the trait, report they can infuse pigs with PRRS resistance.

The Genus procedure, for example, deletes a pig gene that the virus needs as a doorway to the animal's cells. But, as with other gene editing (GE) discoveries, those PRRS remedies can't happen until the U.S. government approves these new animals.

More broadly, researchers are eager for the U.S. and other governments to decide how to use science and risk-based criteria to regulate this new approach to genetic engineering. That's why commercialization is still a few years out.

For now, the Food and Drug Administration (FDA) defines all intentional DNA alterations in animals as drugs, irrespective of how or why they were changed.

"It's a nonsensical position," says Alison Van Eenennaam, a Cooperative Extension Specialist in Animal Genomics and Biotechnology at the University of California-Davis.

"If I didn't know any better, I would think the (FDA) regulations were written by Greenpeace because they are not science-based," she emphasized during a recent speech at the American Farm Bureau Federation. "We can't run all of these animals through a drug evaluation."

But while the regulatory issues are being sorted out, researchers and industry leaders are marching ahead with these new scientific tools, along with ongoing efforts to use current animal-improving practices like artificial insemination, embryo transfer and genetic selection.

Van Eenennaam says there's an "urgent need to ensure the science-based framework focused on these novel products is adopted. Or else it's going to block U.S. ranchers from having access to this technology."

"We're very excited by the potential for gene editing, and not only against PRRS … a devastating disease to the industry," said veterinarian Dan Kovich, speaking for the National Pork Producers Council. "In the future," he said, "looking to other applications for disease resistance, prevention, management – all sorts of traits -- I think the potential is there (for gene editing) traits that can have an impact on animal welfare, reducing the need for antibiotics . . ."

Alison Van Eenennaam, a Cooperative Extension Specialist in Animal Genomics and Biotechnology at the University of California - Davis

"This is very different from the GMOs (genetically modified organisms) that people have talked about in the past," Kovich says, noting that in gene editing, no genes from foreign species are introduced. "I think there are very sound reasons why the marketplace will be accepting of this technology. This is a very precise technology, working within the genome of the pig. It's not transgenics." So, he says, NPPC is "just really excited about where this can go."

Swine-related gene editing discoveries are a small part in the leading edge of genetics research. Even though the new genetic altering procedures such as CRISPR (Clustered, Regularly Interspaced, Short Palindromic Repeats) and TALEN (Transcription Activator-Like Effector Nucleases) are just a few years old, scientists have already invented some more refined versions of even those techniques – for example, modifying the performance of specific genes without actually cutting or removing any genes in the nucleus, as CRISPR and TALEN do.

Scientists around the globe are giving the genetic manipulations test drives: cutting, splicing, deleting and amplifying genes within the cells of everything from viruses and fungi up the biological scale to mammals and the human body.

One significant feature of all the gene editing work is that most of it is taking place not in the cell nuclei of higher life forms, but of the lowest.

Megan Hochstrasser, science communications manager for the Innovative Genomics Institute at UC-Davis, keeps a roster of new research postings of gene-editing products developed with CRISPR-Cas9, the most frequently used new gene-editing system.

"It seems that the microbes section is growing the most rapidly," she said. So far, 88 of 208 posted CRISPR successes are about microorganisms: fungi, bacteria, and protists (molds, algae, etc.). Another 46 are on invertebrates (insects, snails, worms, etc.), 39 alter crops and other plants, and 10 are on fish.

Thus, only a handful of successful genome editing advances– just 9 percent in Hochstrasser's count – directly alter the biological makeup of mammals or birds. But such genome amendments could also be significant to human health.

Megan Hochstrasser, science communications manager for the Innovative Genomics Institute at the UC-Davis

Two years ago, an immunologist in London, for example, was able to extract immunity cells, called T-cells, from the blood of a donor, modify them with a TALEN gene-editing procedure so the body of a young human recipient of the cells would not reject them. The altered T-cells were then used to replace a childhood leukemia patient's own T-cells. The child gained full remission from leukemia.

Most of the gene editing successes have worked within the animal's own genes, not injected ones from other species, and are intended to reduce suffering, infections, and injuries.

For example, Acceligen is working with [Van Eenennaam and scientists at UC-Davis]() to gain government approval for its genetic amendment for polled cattle, which means that they don't grow horns. A few breeds are naturally hornless. Cattle with horns can injure others in the herd or people handling the cattle. Meanwhile, dehorning adds to management chores for ranchers and means pain and blood loss for calves.

Tad Sonstegard, Acceligen's chief scientific officer, explained that isolating precise gene amendments for the polled trait can allow ranchers to make that sole change directly to a herd. Making a single targeted change can relieve the breeder from needing several generations to rebuild the genetic profile of the herd.

Australians found that while European-origin cattle (including American breeds) assimilate Acceligen's polled trait, hot-climate cattle such as Brahman and Bos indicus (zebu) cattle raised in northern Australia, do not. So Australian researchers did their own gene editing to select the polled trait for their breeds, allowing the rancher to breed cattle with double-dominant genes for the "true-polled" inherited hornless trait.

Another pain-reduction application of gene editing on tap in the swine sector is eliminating the need for surgical castration to produce favorable tasting pork. For swine, castration is done to avoid so-called boar taint, an unpleasant odor and taste in pork.

Recombinetics and DNA Genetics, a swine genetics supplier, have joined ranks to perfect a breeding technology that results in male piglets remaining in a pre-puberty state – thus, naturally castrated, in effect. Besides avoiding pain for piglets, GE castration would save labor and avoid risk of infection from the surgical procedure.

"We are trying to make male and female parents both sterile, and they will only be able to reproduce if someone administers spawning hormones to them. So, then the resulting offspring will all be sterile," Auburn University geneticist Rex Dunham said. He notes that some fish breeds are naturally that way, and won't breed unless certain hormones are present at high enough levels in the water.

Rex Dunham, Auburn University

In the aquaculture sector, meanwhile, Dunham has inactivated three genes in catfish cell nuclei, which direct release of reproductive hormones that allow the fish to breed.

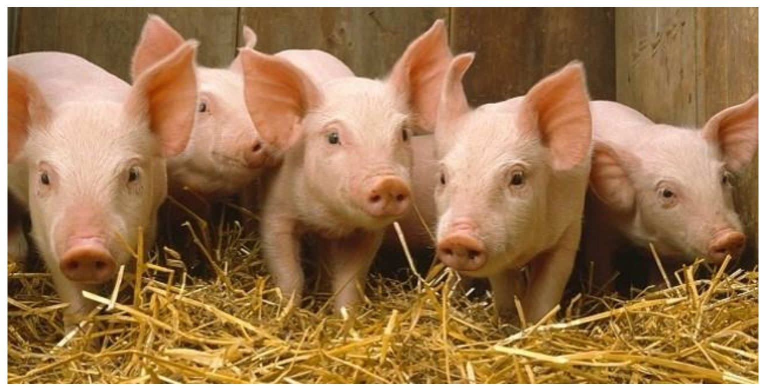
"The primary purpose," he explains, "is to have complete reproductive control over transgenic or other types of gene-edited fish. So, we could almost entirely eliminate any possibility of environmental impact, and completely prevent them from interbreeding (with other catfish)."

His gene editing appears successful so far, Dunham reports. In his first parent generation, “we have really strong evidence that these fish were sterile or had greatly reduced reproductive capacity,” he said, noting that complete adoption is never achieved in the first generation.

Significantly, most of animal-related gene editing to date doesn’t mess directly with the genomes of humans or higher life forms. Nearly all of it involves modifying simpler life forms to improve health for people, farm animals and wildlife, and most of it, like the PRRS remedy, targets diseases or harmful pests.

Gene editing, in fact, portends even more direct benefits to human health. Possible herpes resistance, for example. Nearly all adults are infected with types of herpes viruses, a group that cause cold sores, keratitis, genital herpes, shingles and mononucleosis. The viruses can remain dormant for long periods and then spring up. But scientists now suspect that they can use CRISPR genome editing to inactivate herpes by attacking its genes directly within people’s virus-infected cells.

Meanwhile, gene editing is sure to spell more human medical blessings via farm animals, often called farmaceuticals.



A few such advances, using transgenic procedures, have already been approved, because they offer clear benefits for people. In 2006, for example, the biotech-reluctant European Union (and in 2009, the U.S.) green-lighted a breed of goat modified to produce a human anti-clotting protein, called antithrombin alfa, in its milk. The protein is needed to treat people with hereditary antithrombin deficiency, a rare clotting disorder. Now, it can be harvested from the goat’s blood, instead of from a human donor’s blood, the traditional source. Also, the EU and U.S. have both approved a genetically-modified chicken that lays eggs containing an anti-cholesterol drug.

Kris Huson, communications manager for Recombinetics, explains that her company, like many other biomedical research entities, likes to employ pigs as surrogates for developing health remedies for people.

“Now, particularly with gene editing – and pigs share so much DNA with humans and human diseases – they are just a superior animal model,” she said.

Her company has modified pig genomes so the animals can develop colon cancer, human heart disease, Alzheimer’s, diabetes, and other human disorders and/or resistance to such disorders, giving researchers targets to pursue human health remedies.

Mice have long been used as a model for human health research, she points out. “But it is a big stretch to go from a mouse heart to a human heart,” she says, so pigs, whose organ sizes are similar to human ones, have become highly desired for clinical research.

“The next step is using pigs as an ‘oincubator,’ we are calling it, -- sort of an incubator -- to grow human tissue cells and organs in the context of a pig.” She asks: “Could we one day grow organs for patients: a cornea, lung tissue, hepatocytes, pancreas cells?”

For anyone on a transplant waiting list – and there are plenty – that's welcome news. In the U.S. today, more than 116,000 people are waiting for an organ transplant, according to orgondonor.gov. Roughly 20 people per day die waiting.

Recombinetics is working on pig embryos that had been injected with human stem cells to begin growing organs containing human cells, creating what's known as a chimera, scientists reported in January 2017. Chimeras have cells from two different types of organisms.

Researchers hope they will eventually be able to grow new human body parts for transplantation – a transplant organ that's uniquely made to match a human's DNA. But for now, they need to figure out how to get more human cells to survive inside of their pig, cow or sheep host.

SAB Biotherapeutics, on the other hand, uses cattle as its living factory of antibodies to help people fight disease, in part because cattle are big, thus turn out big volumes of antibodies. Its genetically designed cattle, Tc Bovine, produce a menu of antibodies the same way that other cattle or people make antibodies, except they produce human antibodies instead of cattle ones. Its antibodies can be aimed at "prevention and treatment of diseases from Ebola to cancer, and diabetes to influenza," the company says on its website.

Chicken eggs, too, have been harnessed as factories for biomedical research and health care. Trouble is, a lot of children are allergic to eggs. So, they can't get some immunization shots, since many major vaccines are cultured in chicken eggs.

Australian researchers report, however, that they have altered an egg-white gene largely responsible for the allergy, making the egg hypoallergenic. That suggests hypoallergenic egg-based vaccines may be on the way.

The dynamics of editing animal genomes changes when the editing is done on wild species or when unlimited, worldwide adoption of such an edit is contemplated. As with the Dunham goal of raising sterile catfish, breeders think about the need for a brake pedal, so that a dominant and potentially harmful trait, doesn't enforce itself around the world.

Breeders think about brake pedals

Intrexon, for example, developed male Oxitec mosquitos that pass on to offspring a self-limiting trait wherein the offspring don't live to adulthood. As a result, the local mosquito population declines.

Those mosquitoes have been spread in recent years in Brazil, Panama, the Cayman Islands and Florida in efforts to control the mosquito population that carry Zika, dengue and other dangerous viruses. **A Zika virus infection during pregnancy created alarm for thousands of women when it was identified as a cause of congenital brain abnormalities.**

On the other hand, consider what may be the biggest weapon for controlling organisms in the wild – a gene drive. The genetic tweak employed by Intrexon means female mosquitoes will have dead-end offspring when mating with the mutant males; not when they mate with other males.

The concept is simple. Just ensure the gene or genes for adding or blocking a trait are on both chromosomes – thus, a double dominant modification that will be carried to practically all offspring. For example, a gene drive could single out a species, like pesky rats, to potentially eliminate.

Babies with Congenital Zika Virus Infection have smaller head sizes and other abnormalities. Source: Centers for Disease Control

Scientists working at the University of Edinburgh's [Roslin Institute] are using CRISPR gene editing techniques as a way to spread infertility in rats and mice – similar to the technique that already works for mosquito control – so that an entire population could be eliminated within a few generations.

The gene drive has selling points, and the attraction is clear if the goal is pest control. That is, a gene drive is toxin-free, eliminates a producer's costs and cuts down on the environmental and health risks of using pesticides. What's more, it avoids collateral damage: unnecessary harm to insects, bacteria, fungi, rodents and other critters who are beneficial or harmless.

"The promise of a gene drive, from a technical point of view, is that you can make a very small change at the genome level and release a single mosquito, a single mouse . . . and have a very large change across a whole population," said Jim Thomas, co-executive director of ETC Group, a California based ecological policy consultant, speaking at a UC-Davis conference on gene-editing.

"Here we have an exceedingly powerful technology, and we have to be exceedingly careful," he said.

One solution: Scientists at the University of California, Riverside, have already installed a gene drive in the drosophila that would potentially wipe out the insect over time. Its use would require regulatory approval. Siccing a gene drive on the spotted-wing drosophila, for example, holds the potential for greatly reducing billions of dollars annually in damage that pest unleashes on cherries, berries and other stone fruits in the West Coast region, in Europe and elsewhere. This Asian native fruit fly lays its eggs in ripening fruit – instead of in rotting fruit, as other fruit flies do – making the fruit unmarketable.

Spotted wing drosophila eating a strawberry. Source: Clemson University

On an even larger scale, using a gene drive on another strain of mosquito – the top malaria-spreading one -- might save millions of people in Africa and Asia from illness and death. More than 200 million people get the parasitic disease annually, and nearly half a million die from it, according to the World Malaria Report 2017.

Years ago, UC, Irvine biologist Anthony James found specific genes in Anopheles gambiae, a type of mosquito, that pass malaria-resistance on to offspring. Those with the trait can carry the disease but are not able to spread it. More recently, he and fellow scientists have been funded to develop a gene drive to spread that resistance to all offspring of that strain of mosquito.

Field testing a malaria mosquito gene drive will probably occur in Africa, where malaria is by far the most prevalent. But James tells *Agri-Pulse* that it is "hard to predict" when it will start or in which countries.

"There are significant scientific, regulatory and social challenges to be met" before breeding such mosquitoes in the open, he says. He notes that the hosting countries will have to approve the work.

What's more, scientists at Imperial College London have prepared their own mosquito gene drive: this one to impose infertility on female offspring of the same malaria mosquitoes.

As with most biotech advances, there is fear about possible outcomes, especially, in this case, runaway unintended results in the wild.

The Broad Institute of the Massachusetts Institute of Technology and Harvard, which hold the patent for CRISPR-Cas9, discourage its use for gene drives. Also, a year ago, environmental advocates pressed world governments at their meeting as the United Nations Convention on Biodiversity, to initiate a ban on gene drives. Scientists there persuaded the gathering to reject that move, but the technology will undoubtedly face a lot of opposition worldwide.

Ryan Phelan, a wildlife conservationist and executive director of Revive and Restore

Ryan Phelan, a wildlife conservationist and executive director of Revive & Restore, agreed at the UC-Davis, CRISPR conference that the gene drive concept "does bring up huge issues" when it comes to protecting wildlife, especially threatened species. She asks: "Who gets to decide how you adopt something that knows no bounds?"

Yet, she says, in trying to protect wildlife, "there are some problems that are so intractable," and she thinks gene editing may have to be one of the remedies pursued while mankind still has an opportunity to try solutions.

Phelan points, for instance, to an invasive strain of mosquito in Hawaii that spreads avian malaria, which has already killed off some wild species of birds there and threatens the survival of others, including the native honeycreeper. Plus, the impact on the birds is worsening as climate change brings higher temperatures – and more avian malaria mosquitoes – into Hawaiian mountain habitat.

"Gene drive could indeed be part of an ultimate solution," Phelan opines. "But gene drive technology needs more iterations before we could safely test gene drives in the wild," and she says, and applying the technology would have to be “fully transparent and closely engage local and indigenous communities in Hawaii.”

On the other hand, she says, “avian malaria should not be in Hawaii.” Plus, the islands there have their own native mosquitos; the avian malaria one has no value in the food chain. So, she asks, “Do we have a right, or a moral obligation, as stewards, to remove those mosquitoes” via a gene drive or other biotech remedy?

It’s important to always remember that, “science and innovation always outruns law and policy. These ethical and moral questions are not new,” says Bill Even, CEO of the National Pork Board. “They arise every time a new technology emerges.”

“I would have these same discussions when I worked at Pioneer,” Even recalls about some of the Iowa-based seed company’s early research aimed at improving corn yields.

“When Henry Wallace pioneered the use of hybrid seed corn in the 1920’s, there were all sorts of people saying ‘the sky is falling,’ ‘you’re messing with God,’ and this is ‘not the natural way things should happen.’ There was all this fearmongering.

“Now, it’s viewed as one of the most successful improvements in agriculture and modern history. And people assume it’s natural and they welcome they welcome it,” Even adds.

THE BREEDING EDGE

Will the breeding evolution lead the next green revolution?

Chapter 5

Will new regulations stifle innovation in plant and animal breeding?

By Philip Brasher and Steve Davies

Gene editing is touted as a promising new way of altering the DNA of plants or animals to speed their growth, enhance flavor, extend shelf life or combat viruses. But those who see it as a key component of agriculture's future want to make sure that the regulations written for it do not stifle its promise.

"What we need to have is an environment where the regulation fits with the risk, and regulation fits with the application" of the technology, says Kevin Folta, professor and chairman of the Horticultural Sciences Department at the University of Florida. "Let's not bottleneck innovation."

New methods such as CRISPR may allow plant breeders to avoid the regulatory bottlenecks. Harry Klee, a colleague of Folta's at the University of Florida who is working to improve the taste of tomatoes, says he's using CRISPR instead of "traditional genetic engineering" that uses foreign DNA.

"We are hopeful that a clean gene-edited mutation with no foreign DNA that is equivalent to a natural mutation will be free and clear of regulations," he said in an email. "**Being able to take such a mutation and introduce it widely into many elite breeding lines without the need for extensive backcrossing is a potential game changer in breeding programs."**

So far, the odds are good that Klee will get his wish. All of the gene-edited products APHIS has reviewed thus far under its "Am I Regulated" process have received "no" answers – including a non-browning mushroom developed at Penn State using CRISPR/Cas9.

But that's not the case for gene-edited animal products.

For now, the Food and Drug Administration (FDA) defines all intentionally altered genomic DNA as a new animal drug, irrespective of how or why they were changed. For example, firms that use gene editing to develop pigs with PRRS resistance, would have to go through FDA's process for evaluating a drug.

"It's a nonsensical position," says Alison Van Eenennaam, a cooperative extension specialist in animal genomics and biotechnology at the University of California-Davis who has conducted extensive research on gene editing with cattle.

"Regulatory processes should be proportional to risk and consistent across products that have equivalent levels of risk," she explained. For now, "GE animal regulatory burdens are disproportional high and associated with unaccountable delays and considerable uncertainty. These regulatory burdens are not justified by scientific evidence or experience," she adds. And they require elaborate and costly studies, not to mention time delays.

So, for many industry stakeholders, there is still a great deal of regulatory uncertainty. And it's not clear how the regulations might change in the future.

Mike Firko, deputy administrator for APHIS' Biotechnology Regulatory Services, said at BRS's recent annual stakeholders meeting that the track record so far isn't necessarily predictive of the future.

"We decided that those particular products that were put before us would not be regulated," he said. "CRISPR is a very powerful tool. It may very well be possible that we will soon see a product produced with CRISPR technology where we would say, 'Oh, yes, that falls within our regulatory authority, under our current regulation.' So, I want to make it clear that our decisions haven't been about all products produced with CRISPR technology – only those that we've seen so far."

It may be some time before there are clear answers on where the federal agencies responsible for regulating genetically engineered products draw the line. Given the fractured nature of the current regulatory framework, that will take a lot of meetings, talk and compromise.

There are also major trade concerns to address: Exporters of grains, oilseeds and other commodities have raised concerns about an overhaul of U.S. regulations, saying that any changes need to be coordinated with importing countries to make sure that shipments of gene edited products will be accepted. Many countries, from the nations of Europe to Japan and New Zealand, are in various stages of assessing how they will regulate products of various gene-editing techniques.

As for the effort to modernize U.S. regulations, "My feeling is it's pretty stalemated," says Nicholas Jordan, a professor at the University of Minnesota who has studied and written about biotech regulation. "There are such divergent ideas about what the future regulatory system should look like."

Nicholas Jordan, University of Minnesota

That's not just because of rapidly advancing technology, which makes it difficult – if not impossible – for regulators to keep up. It's also because of the three-legged approach used to regulate biotech in the U.S.

Currently, the USDA's Animal and Plant Health Inspection Service regulates genetically engineered plants if they are deemed to pose a plant pest risk. APHIS also reviews petitions to determine whether new genetically engineered products pose enough of a safety risk to even warrant regulation.

Also in the regulatory mix: The Food and Drug Administration, which regulates plant and animal food products derived from conventional breeding techniques or genetic engineering. Finally, the Environmental Protection Agency regulates plant-incorporated protectants – "plants that have been genetically altered to produce proteins that are harmful to certain insect pests," EPA explains, citing as examples Bt corn and Bt cotton, engineered to resist pests such as corn rootworm and cotton bollworm.

Despite their best efforts to coordinate oversight of gene edited products, the arrangement has occasionally led to confusion, as with Oxitec's attempt to release male mosquitos with a "self-limiting" gene that causes offspring to die before reaching adulthood. Initially, FDA performed an environmental assessment on the mosquitos based on its conclusion that they were "animal drugs," but later it kicked the regulatory responsibility to EPA after both agencies concluded that "articles or categories of articles that control the population of mosquitoes are most appropriately regulated as pesticides."

"When you're mostly trying to eliminate a pest population, why not just let one agency do it?" says Jaydee Hanson, senior policy analyst at the Center for Food Safety, who pushed for the change.

Everyone in the biotech realm – including government officials, private industry, non-governmental organizations and plant breeders – agrees that clear lines of responsibility are essential. **The recent report from the Trump administration's Rural Prosperity Task Force calls on the administration to "develop a streamlined, science-based regulatory policy" that ensures products are assessed "in a risk-based manner, providing predictable pathways for commercialization."**

President Trump signed the executive order creating the Rural Prosperity Task Force on Ag Secretary Sonny Perdue's first day on the job.

"We're hopeful that perhaps that's kind of the hook that USDA will start taking some leadership in getting other agencies to move in a similar direction… and with some leadership from the White House as well," says Clint Nesbitt, director of regulatory affairs for food and agriculture at the Biotechnology Innovation Organization (BIO).

"It is important that you have a regulatory system that does give consumers the reassurance that these products have had the appropriate amount of regulatory oversight," Nesbitt says. "That doesn't mean that every single thing necessarily needs one-by-one review."

But every sensible recommendation comes with a caveat. In order to fashion a coordinated approach, there must be people in place to do the coordinating. And the Trump administration has been slow to get its political appointees in place.

The Rural Prosperity report, for example, recommends that the White House Office of Science and Technology Policy coordinate interagency biotechnology efforts. In addition, the report recommends the administration "create a forum led by (OSTP) that connects regulators with the funding and R&D agencies to increase awareness and speed the safe commercialization of novel biotechnology products."

Building on the task force report, USDA and FDA announced a joint agreement Jan. 30 that pledges to carry out a 2015 strategy, spearheaded by OSTP under the Obama administration, for modernizing the government's regulatory process for biotechnology.

Just one problem: OSTP still doesn't have a director, more than a year after President Trump took office. It's the longest stretch the position has been vacant since OSTP was established in 1976.

Nesbitt says that BIO hasn't yet had detailed discussions with agency regulators about biotech, citing in part the dearth of officials in positions of responsibility.

One area that is of particular interest encompasses what are known as the Part 340 regulations, which govern how APHIS deals with plant pests. APHIS released a proposal to rewrite those regulations the day before President Obama left office.

The proposal, which would have required upfront analysis of the risks of GE organisms, was roundly criticized by stakeholders on all sides of the issue and was eventually withdrawn in November.

Firko called the comments on the Part 340 changes "a head-scratcher" at BRS's annual stakeholder meeting in November. One group of commenters said, "Oh my gosh, this is so much more regulation," Firko said. "And then there's a whole 'nother bunch of people (who called it) dramatic under regulation."

"Somewhere in our communications, different groups of people are interpreting our proposed rule very differently," Firko said. "So, that's why we need to engage with stakeholders more... try to figure out what was going on there, why different groups of people viewed our proposed rule so dramatically differently. And that's what the new stakeholder engagement is intended to address."

So far, however, there have been no announcements about stakeholder engagement.

"As of this moment, we don't have any specific dates for public meetings and things like that," said APHIS spokesman Rick Coker.

Nesbitt said early stakeholder input is crucial so APHIS understands the concerns with the proposal. "Fairly widely within the industry, we all felt that the proposal was confusing, complicated, created a lot of unanticipated problems that they didn't intend," he said.

The American Soybean Association, for example, said the "upfront risk analysis" sought in the proposal "all but guarantees that only the largest companies would have the time and resources to undergo this lengthy and costly process. Furthermore, it would deter much needed research at our universities and elsewhere."

"If the U.S. is to be at the forefront of innovation and advancements in technology, we cannot create a regulatory system that stifles this goal and leaves U.S. businesses at a competitive disadvantage," ASA said.

Grain and oilseed exporters, however, repeatedly raised concerns with the Obama administration about streamlining the Part 340 regulations without first making sure that the changes would be accepted by the importing nations. The exporters repeated the concerns to the Trump administration, urging APHIS to withdraw the proposed rule.

The exporters are still smarting from the 2014 disruption in shipments to China when Syngenta commercialized its Agrisure Viptera corn seed in the United States prior to getting import approval from China. The industry estimates the disruption cost it $1.5 billion to $4 billion during the 2013-14 marketing year.

In joint comments filed with APHIS, the Corn Refiners Association, National Grain and Feed Association, National Oilseed Processors Association, North American Export Grain Association and North American Millers' Association called for the administration to pursue a "comprehensive engagement strategy" with other countries in order to "build international regulatory compatibility and acceptance around a new, more science- and risk-based approach" to regulating genetically modified plants.

"To be clear, this does not mean that other countries need to adopt a regulatory approach to new plant-breeding techniques that is identical to a future approach employed by APHIS. But, emphatically, it does mean that APHIS's regulatory approach must be recognized by, and acceptable to, government regulatory authorities in U.S. export markets so as not to trigger regulatory action against U.S. commodities produced using genetic engineering or plant-breeding innovation, including gene-editing, in international commerce," the groups said.

Bobby Frederick, director of legislative affairs and public policy for the National Grain and Feed Association, applauded USDA's decision to withdraw the Part 340 rule, saying it gave APHIS time to pursue the internationally coordinated approach that the exporters have been seeking. "You can't guarantee seamlessness, but you can line things up where they are more clear," he said.

The exporters are simultaneously urging the FDA to require companies to notify the agency before they go to market with new gene edited products. Not only is that premarket notification necessary to protect consumer confidence, it also would be "invaluable in facilitating the domestic and international marketability of crops utilizing these new techniques, thereby benefiting agricultural producers, the value chain, consumers and ultimately plant breeders," the groups wrote in their joint comments.

Gene editing also has raised trade issues in the meat and dairy sectors. Industry groups are worried that FDA will move ahead with regulating all intentionally altered genomic DNA as a new animal drugs, the approach the agency now uses for all genetically engineered animals.

"Treating these animals as drugs also will likely have a negative impact on domestic and international trade," the North American Meat Institute said in comments to FDA. NAMI noted that some foreign countries may not regulate gene-edited animals.

National Pork Producers Council President Ken Maschhoff says it would be "indefensible" for FDA to regulate all gene-edited animals as animal drugs.

"We ask the FDA to acknowledge that not all gene editing applications to animals require approval as new animal drugs under the Food, Drug and Cosmetic Act (FD&C Act)," Maschhoff wrote in public comments to FDA in June 2017. "Such an interpretation of the FD&C Act—especially as applied to many potential uses of gene editing techniques in livestock— mischaracterizes resultant edited genomes as an 'article,' creates potentially insurmountable practical barriers to enforcement by the FDA and utilization by industry, and is not in keeping with federal policy and precedence - and indeed, global regulatory trends—concerning the use of biotechnology in agriculture."

Ken Maschhoff, NPPC

The Foreign Agricultural Service has been tracking developments globally and there are signs of some consensus developing on approaches to gene edited products. As with the United States, however, countries are still in various stages of developing or revising policies. Some countries are even ahead of the U.S. government, according to an FAS survey obtained by *Agri-Pulse*.

Chinese scientists have published dozens of papers on their use of CRISPR techniques, while the agriculture ministry is finishing work on its regulations for gene editing, according to FAS.

In Japan, the government has been handling gene edited products "on a case-by-case basis," according to the FAS report. "Consequently, researchers have been taking a relatively conservative and cautious position towards R&D." South Korea, which hasn't decided how to regulate gene editing yet, "is closely watching developments in other countries to determine how they can regulate innovative technologies," FAS said.

But in South America, Argentina and Chile have decided that some plant products of certain gene editing techniques as well as conventional breeding may not be regulated as GMOs when the traits could be obtained through conventional breeding or mutagenesis, according to the FAS survey. Products will have to be considered on a case-by-case basis, however.

Brazil's agriculture ministry has not finalized that country's policy. However, based on what has been proposed so far "it seems that all these three countries are going in the same direction," said Diego Risso, executive director of the Seed Association.

The industry may have to live with the idea that products will be assessed on a case-by-case basis, however. "We need to accept that it's the way the regulators feel more comfortable," Risso said, speaking at the American Seed Trade Association's annual meeting in December.

The South American countries' policies are also expected to apply to gene editing of animals, according to the FAS survey.

Even in Europe, where there has been strong resistance to biotechnology, some governments want to take a different approach to regulating gene editing, at least when it comes to plants.

Several members of the European Union are awaiting a European Court of Justice opinion clarifying whether products of gene-editing techniques that are considered mutagenesis would be regulated as GMOs. A non-binding advisory opinion provided by the court's advocate general in January was mixed. A final decision by the court is expected this summer.

The Netherlands, meanwhile, has proposed to the European Commission that many gene edited products be exempt from regulations as GMOs if the same modifications could be achieved through conventional breeding. FAS cautions that a decision by the Commission could take years.

Back in the United States, the proposed Part 340 revisions also came under fire from groups traditionally critical of biotechnology. The Center for Food Safety (CFS) criticized APHIS's proposal to exclude three classes of organisms from regulation, saying that "would allow GE organisms that could cause (Plant Protection Act) risks to entirely escape review and regulation by APHIS."

"Any type of change in a gene sequence can potentially cause phenotypic changes that have significant consequences, whether the change could occur naturally or not," CFS said in its comments. "Moreover, genome editing methods are still in early development, and risks of their use are not known well enough to predict impacts" without actually observing how they work in nature.

BIO, meanwhile, "believes APHIS could go a long way in improving the current regulatory system in the near term without revising the regs while still moving forward with rulemaking on a parallel track in the longer term."

“They could push the boundaries a little further in terms of deregulating categories of things similar to things they’ve already seen,” BIO's Nesbitt said. In its comments to the agency on the proposed 340 changes, BIO said, “We believe that there will be many instances in which it is more scientifically justifiable to assess the regulatory status of whole categories of organisms, such as those of the same combination of species and trait.”

Greg Jaffe, CSPI

Greg Jaffe, director of the Project on Biotechnology at the Center for Science in the Public Interest, said he has been advocating a “nuanced approach” to regulation. **“There should be risk-based analysis where you’re looking at the different crops, the different possible gene edited products and putting them into buckets. Some of those buckets may involve little or no regulation, and others may involve a full risk assessment and a mandatory pre-market approval process. It would depend on what the potential risk profile is for those.”**

Paul Schickler, former president of biotech seed giant DuPont Pioneer, has cautioned the biotech industry against using gene editing to avoid regulation. Speaking at The World Food Prize’s Borlaug Dialogue in 2017, he said, **“If we do position gene editing as an end-around of regulatory systems, it will be the failure of gene editing as a technology.”**

But clearly, something has to give on the regulatory front if researchers and investors are going to advance both plant and animal agriculture.

FDA has approved one genetically engineered animal for food use: AquAdvantage salmon, genetically engineered by AquaBounty to grow much faster than non-GE farm-raised Atlantic salmon. But it took 20 years for that process to play out, “so that’s not a working regulatory system,” Nesbitt said. And the salmon have yet to be sold in the U.S. because of barriers imposed by Alaskan lawmakers.

“All kinds of animal companies are working in the space, but there’s just not a clear path forward, and clearly the process at FDA is not working,” he said.

FDA says the regulatory process it went through with AquAdvantage salmon does not necessarily provide lessons for future animal applications.

In response to questions posed by *Agri-Pulse*, FDA said, “Application of the regulations to individual products is highly case-specific so the experience with one application, such as the one for the GE salmon, may not be relevant to other applications.”

But Karen Batra, a BIO spokesperson, says that “A lot of our member companies are looking to that as kind of the model. They’re saying, "Well, I’m going to go to Brazil, I’m going to go away from this regulatory system. I’m not going through what (AquaBounty) went through.'”

Nesbitt adds, “For those things where there’s not a clear path to market, they just won’t even invest in the research in the first place, so until we have these clear paths, a lot of these companies won’t even be investing in this space.”

J.R. Simplot Co. also commented on the chilling effect that the draft FDA guidance could have on new opportunities for agriculture.

If FDA “were to regulate animals with intentionally altered genomic DNA as a new drug, the regulation would be disproportionate to the risk and not based in science. In fact, it would regulate the identical animal differently depending on whether the altered genomic DNA occurred naturally or by genomic editing," Simplot wrote.

"The consequence that follows is that it will become nearly impossible to effectively use promising and emerging technologies in animal genetics.”

At this point, it’s not clear where FDA will end up. It did not say when it will release its final guidance.

Chapter 6
Are consumers ready to accept gene editing?

By Ed Maixner

Americans are easily riled about genetically modifying crops, animals and foods, even though research indicates they know little about how GMOs are produced. So, what about consumer understanding of a new and much different approach to precision breeding called gene editing?

There's a big difference between what are usually referred to as GMOs and products produced through gene editing, but that has not stopped some activists from tying the two types of technologies together on social media and creating unnecessary fears. Add that to a general public misunderstanding of science and food production, and you have a budding recipe for confusion and misunderstanding.

For example, when the Washington Post ran an opinion piece recently by Purdue University President Mitch Daniels, a former Indiana governor, extolling genetically modified (GMO) crops and saying it is immoral for society to prohibit them, the article generated 1,630 reader comments within two days.

The remarks were typically emotional, angry or sarcastic and frequently displayed ignorance about genetic modification processes and products.

But even though Americans get their dukes up over GMOs, they may not understand why, says Michael Specter, veteran science writer for the New Yorker.

"I don't think there are many people in this country who get up in the morning and say, 'Oh, my god, I'm so happy we have GMO corn,'" Specter told people attending a gene editing conference, called CRISPRcon, last August at the University of California-Berkeley. Herbicide- or insect-resistant corn, he says, don't hit people where they live, as would biotech remedies to wipe out malaria mosquitos and save lives.

"What matters is a connection to people on something they care about," Specter said.

In general, Americans don't like the idea of GMOs, even if they don't know what they are. In a 2015 national survey, the Pew Research Center found that 57 percent of adults, including two of three women, consider food products with GMOs to be unsafe. About half – especially women and folks under 30 years old – say they watch for GMO listings on labels at least sometimes.

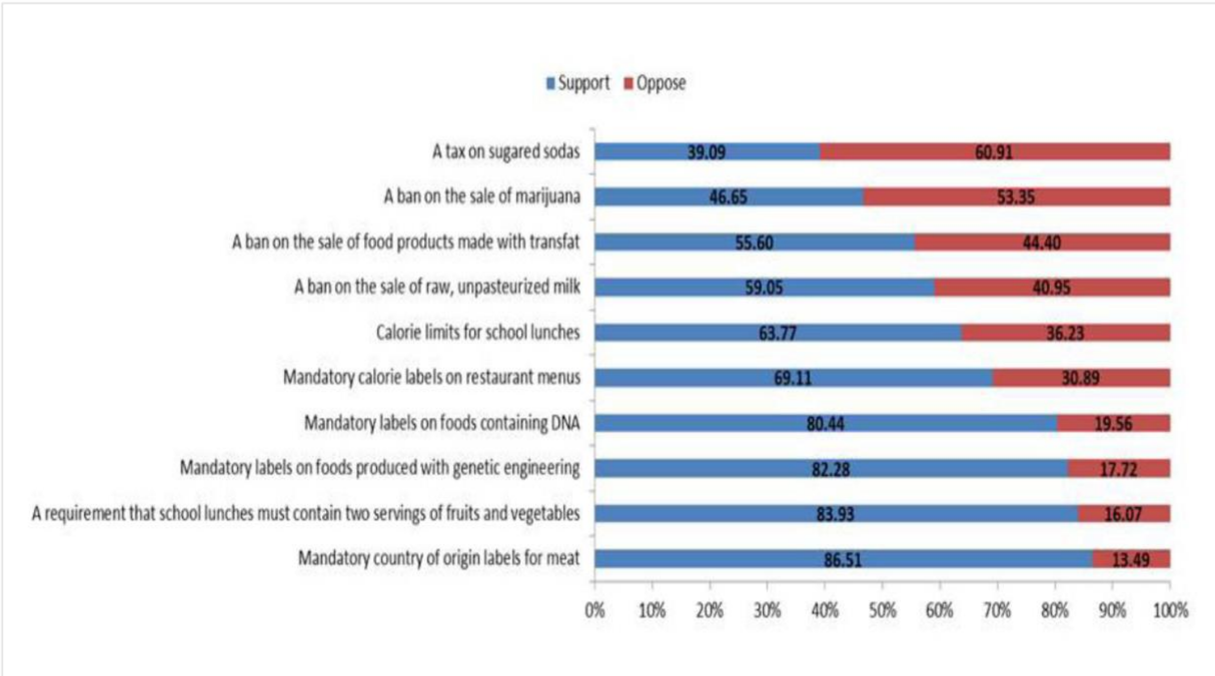
What's more, it seems that nearly 90 percent of U.S. adults want food labels to indicate the presence of GMOs, according to a survey sponsored by Just Label It, a leading proponent of such labeling. Last year, in fact, Congress ordered that products must disclose scannable or printed GMO information, and regulations mandating such labeling are due out from USDA by mid-summer.

But those snapshots of Americans' opinion on GMOs tell little about their understanding of food products and how they are created.

A Michigan State University Food Literacy and Engagement Poll asked 1,059 U.S. adults last summer, for example, if this statement is true: "Genetically modified foods have genes and non-genetically modified foods do not," and 37 percent agreed, showing that many people just don't know what genes or GMOs are.

Indeed, American consumers' knowledge in biotechnology is hit and miss, agrees Dietram Scheufele, a professor in the Department of Life Sciences Communication at the University of Wisconsin. He noted that Oklahoma State University pollsters, in their 2015 Food Demand Survey, asked a thousand Americans if they want mandatory labeling of DNA in foods. The result: 80 percent said yes (See chart on right.) Obviously, those respondents did not understand that DNA (deoxyribonucleic acid) is an essential part of all living organisms - organic, non-GMO or GMO.

Support or Oppose of Government Policies



Source: Jayson Lusk, Oklahoma State University

Note too, that the references here to Americans' lack of knowledge about GMOs relate to transgenic cell modifications (between separate species), which have been happening in commercial products for decades. So, don't look for Americans to have any vast awareness of the new non-transgenic techniques, like gene editing, which involve modifications strictly within a cell's nucleus.

Dietram Scheufele, Univ. of Wisconsin

Nonetheless, when people see potential or assured benefits from biotech for their own bodies, they become more accommodating, Scheufele and others found in their 2017 survey, which questioned 1,600 adults about new human gene editing remedies in medicine.

"We found that 59 percent of respondents expressed at least some support for human genome editing to treat human medical conditions or restore health," Scheufele said. Note, however, that only a third expressed even cautious support for such techniques if used to enhance or improve human mental or physical abilities or appearance.

But, he said, "with CRISPR (a popular new gene editing process in the laboratory), "we're seeing some of those issues come together in people's minds . . . the idea of having a more technically safer and efficient solution for editing than we've had in the past … and that is exciting."

Can Scheufele's findings in the medical sphere be equated with gene editing in agriculture?

"There are certain parallels and certain clear differences," he said. Research shows people regard "their own bodies much differently than they would treat production agriculture." On the other

hand, he said, "with food, as soon as they start putting something into their bodies, it relates much closer to some of the findings that we've had" in research on human genetics.

Some factors emerge as important when people consider what is acceptable in any kind of genetic modification, whether gene edited or transgenic manipulations, Scheufele said. One is the respondents' "religiosity," which is offended when the scientist is seen as "blurring the lines between God and man." People also commonly oppose genetic alterations they see as "unnatural," he said.

Along with those sort of root moral and ethical criteria, the flow of information from news, internet communications, family and friends tends to shape views of biotechnology, Scheufele said. The consumer's view of GMOs "is what popular discourse looks like," he said. If popular discourse is dominated by references to a certain meat product as "pink slime" or a biotech strain of fast-growing salmon as "Frankenfood," then "that is going to determine my decisions about GMO or GE products," he said.

However, what do people familiar with biotech issues think about CRISPR and other gene editing laboratory tools?

Last summer's CRISPRcon conference featured some instant polling of such a biotech-savvy crowd. Its more than 300 participants came from the life sciences, food and agriculture sectors, environmental and wildlife organizations, or were science news writers and so forth.

Given a multiple choice, 43 percent of participants who responded agreed that gene editing is "a tremendous tool to benefit mankind." But they could select more than one answer, and 47 percent agreed that gene editing is all three of these things: "a tremendous tool . . . a technology that is poorly understood and needs more research . . . (and) a dangerous tool that could easily be used to the detriment of mankind."

Not surprisingly, many big farm groups, food makers and retailers believe gene editing holds a lot of promise. In late 2016, several groups who were already members of the Center for Food Integrity (CFI) formed the Coalition for Responsible Gene Editing in Agriculture to promote gene editing to Americans as a smart direction toward efficient food production, healthy eating and environment. The coalition puts a premium on earning the trust of consumers for any gene editing process or products, said Roxi Beck, CFI's consumer engagement director.

Traditionally, said CFI Chief Executive Charlie Arnot, "the assumption has been, if the science is sound and the regulatory process is valid, there will be social acceptance" of new biotech products. "But we know from experiences with GMOs that isn't the way it works in today's environment."

CFI Chief Executive Charlie Arnot

CFI's research on consumer attitudes, Beck said, found that, although it's still a priority in the coalition's guidelines for biotech developers to present the facts around their discoveries clearly and honestly, "you don't change (consumers') ethics and beliefs with science and facts alone."

In fact, she said, CFI's research shows that "the facts are three to five times less important than connecting (with consumers) through shared values." So, she said, biotech developers and food companies with a new product in the works need to ask, "Should we be doing this?" rather than, "Can we do this?'

As might be expected, the coalition defines gene editing in very attractive terms: "Gene editing makes precise, intentional and beneficial changes in the genetic material of plants and animals used in food production, which can improve their health and sustainability. This often mirrors changes that could occur in nature or through traditional breeding. Gene editing helps farmers keep pace . . . while using less water, land, nutrients and other resources."

On the other hand, Arnot expects the coalition's guidelines may change as its membership broadens, and he thinks public acceptance for GE won't be easy.

"Our next step is to engage the food system … to begin to answer concerns that food companies may have about gene editing, what it might mean to their supply chain … to bring forth products with potentially improved shelf life or nutrition," he said.

On the opposite biotech policy front, nearly all advocates of organic food and farming, many of them versed in biotechnology, are opposed to new GE techniques, just as they've been to earlier transgenic plant and animal products – at least in terms of any such biotech in organic production.

"As an organic community," said Abby Youngblood, executive director of the National Organic Coalition, "we have a framework for looking at what is and what is not prohibited in organic, and we have definitions of what we mean when we talk about genetic engineering that align with international definitions that have been adopted by (world plant regulating bodies)."

Also, the U.S. organic program has "equivalency agreements with other countries," Youngblood says, and "there is really broad agreement that gene editing is prohibited in organic production."

Scott Faber, Just Label It

Just Label It and others who don't like GMOs agree with the organic folks and see gene editing as just another GMO category that ought to be indicated on labels. In its upcoming regs on GMO labeling, USDA "should consider the terms 'genetic engineering,' 'genetic modification,' and 'biotechnology' as interchangeable with 'bioengineering,'" JLI wrote to USDA.

"This should be simple," said Scott Faber, JLI executive director. "Any product of genetic engineering, whether it's transgenic or gene editing should be disclosed. Hiding information from consumers is a recipe for more mistrust" from consumers, and that isn't in food industry interests, he said.

Youngblood, meanwhile, notes her coalition is also concerned that exclusive patents granted for gene editing laboratory processes or products might end up making valuable plant and animal genetics out of bounds to researchers trying to breed new varieties for organic production. "We have a concern about genetic material (not) remaining in the public realm," she says.

"We are concerned about exclusive rights in that area, too," from a global perspective and beyond the implications for organic production, said Jamie Love, director of Knowledge Ecology International. He said KEI and others with a stake in agriculture worldwide "think it's a mistake to associate exclusive rights with that sort of technology." GE technology "should be used as freely as possible … for research, but also so that people can actually benefit from it," he says.

The odds of gene editing gaining favor in America any time soon aren't clear. It is good to remember that acceptance of scientific discoveries can take what seems like ages. **Galileo, for example, was convicted by a church Inquisition in 1633 for teaching that the Earth circles the sun and spent the last nine years of his life in house arrest because of that supposed heresy. Yet, today, only 76 percent of American adults believe the Earth circles the sun, and only 49 percent believe in human evolution, according to the 2016 National Science Board's survey on *Public Knowledge about Science and Technology*.**

Mindful of the deepening polarization over gene editing and GMOs in general, scientists at the University of Minnesota are trying what they call "cooperative governance networking" to tease out a public policy consensus on whether, when, and how GE could be used and developed in ways that would be acceptable to society.

UM Agronomy and Plant Genetics Professor Nicholas Jordan said his team began with the assumption that folks who've long objected to biotechnology in agriculture and "want to hold up genetic engineering … hold the upper hand."

So, Jordan reasoned, "we will try to engage them in something they care about," and thought gene editing in cover crops might be a place to start. There has been paltry investment and not much progress in cover crops, he said. But with climate change threatening, genetic advances are needed for winter-hardy grains, oilseeds and other crops to help them flourish.

He is working to make those advances happen with the Foundation for Food and Agricultural Research, environmentalists, soil scientists, plus venture capitalists who might consider developing new cover crop traits. He has also applied for a USDA grant.

Jordan said his project provides "an opportunity to take a look at the value of gene editing" from various perspectives. For investors, he said, getting favorable consensus from across the spectrum of stakeholders is a good way to reduce financial risk.

So, what is the future of precision breeding tools in the U.S.?

Nina Fedoroff

Nina Fedoroff isn't all that optimistic. Fedoroff, a molecular biologist with Penn State University and other institutions for nearly a half century, calls herself "one of the few people left who have been around since the beginning" of cell nucleus manipulation in the laboratory. She points to the 2016 National Academies of Science findings of no health or safety risks linked to biotech crops.

“The point is that, today, we know that the methods are not dangerous. People have been looking for problems associated with simply using molecular techniques for 30 or 40 years now and haven’t found them,” she declared.

She believes Americans have “tied ourselves into unbelievable irrational knots” over policy on biotech crops. She said she watched what she considered a slow but reasonable path toward public review and acceptance of transgenic crop enhancements for many years.

But, Fedoroff said, “in about the last decade it has become something different. Basically, the organic marketers decided that they could gain market share by vilifying GMOs, and that’s what they started to do.”

She says Greenpeace and other environmental and consumer groups have picked up the practice of condemning biotech foods primarily to raise money and without regard to science. She said she doesn’t know if the resulting tide of opinion against biotechnology leaves room for gene editing to advance in years ahead.

Specter, the New Yorker writer, favors gene editing himself and generally agrees with Fedoroff that biotech in agriculture has suffered from campaigns of untruth.

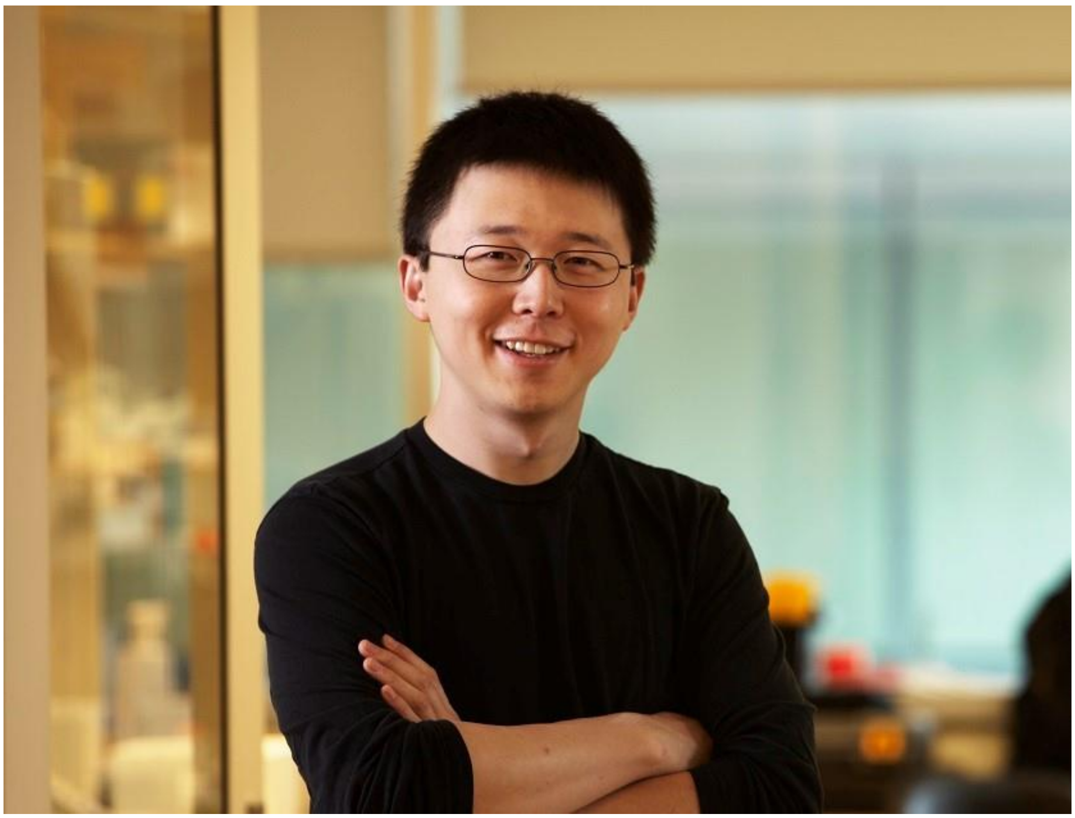
“People are paid for, and attracted to, extremely engaging and horrifying stories. It’s cheap and it’s easy,” Specter said. His solution: “You have to fight misinformation and lies with the truth. I don’t know any shorthand for that.”

THE BREEDING EDGE

Will the breeding evolution lead the next green revolution?

Chapter 7

Who is leading the charge for new precision breeding tools?



By Sara Wyant

Historically, farmers and ranchers have looked to USDA and their universities to develop the “next big thing” in plant and animal breeding.

They still do, even though the private sector has emerged as a key source of delivering innovation to the farm, while the public sector – confronted with declining investments from taxpayers – has been more focused on basic research.

That’s why the “troops” behind a new revolution in agricultural technology and precision breeding represent some familiar faces, as well as many new and perhaps untraditional players.

For example, from 2010 to 2016, the National Institute of Food and Agriculture (NIFA) funded over 75 gene editing research projects, investing over $76.5 million.

One of the NIFA grants helped drive research at Recombinetics, an animal gene-editing company based in St. Paul, Minnesota. The firm is now a leader in "naturally hornless" (polled) and "naturally cool" (heat-tolerant) cattle. Both types of animals were bred by copying naturally occurring traits already found in cattle.

But even with NIFA's new focus, "we've got fewer dollars and we just can't keep up with demand," said NIFA Director Sonny Ramaswamy. "Because of the continued disinvestment in the public sector at the federal and state levels, public dollars are fewer and fewer and it requires more private investment."

Where are the new research funds going to come from? Farmer-funded commodity checkoffs, which Ramaswamy described as "critically important" to land grants and other university research programs, are one source. Donations from leading crop and livestock companies are also key.

At the same time, newer companies like Caribou Biosciences, TargetGene Biotechnologies, Cibus, Calyxt and Benson Hill Biosystems, funded by venture capital funds, are sprouting up with promises to deliver significant breeding advancements for farmers and ranchers around the globe.

Industry observers say the growth in new start-ups is fueled, in part, by the precision, the lower cost and shorter development time available with innovative gene editing systems like CRISPR.

"Gene editing is a transformative game-changing type of approach that is going to allow us a step increase," Ramaswamy said.

"Gene editing, gene drive and things like that have really democratized research" so that companies of all sizes can participate, he added. "With gene editing, one can do it in your garage. Now companies are trying to figure out what their best action is."

NIFA Director Sonny Ramaswamy

Gregory Graff, an associate professor in agricultural economics at Colorado State University, who tracks start-ups in ag technology, agrees that opportunities are opening up for new companies and new types of crops, including some that have lacked research focus in the past.

"For many years now, with transgenic crop traits, the big agricultural business companies with corn and soybeans were the only game in town because of the cost of commercializing," said Graff.

The whole sweet horticultural sector – everything from tomatoes to sweet corn to grapes, you name it – never "really saw any applications of the technology at all," he added.

"It was just the 'billion-dollar crops' like corn and soybeans that provided a big payoff for research and development," Graff said.

Changes in technology have dramatically lowered the cost to enter the market, said Brett Morris, a principal with Technology Acceleration Partners (TechAccel).

"This is especially true in ag-biotech, where a range of once-daunting capital requirements have plummeted in cost. The cost of genome sequencing has declined from $100 million per genome in 2001 to approximately $1,000 per genome in 2015," Morris wrote in a blog for GlobalAg Investing.com.

"Cheap cloud storage now allows companies to host, analyze, and reproduce huge data sets. Computing power has significantly increased and enabled advances in artificial intelligence and machine learning. As a result, companies are observing phenotypic characteristics in the lab versus the field, and continuously improving outcomes by feeding more data to smarter algorithms. And the insights these start-ups are producing are moving into development faster, thanks to high-throughput automation. In simple terms, this means it's easier to start a company today than ever before."

Still, much of the basic research starts on university campuses and then, through business incubators, collaborations with other companies and licensing agreements, the research into both agriculture and human health, spreads like a spiderweb – ultimately connecting in myriad directions and levels of investment.

Biochemist and Professor Jennifer Doudna

Consider the case of Biochemist Jennifer Doudna at the University of California-Berkeley. She, along with Emmanuelle Charpentier from the University of Vienna and others, are widely credited with being the first to propose that CRISPR-Cas9 could be used for programmable gene editing.

In plants, this editing capability can be applied to promote drought tolerance and disease resistance to protect plant health and increase crop yields. It also can provide direct consumer benefits like the removal of food allergens and the improvement of the nutrient composition of foods and oils.

Researchers discovered they could harness CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) systems to more precisely cut and edit DNA in plant, animal, and human cells. And it didn't take long to realize that this new technology could potentially be a game changer in providing benefits for both agriculture and human health.

In 2011, Doudna teamed up with researcher Rachel Haurwitz, Martin Jinek, then a postdoctoral researcher, and James Berger, then a professor at UC-Berkeley, to found Caribou Biosciences. The name "Caribou" is a combination of Cas, a term meaning CRISPR-associated, and ribo, as in ribonucleic acid, or RNA. Doudna remains on the firm's scientific advisory board.

But in addition to working as a professor of molecular and cell biology and chemistry, Doudna and colleagues at Berkeley and UC-San Francisco (UCSF) launched the Innovative Genomics Institute in

2014 to perfect the CRISPR/Cas9 tool and develop new human disease therapies. Some of their early achievements included improving efficiency of gene replacement and a new approach toward a treatment for sickle cell disease. That promising approach generated a $4 million investment from the California Institute for Regenerative Medicine, the Berkeley News reported in 2017.

Armed with significant new funding, IGI, led by Doudna as executive director, relaunched with plans to use a portion of the funding to solicit research proposals in the areas of human health, agriculture and microbial ecology.

Just last month, IGI selected 24 new research projects across three academic institutions (Berkeley, UC-San Francisco, and UC-Davis). The agriculture program area is seeing the largest growth, with the addition of 11 new projects, IGI reported.

In the area of human health, IGI also plans to work with the Bay Area's Chan Zuckerberg Biohub, a collaboration among UC Berkeley, UCSF and Stanford University launched in 2016 with $600 million in funding from Facebook CEO and founder Mark Zuckerberg and his wife, Priscilla Chan, who is a UCSF pediatrician. Doudna is a member of the organization's Science Advisory Group.

Researchers anticipate that new precision tools and systems will be crucial in solving human health problems, but in the short term, many of the advancements could apply to agriculture.

"I think there are going to be as many or more applications of CRISPR-Cas9 in plants than there are in the biomedical area," Brian Staskawicz, a UC-Berkeley professor of plant and microbial biology who will direct the agricultural arm of the IGI, said in an interview with the Berkeley News. **"In California alone, we grow 300 different crops, many of them improved by standard cross-breeding, which introduces all sorts of undesired traits along with the one you want. CRISPR-Cas9 allows us to introduce genes with a level of precision that we have not been able to do in the past and potentially cut four to five years off breeding cycles.**"

IGI is also working with crops at risk around the globe, like the cacao beans used to make chocolate. Over half of the world's chocolate now comes from two West African countries: Côte d'Ivoire and Ghana where the cacao trees are at risk from disease and climate change, according to Climate.gov. In an effort supported by food and candy company MARS Inc., IGI scientists are developing CRISPR editing technologies to alter cacao trees that will be resistant to both viral and fungal diseases.

Meanwhile, some of the biggest plant breeding companies for major crops like corn, soybeans and wheat are investing in their own proprietary research as well as collaborating with Doudna and others.

For example, in 2015, DuPont and Caribou Biosciences jointly announced a strategic alliance, allowing both firms to cross-license their respective patent portfolios. As part of the deal, DuPont received exclusive intellectual property rights for CRISPR-Cas technology applications in major row crops, and non-exclusive rights in other agricultural and industrial bioscience applications.

The alliance between DuPont and Caribou involves a multiyear research collaboration with scientists from the two organizations focused on enhancing the breadth, versatility and efficiency of the core CRISPR-Cas toolkit. DuPont also has made a minority equity investment in Caribou to further strengthen the working relationship.

But Doudna and the folks at UC Berkeley aren't the only game in town when it comes to developing CRISPR and other precision editing tools. In fact, there are ongoing legal disputes over

who owns the patent to the technology – and some countries view the patent ownership differently than others.

In May 2012, Doudna and Charpentier respectively filed for patent claims in the U.S. In December 2012, a team led by bioengineer Feng Zhang from the Broad Institute of MIT and Harvard also filed patent claims. But the U.S. Patent and Trademark office reviewed Zhang's filing first because Broad paid for a fast-track review process. In 2014, the Patent and Trademark office granted the first CRISPR patents to the Broad Institute for showing that the CRISPR system could be used to edit more advanced, eukaryotic cells, including animal and human cells.

UC-Berkeley asked for an "interference proceeding" to re-assess and determine who was the first to invent the genome editing tool.

However, in February 2017, the US Patent Trial and Appeal Board (PTAB) again ruled that the Broad/MIT group could keep its patents on using CRISPR-Cas9 in eukaryotic cells. In essence, the PTAB said there is no interference between the two groups' patent claims because the patent issued to the Broad/MIT group is sufficiently different from that filed by the UC team, including Doudna and Charpentier who said they had first successfully demonstrated the technology.

The University of California is now appealing this decision. In a brief, the UC team asserted that the PTAB "ignored key evidence" and "made multiple errors" when assessing whether CRISPR/Cas9 gene editing in eukaryotes was an obvious extension of the UC invention.

In response, the Broad Institute said that PTAB's judgment was supported by substantial evidence and in full conformity with the law. **"We are confident the Federal Circuit will affirm the PTAB's judgment and recognize the contribution of the Broad, MIT and Harvard in developing this transformative technology," the Institute noted.**

Bioengineer Feng Zhang, Broad Institute

Further muddying the patent and legal environment is a recent decision by the European Patent Office (EPO) which revoked the first of several patents obtained by the Broad Institute, citing a lack of novelty.

That decision was heralded by ERS Genomics, which was co-founded by Charpentier.

"We have always felt confident that the Broad's patents would be overturned in Europe based on lack of novelty in light of the Charpentier/Doudna patent filings, but the simple confirmation of this technical deficiency in the Broad's priority claims made it unnecessary for the European Patent Office to pursue this line of questioning in their revocation decision," said Eric Rhodes, CEO of ERS.

"The technical fault was sufficient for the Patent Office to quickly come to a determination that the Broad claims to their earlier priority dates were not legitimate and therefore their claims lacked novelty in light of several publications which predated their allowed priority date."

For its part, the Broad Institute said the EPO's decision was based "on a technical formality" and "the decision does not involve the actual scientific merits of the CRISPR patent application."

"The Broad Institute will appeal the decision to EPO's Technical Board of Appeal, which is expected to use this case as an opportunity to review and resolve this international inconsistency, not just for CRISPR patents, but for a wider range of European patents and applications that originated as U.S. provisional applications."

Little wonder then that U.S. agribusinesses are keeping a foot in more than one camp, trying to make sure that innovations and licensing opportunities can continue in as many laboratories as possible.

Neal Gutterson, DuPont Pioneer

For example, DuPont Pioneer and the Broad Institute of MIT/Harvard reached an agreement to jointly provide non-exclusive licenses to foundational CRISPR-Cas9 intellectual property under their respective control for use in ag research and product development at universities and nonprofit organizations for academic research.

"The promise of CRISPR-Cas9 technology in the hands of many will result in a wide array of benefits for the global food supply, ranging from higher and more stable yields of grains, fruits and vegetables for farmers; more nutritious, healthier and affordable foods for consumers; and improved sustainability of agricultural systems for society," said Neil Gutterson, vice president of Research and Development at DuPont Pioneer.

Other major agribusinesses are taking similar approaches, with multiple licensing agreements and investments in precision breeding.

Early in 2017, Monsanto Company announced that it had reached a new global licensing agreement with the Broad Institute of MIT and Harvard for the use of the novel CRISPR-Cpf1 genome-editing technology in agriculture.

"This system has potential to be a "simpler and more precise tool for making targeted improvements in a cell's DNA when compared to the CRISPR-Cas9 system," the company said in a release. "In addition, the smaller size of the CRISPR-Cpf1 system provides researchers with more flexibility to use the genome-editing technology across multiple crops."

"Gene editing actually covers a range of technologies that can be used to make precise targeted modifications of genes in the genome," explains Dr. Larry Gilbertson, a molecular biologist with Monsanto. "We believe that to be successful in this area it will require a combination of technologies and tools and our licensing strategy reflects that."

Other recent Monsanto investments and joint agreements include:

- A non-exclusive global option and licensing agreement on Dow AgroSciences' EXZACT Precision Technology. The EXZACT technology, which Dow AgroSciences has developed under an exclusive license and collaboration agreement in plants with Sangamo BioSciences Inc., (now Sangamo Therapeutics Inc.) facilitating the creation of crop varieties and lines having improved traits. An agreement with Nomad Bioscience GmbH, based in Munich, Germany, whereby Monsanto obtained rights to apply Nomad's proprietary technology to its genome-editing projects. The licensed technology enables more efficient development of edited traits and may be applied across a broad range of genome-editing technologies and project types, Monsanto said.

- An exclusive license to TargetGene's novel and proprietary "T·GEE" (Genome Editing Engine) platform to deliver continuous improvements in agriculture. Monsanto also established an equity position in the private Israel-based company.
- A global licensing agreement for the use of South Korean-based ToolGen's CRISPR technology platform to develop agricultural products.

"It's one thing to be able to edit but another thing to know what to edit and where to get the most value," adds Gilbertson. "And that requires a lot of knowledge of the genes, the genomes and having the information and the data science. That's where we have a lot of strength."

Plenty of precision breeding innovation

Given all of the various investments and licensing agreements, it's difficult to identify just one industry leader at the top of the precision breeding space – partly because investors range from small individual start-ups to large agribusinesses which may be investing or partnering in start-ups and technology companies on their own.

For example, Syngenta expanded its own direct investment vehicle, Syngenta Ventures, in 2009, to become one of the first venture capital teams dedicated to the agricultural space. Monsanto launched its own Monsanto Growth Ventures to invest in promising new ventures dealing with agricultural productivity, digital agriculture and biologicals. And Bayer's LifeScience Center (BLSC), operates as a strategic innovation unit within the company to uncover, encourage and activate fundamental breakthroughs by creating or partnering with other companies.

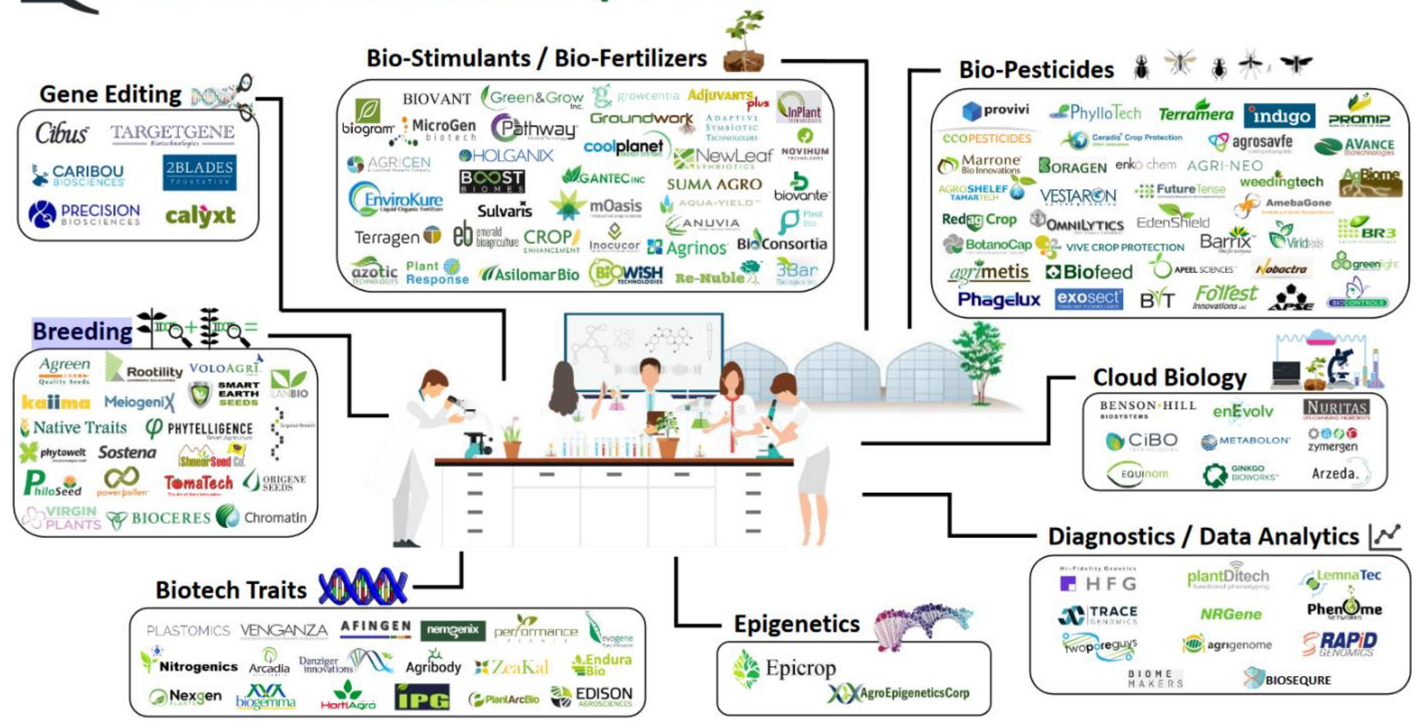
Jim Carrington, president, Donald Danforth Plant Science Center

"Here's the thing about gene editing. Everybody is using it. It is a ubiquitous tool in any company that is doing crop improvement. It's a ubiquitous tool in all research institutes and universities that work on or have a focus on plant science," said Jim Carrington, president of the Donald *Danforth* Plant Science Center in St. Louis. "But everyone uses it, because the tools are available to everyone and they're simple to use. And they work really well. So, it's hard for me to say who the leaders are."

Several venture capital and research firms are tracking the leading firms and their interrelationships. For example, TechAccel is a venture and technology development organization which tracks both the plant and animal investment landscapes. As can be seen from the diagrams below, gene editing is just one part of a much larger "pie" of technological advancements underway.

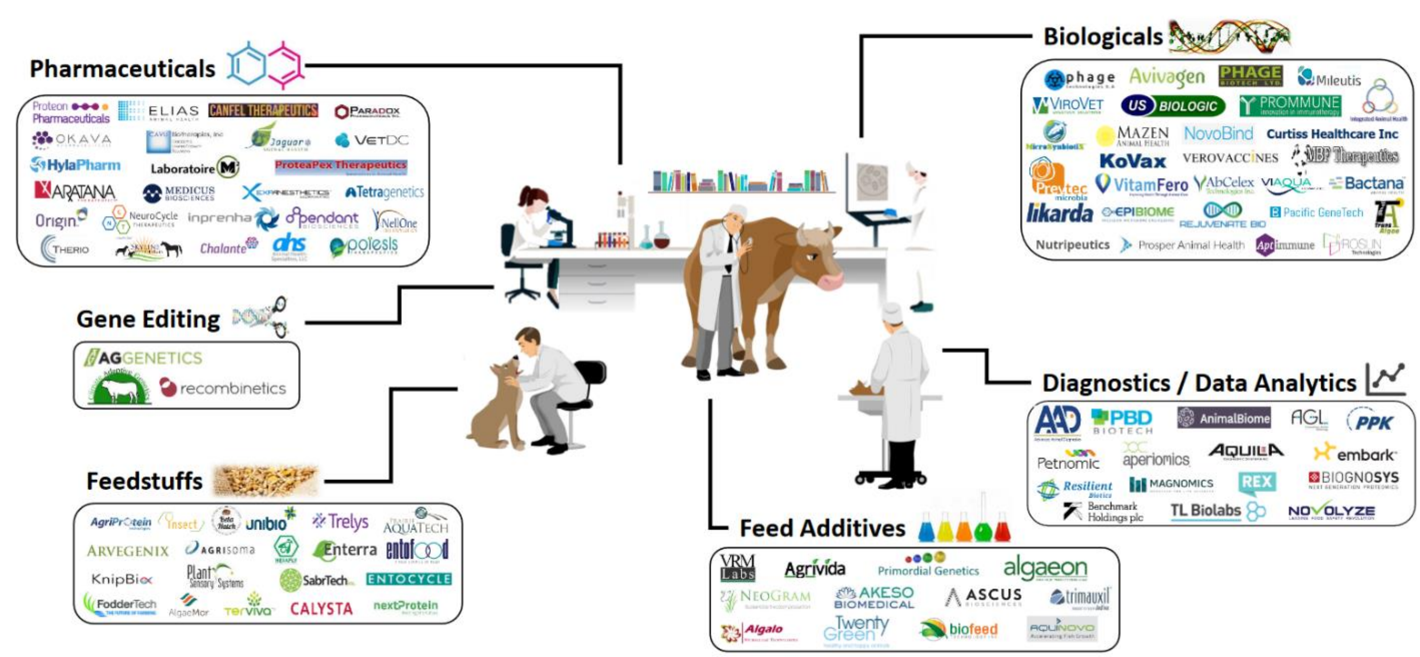
Plant Biotech Landscape 2017

TechAccel Technology Acceleration Partners



Animal Biotech Landscape 2017

TechAccel Technology Acceleration Partners



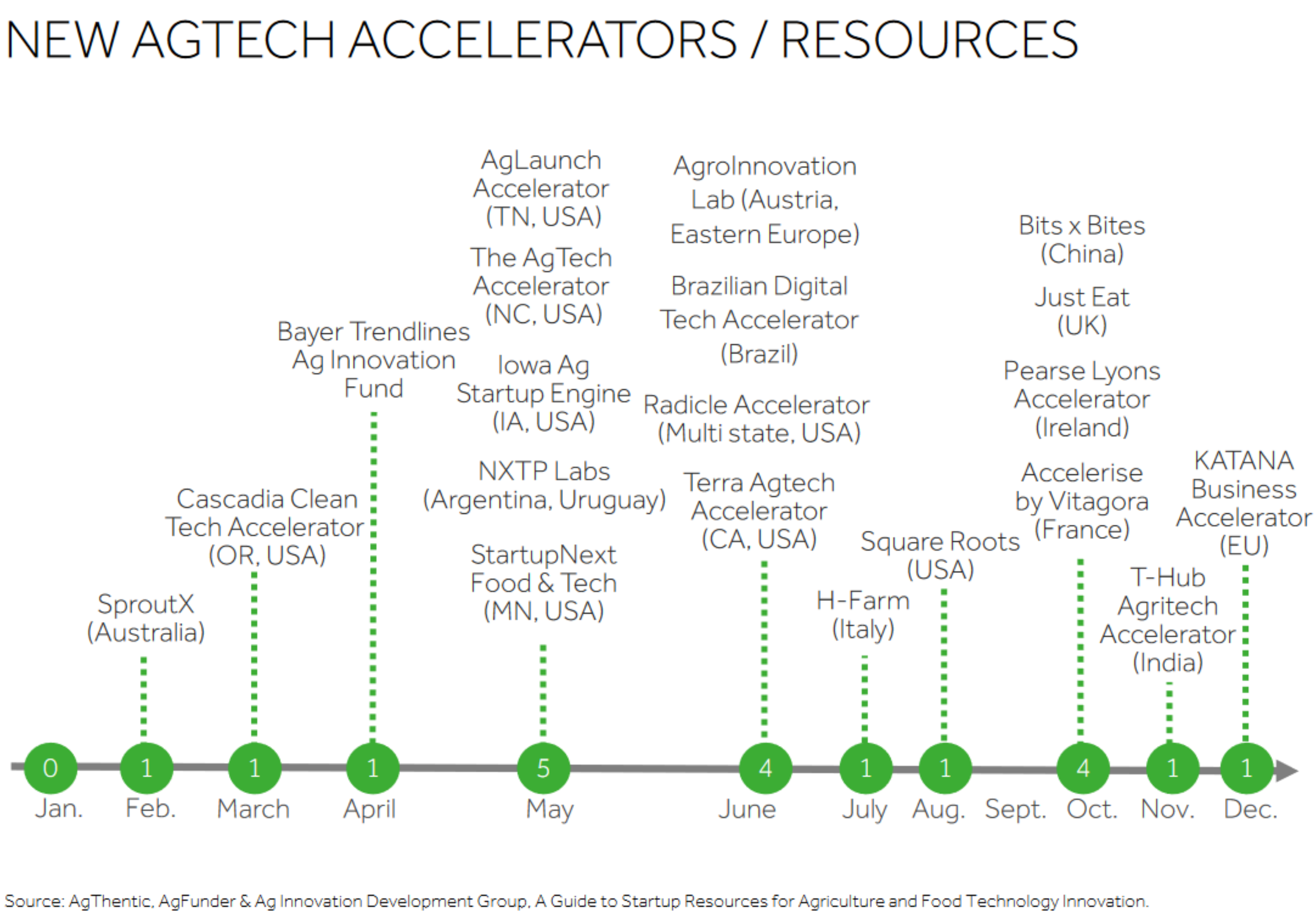
Even though there's no shortage of investors and firms looking to see which technologies are likely to deliver the next financial blockbuster, there have been shortages of investment funds for ag tech at some entry levels, said TechAccel's Brett Morris. It's a funding gap that he noticed early in 2017, when he was approached by several CEOs seeking additional funding for their start-ups.

TechAccel's Brett Morris

Morris said he noticed agtech-angel/seed funding was recently on the rise, while more valuable Series A funding showed a sharp decrease in 2016 from the previous year.

"This is nothing out of the ordinary for venture professionals, as we see hundreds of deals come across our desk annually. These conversations, however, were different from the previous year. Most of the entrepreneurs were struggling to find investors with an appetite for early-stage risk," Morris wrote in a blog for GlobalAgInvesting.com.

"More start-ups competing for a limited amount of funding at the next stage presents a problem for many founders," he added. There were also more entities helping start-ups get established, as AgFunder reported in their 2016 Annual Report (See below).



Another issue: Morris said that "generalist investors and individuals tend to shy away from early-stage risk if they don't understand the industry or underlying science.

"If generalist funds are hesitant to back a play because they don't understand the science behind it, then companies that can perform financial and scientific due diligence have an advantage," Morris said.

"Corporate venture models, such as Monsanto Growth Ventures and Syngenta Ventures, can efficiently de-risk opportunities because they have large R&D departments and scientists to help with diligence. They also have large-scale plots of land to perform high-powered statistical proof-of-concept studies to validate technologies. And by providing exit opportunities and ongoing business relationships with the start-ups in which they invest, these corporate ventures are able to further reduce risk."

Who has the IP?

Another technology research firm that specializes in patent licensing and litigation, iRunway, recently analyzed 4,336 IP (Intellectual Property) assets in CRISPR filed over the last 20 years across the globe, identifying leading patent holders and focus areas of development.

In iRunway's recent report, they found that research related to genome editing involving zinc finger nucleases (ZFNs) and TAL effector nucleases (TALENs) remained the primary area of focus until 2012. But that changed with new research and publication of three studies in January 2013 – demonstrating CRISPR-Cas9 to be an efficient tool to edit the genomes of human cells. That's when research involving CRISPR Cas9 became a revolution, iRunway wrote.

Research publication and patenting filing activity describing CRISPR gene editing increased by leaps and bounds after those studies were published but has declined in the last two years.

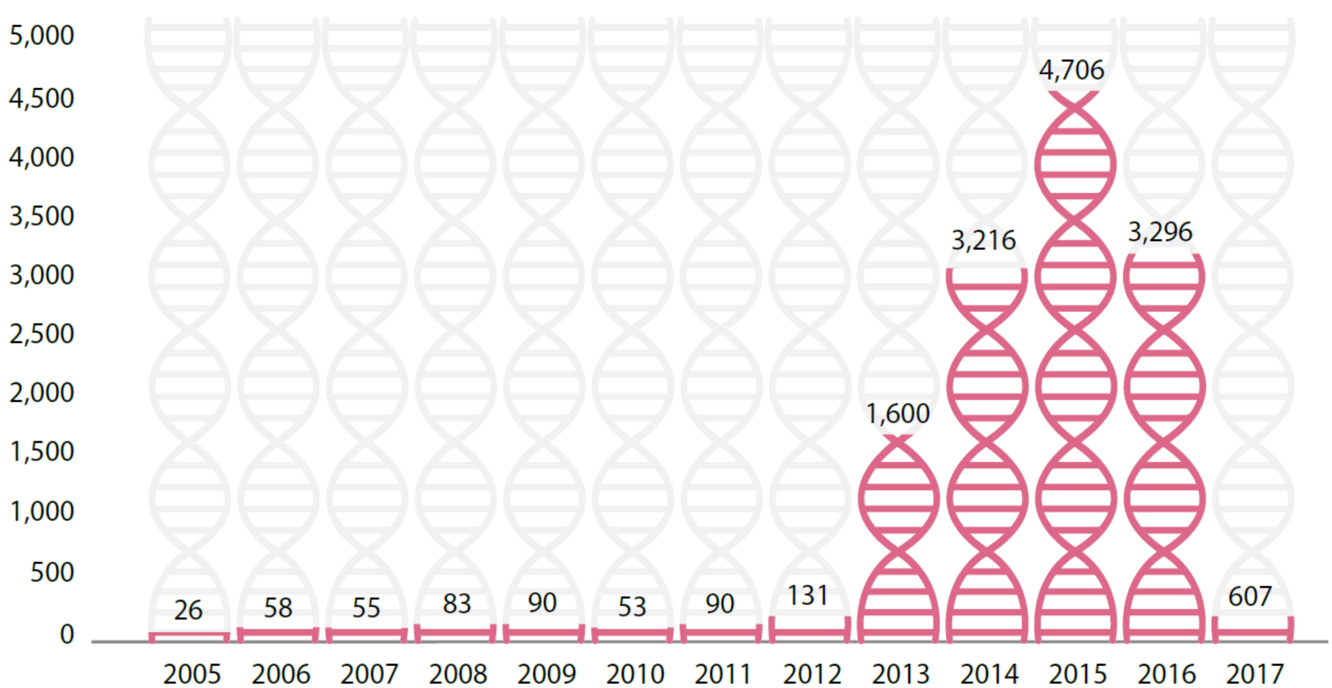


Figure 4: Patent Filing Trend in CRISPR
(Source: iRunway Analysis based on patent data from Innography.)

iRunway's study also found DowDuPont leading the CRISPR global patent landscape with 514 IP assets. This includes 216 patents filed by Dow Agrosciences (a wholly owned subsidiary of Dow Chemical Company). DuPont filed 122 IP assets, while its subsidiaries Pioneer Hi-bred International Inc. and Danisco filed 82 and 69 patents, respectively.

Harvard University stood second with 343 IP assets, of which it filed 231 patents independently and 112 patents filed in collaboration with Broad Institute, with most of its patents co-assigned to Massachusetts Institute of Technology and Harvard University.

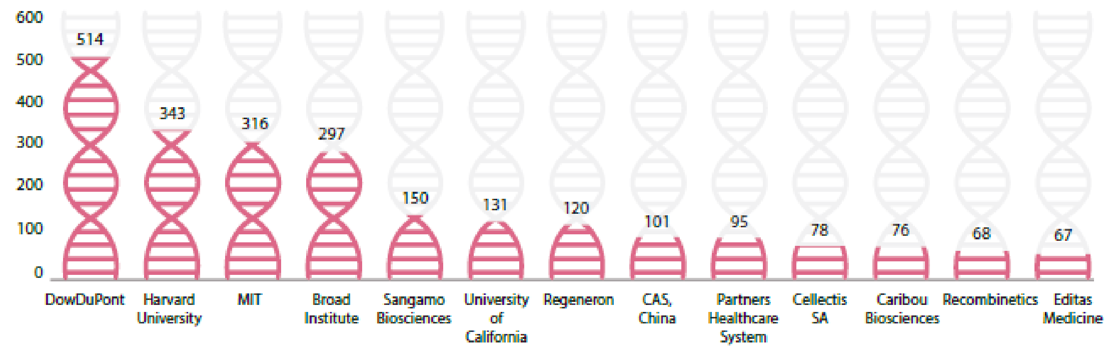
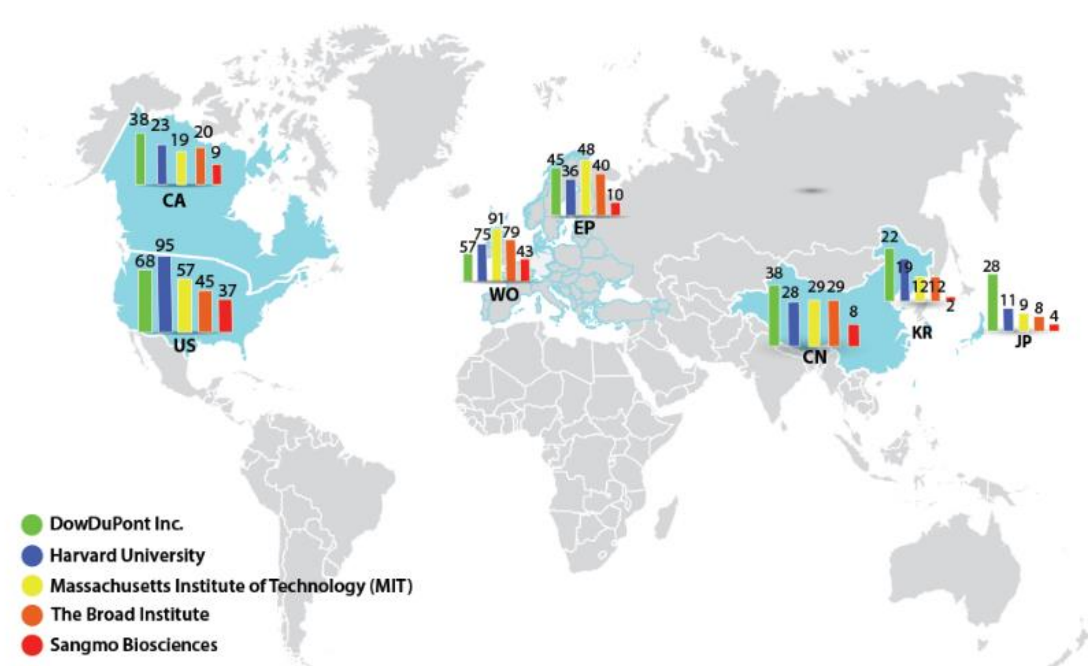


Figure 13: Leading Assignees of CRISPR Patents
(Source: iRunway analysis based on patent data from Innography.)

The report also noted that the technology areas of focus of all the leading assignees was very diverse. While DowDuPont has focused its research in discovering bacterial sources for CRISPR array, a large share of Broad Institute's R&D is regarding engineered guide RNA and delivery vectors. And there is a lot of diversity of IP assets across the globe.

Distribution of CRISPR Patents of Top 5 Assignees in Leading Patent Offices



And when it comes to the inventors who are leading the CRISPR revolution, iRunway reported that the Broad Institute's Feng Zhang is clearly ahead in terms of intellectual property assets, with 355.

These inventors obviously have a head start, but many others are expected to jump into the precision breeding community.

Citing market research, iRunway predicts that the genome editing/genome-engineering market will double to $6.28 billion by 2022 from $3.19 billion in 2017, at a compound annual growth rate of 14.5 percent.

Inventors	Assignee	No. of IP Assets
Feng Zhang	Broad Institute	355
Steven Robert Webb	DowDuPont	150
Phillipe Horvath	DowDuPont	126
Lakshmi Sastry-Dent	DowDuPont	124
Le Cong	Broad Institute	113
Rudolphe Barragou	DowDuPont	100
Zehui Cao	DowDuPont	99
Christophe Fremeaux	DowDuPont	90
Dennis Romero	DowDuPont	82
Andrew Paul May	Caribou Biosciences, Inc.	80
Jennifer Doudna	University of California	71

Table 2: Leading Inventors of CRISPR Technology
(Source: iRunway analysis based on patent data from Innography.)

"This will be driven by rising government funding, university research, growth in the number of genomics projects, quest for finding sustainable treatment for infectious diseases and cancer, increasing production of genetically modified crops and growing application areas of genomics," iRunway's report noted.

But others aren't so sure that U.S. government funding will play much of a role. Already, U.S. federal investment has been declining while countries like China are dramatically ramping up their research efforts.

"America is still ahead of the game, but we are reaping the investments made over the last many years," said NIFA's Ramaswamy. "The Chinese invest between 5.5 and 6 percent of their gross domestic product (GDP) on public research. In the ag and food space in America, it's less than 2 percent of GDP. So, the Chinese and others are surpassing us."

That's a trend that many land grant universities and NGO's hope to reverse in the upcoming farm bill debate, as they advocate for more federal research dollars. Last fall, over 60 organizations signed a consensus document, calling for an annual $6 billion goal for USDA food and agricultural research over fiscal year 2019 to 2023.

"The U.S. has been second to China in total public agricultural research funding since 2008. By 2013, China's spending on public agricultural R&D became nearly double that of the U.S.," the letter noted. "Though public funding for other forms of domestic research has risen dramatically,

the U.S. agricultural research budget has declined in real dollars since 2003. This is an area of R&D where return on investment is estimated at 20 to 1."

Carrington, with the Danforth Center, agreed that more federal funding is needed for the U.S. to remain competitive.

"Back to the middle of the 20th century, research and development was largely in the public domain," said Carrington. "Now, while there still may be some public activity in corn improvement, most of the corn is going to be bought from the big agribusiness companies.

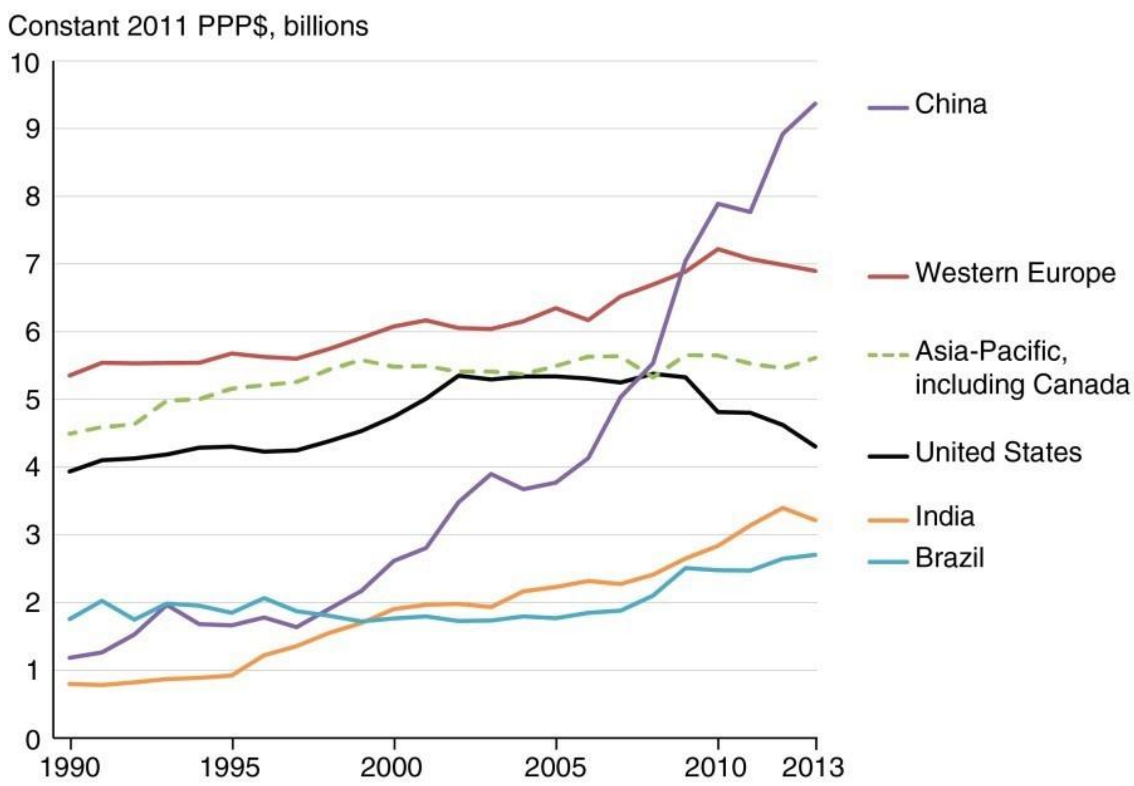
"It's really a shifting landscape of roles," he added. "At the very fundamental research level, it's still being done in universities and research institutions like the Danforth Center.

"But then in turning those innovations into actual products that can be sold, the role of the private sector has really increased."

Yet, Carrington said he doesn't want to "give the impression that there's not an extremely important role for R&D at the federal level.

"Ultimately that's what fuels innovation on the farm."

U.S. public sector funding for agricultural R&D falls as spending by China and India rises



PPP = purchasing power parity.
Source: USDA, Economic Research Service and Agricultural Science and Technology Indicators (ASTI), Organisation for Economic Cooperation and Development.

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