Mastering the Science of Genome Editing

WHERE IS CORTEVA AGRISCIENCE TODAY AND WHAT DOES THE FUTURE HOLD?

A Q&A WITH KEVIN DIEHL,
GLOBAL GENOME EDITING REGULATORY ADVOCACY DIRECTOR

In 1787, Thomas Jefferson, then U.S. minister to France, wrote that, "the greatest service which can be rendered any country is to add a useful plant to its culture." Jefferson, a lifelong scientist-farmer, advocated for the development of new tools and techniques to advance plants, and agriculture as a whole.

Corteva Agriscience scientists devote resources to expanding their toolbox to develop valuable new seed products for customers. Genome editing is helping Corteva deliver on that promise.

To help us better understand the science of genome editing and to get the Corteva perspective, *Groundwork* recently spoke with Dr. Kevin Diehl, Global Genome Editing Regulatory Advocacy Director, Corteva Agriscience. In his work, he engages with regulators, legislators, customers and industry and advocacy groups to help them better understand the innovative science fueling Corteva's corn and soybean genetic improvement. Kevin holds a PhD in plant physiology and weed science from the University of Illinois and is a 30-year veteran of Corteva.

Q Plant genetics and breeding have come a long way since Jefferson boasted of small grain yields of "15 to 20 bushels per acre." Can you describe the historical backdrop that's brought us to today?

A Seed people know that genetic improvement of maize and other crops dates back thousands of years. This technique – selecting and retaining the best plants – eventually helped bring us modern, high-yielding corn hybrids 100 years ago. Little did the ancient agriculturalists, or even their early 20th century peers, know they were making DNA selections.

The 20th century trailblazer Henry A. Wallace, founder of the Hi-Bred Corn Company, later Pioneer Hi-Bred International, advanced these scientific breeding and selection methods. Then, in the late 1980s and early 90s came a game changer – biotechnology. This allowed for adding specific, desirable genes into a plant. The genes could come from other species of plants and even microorganisms. This science was using "transgenes" (genes from other species) and it enabled development of improved crops with herbicide tolerances and insect pest resistance. We still use these methods today, and they remain very important. In addition, genome editing, with its famed CRISPR tool that pioneered in 2012, now lets us modify a plant's own DNA in a targeted, precise and efficient manner without introducing any transgenes to the crop.

Q What is genome editing? How does it work, and what makes it more precise and efficient?

A Genome editing is a set of tools that allows for the modification of a plant's own DNA. Plant DNA (also called its genome) is comprised of numerous building blocks – nucleotides – that form genes controlling various biological functions. There are roughly three billion nucleotides in the corn genome alone. Changing the sequence of nucleotides (for example, deleting or substituting certain nucleotides) can lead to disabling, increasing or otherwise changing the corresponding gene's function.

Genome editing allows for specific changes to be made in the DNA in a precise location in order to create a desired outcome. But being able to make these changes is only part of the science. Genome editing is grounded in an understanding of the plant's DNA and the function of its individual genes. Continuously accumulating a vast amount of knowledge is largely responsible for the development of DNA sequencing technologies – powerful tools that "zoom" into a genome's nucleotide sequence and compare plants with different nucleotide sequences in the same gene. This foundational knowledge is also a significant part of Corteva Agriscience's research efforts and is key to making the science work for our customers and, ultimately, the entire food chain.

Q How does genome editing compare and contrast with other methods?

A As a modern biotechnology method, genome editing requires laboratory assembly of genome-editing tools and their delivery into the plant through genetic transformation — much like the original biotechnology to develop transgenic crops (commonly called "GMOs" or genetically modified organisms). However, genome editing tools do not have to stay in the plant. Once DNA editing is completed, they can be removed by various methods. This means that, unlike transgenic biotechnology, genome editing yields plants that do not carry "foreign" DNA. In that respect, they are similar to conventionally bred plants. DNA changes from genome editing are similar to changes that occur spontaneously in plants naturally or can be obtained through conventional breeding techniques, albeit in a much less precise and efficient manner.

Likewise, development of genome-edited crops is more streamlined as compared to development of GMO (transgenic) crops, because the precision and efficiency of the process helps save several breeding and evaluation cycles. Another element to consider is the cost and timeline associated with the regulatory approvals of GMO crops. This cost is significant and continues to grow, despite the massive scientific evidence of their safety. The number of governments around the globe that acknowledge the similarity between genome-edited crops and conventional crops continues to grow. That means that genome-edited crops are not "GMOs" from the regulatory perspective, but are as safe as conventional crops, and the same regulatory rules should be applicable to genome-edited and conventional crops.

Q Are genome editing and gene editing the same thing?

A "Gene editing" and "genome editing" terms are used in scientific literature and media interchangeably. The way we prefer to differentiate these terms at Corteva is based on the target (scale) of edits that are being made. For example, if DNA editing modifies the function of a single gene or just a few genes — this is gene editing. Today, we can also make edits that cover stretches of DNA far larger than one or a few genes. For example, inverting part of the chromosome or simultaneously editing multiple genes located in various parts of the genome. This would be more accurately described as genome editing. Regardless, the fundamentals of gene or genome editing are the same — targeted and specific changes to a plant's own DNA.

Q Today, we hear lots of talk about CRISPR-Cas9. Is it the same thing as genome editing?

A Genome editing is a process, and CRISPR-Cas9 is a tool used to perform that process. Here's an analogy: Surgery is a process; a scalpel is a tool used to perform surgery.

So, what type of genome-editing tool is CRISPR-Cas9? It's a tandem of two actors: first, a protein enzyme (Cas9) that functions as "molecular scissors" to make a break in a plant's DNA, and second, a small molecule called "guide RNA" which is complementary to the part of DNA targeted for cutting and helps bring the Cas9 enzyme there. In other words, guide RNA is responsible for the location of the DNA break, while Cas9 does the actual job. As science continues to advance, new Cas enzymes are being discovered that give our scientists the capability to edit nearly all the DNA sequences in a plant.

You might ask, "And what about CRISPR?" CRISPR is a complex acronym referring to the native immune system. It was discovered in bacteria to fight viral infections and is where the CRSPR-Cas9 genome editing system originates. This bacterial system, based on the principle "find and cut," has been repurposed to become a genome editing tool for plants, animals and various applications in medicine.

Q Where does Corteva focus its genome editing efforts? Which crops?

A Corteva science is focused on corn and soybeans as our primary crops. As an aside, many people know Corteva is the #1 developer of genetics for these species and that Corteva's 5000-plus-member R&D team is driven to remain at the top. Our extensive genome editing work is focused on doing just that.

Q How does the genome editing process work?

A Let's start with the basic types of DNA modifications that can be done with genome editing: gene deletion, gene editing and gene addition. Cas9 enzymes make DNA break at a targeted location of interest. DNA breaks happen in plant genomes all the time as a result of various biological processes. But DNA cannot remain cut, so it is repaired by one of the plant's native DNA repair mechanisms. The exact same native repair mechanisms are relied upon in genome editing. The mechanism of repair also determines the type of DNA modification.

With gene deletion, a scientist can "knock out" a gene. In doing so, this causes something to *not* happen. An example would be where two DNA cuts are made to remove a gene that makes a certain protein so that the protein isn't produced. Corteva was the first company to develop CRISPR-Cas9 gene-edited corn — waxy corn — where our scientists removed the gene responsible for production of the enzyme involved in starch biosynthesis so that it changes the composition of cornstarch.

Editing can also take the form where the plant is given instruction on how to change a DNA sequence during the DNA repair to produce a desired result. An example would be replacing the disease-sensitive form of the northern-corn-leaf-blight-resistance gene with the disease-resistant form of the same gene.

The next level of complexity is gene addition. For example, we can use genome editing tools to add native disease resistance genes to enhance plants' ability to fight the respective diseases.

Over the past decade, advancements have been made to allow for higher efficiency and even greater precision in genome editing. Simultaneously, considerable efforts are being made to identify genes of interest and understand their functions at a very detailed level. What I just described are the basic, original applications of genome editing — Genome Editing 1.0. Today, Corteva scientists are already at the front lines of Genome Editing 2.0, where we are able to operate with larger parts of genome while still replicating the native types of DNA modifications that spontaneously occur in plants. It's a very exciting time to be a witness to this scientific progress!

Q Farmers and even some consumers know that GMO biotechnology has been tailored to develop crops with insect pest resistance and herbicide tolerance. Is that the extent of what can be done through genome editing?

A Those two achievements were the first and the major areas where biotechnology produced beneficial GMO traits for the farmer. Corteva continues to do work in these areas, and here's how things seem to be shaping up.

Purely from a technology standpoint, genome editing is not limited to what types of traits could be developed. But since genome editing relies on the power of a plant's own DNA, a potential bottleneck is in whether the plant genomes are sufficiently equipped to provide such traits without a need to resort to transgenic sources. This could be a question of plant biology — but could also be a matter of the limits of today's knowledge.

So far, we are not seeing corn and soybeans possessing much native DNA for robust insect resistance. As a result, insect resistance work will likely continue to be done with transgenes.

When it comes to herbicide tolerances, plants do contain some native herbicide-tolerance genes that could be edited. Therefore, the future of herbicide tolerance could involve a combination of genome editing of native DNA and transgene addition.

Disease resistance is present naturally within plants, so it looks like new traits could be available from within a species. This is the case with northern corn leaf blight (NCLB). NCLB resistance was found in an inbred in Brazil and many generations of back-crossing brought it into today's hybrids. Going forward, gene editing could speed up this work. Multiple native disease resistance genes can also be incorporated into a plant at the same time, and such work is ongoing at Corteva. Things get even more exciting in frontiers beyond pest resistance and herbicide tolerances. And Corteva is exploring these in its genome editing work in several ways.

Q Any closing thoughts?

A We at Corteva are very excited about technical opportunities for genome editing for developing new varieties that benefit farmers and consumers. However, any scientific innovation can make a meaningful societal difference only if it is supported by two other fundamental enablers: appropriate regulatory policies and public acceptance of the technology. Therefore, the focus of our company is not only to continue mastering the technology, but also to use our world-class expertise to advocate for risk-proportionate regulatory frameworks and engage with diverse value-chain stakeholders in addressing their questions or concerns and promoting the benefits of genome-editing technology.

The overarching goal of Corteva is to continue to be an innovator and provider of valuable seed technologies over the long term. This means bringing new ideas and technologies forward to address the needs and desires of Corteva customers: licensees, farmers and consumers. We're out applying our scientific capabilities to solve production problems, address changes in climate and growing environments, and, ultimately, develop seed technologies that produce more stable and profitable yields regardless of the farming environment. New R&D methods such as genome editing will help us achieve these results.