CRISPR-Cas Advanced Plant Breeding

By Jeffry Sander, Ph.D.¹ and Mark Jeschke, Ph.D.²

Summary

- Many bacteria protect themselves from invading viruses by using adaptive immune systems called CRISPR-Cas. These CRISPR-Cas systems can recognize genetic sequences specific to that of invaders and cut them.
- CRISPR-Cas can be repurposed as molecular scissors to make cuts at specific locations in a plant genome. Subsequent repair of the cut by the cell’s endogenous repair mechanism can introduce precise changes at the specific cut site.
- CRISPR-Cas as an advanced plant breeding tool represents tremendous promise and potential. It can facilitate precision crop breeding by working with the native characteristics available within the crop, a process often called “genome editing”.
- CRISPR-Cas can also be applied to produce transgenic crops.
- DuPont Pioneer is a world leader in CRISPR-Cas advanced breeding applications in agriculture and is developing its first CRISPR-Cas enabled commercial product, a next generation of waxy corn, to be the pioneer agricultural product on the market developed with CRISPR-Cas.
- Pioneer welcomes the opportunity to collaborate with others to realize the full potential of the CRISPR-Cas advanced breeding technology. As an example, it has established a public-private partnership with the International Maize and Wheat Improvement Center (CIMMYT) to apply CRISPR-Cas technology to address the needs of smallholder farmers around the world.

Introduction

Throughout its over 90-year history, Pioneer has been a leader in driving increased agricultural productivity through crop improvement. Following its founding in 1926, what was then known as the Hi-Bred Corn Company led a revolution in corn breeding that used hybridization to dramatically increase yields. With the introduction of agricultural biotechnology in the 1990s, it was demonstrated that desirable traits from non-native sources could be introduced into crops species. For example, the introduction of Bt traits from soil bacteria provided corn the ability to protect itself from damaging pests, thus improving the quantity and reliability of corn yields. Now, breakthroughs in the field of genome editing are bringing forth a third revolution to crop improvement to be used alongside existing technologies. Genome editing is the process of making targeted and precise changes to the strings of DNA made up of sequences of four nucleobases (cytosine, guanine, adenine, and thymine) that comprise genes and other genomic features determining plant characteristics and diversity. While the actual implementation of CRISPR-Cas is more complex, it is conceptually similar to editing a text document using a word processor. In this analogy the CRISPR-Cas tool is the cursor that can be pointed to the desired location within the text. Placement of this cursor enables one to delete, change or insert letters or even words at the selected location thereby improving the text. In the same way a plant’s own genetic sequences can be targeted using CRISPR-Cas and purposefully changed to provide desired characteristics. This ground-breaking technology is expected to help scientists to develop innovative and sustainable solutions for growers similar to those realized through conventional plant breeding practices, but with even greater quality, accuracy and with more efficient development timelines.
Much of the excitement in genome editing is centered around CRISPR-Cas, which has been rapidly adopted due to its advantages over other genome editing tools in quality, efficiency, and technical flexibility. CRISPR-Cas has many potential applications extending well beyond agriculture, and has garnered wide mainstream media attention in the past few years as research in this area has exploded (Figure 1). Pioneer is the agricultural industry leader in CRISPR-Cas advanced plant breeding and has announced intentions to bring the first commercial agricultural product developed through the application of CRISPR-Cas to market within five years, pending field trials and applicable regulatory reviews. The purpose of this Crop Insights is to provide a brief overview of the history of CRISPR-Cas, its use as a genome editing tool, and how Pioneer is using this technology to facilitate a new era of crop improvement.

**Figure 1.** CRISPR-related scientific publications in PubMed, 2005-2016 (as of 12-9-16, www.ncbi.nlm.nih.gov/pubmed).

### The History of CRISPR-Cas

The initial discovery of what would later become known as CRISPR-Cas was reported in 1987 by researchers in Japan studying a region of the *E. coli* genome. They identified the presence of five identical DNA sequence repeats separated by identically sized non-repetitive DNA sequences. At the time it was noted as a curiosity, but left unexplained (Ishino et al., 1987). As more genomes were sequenced, these repeat features were observed in numerous species of bacteria, including the bacteria used to make yogurt and cheese, and bacteria residing naturally in the human gut. To add to the mystery, these regularly interspaced repeats were observed to be accompanied by a common group of genes. The repeat regions were eventually named “CRISPR”, an acronym for “clustered regularly interspaced short palindromic repeats”, and the accompanied genes were labeled as “Cas” genes, short for “CRISPR-associated genes” (Jansen et al., 2002). Eventually, it was realized that the sequences between the repeats shared identity with viruses known to infect those bacteria, akin to an immunization record, and that some of the associated Cas genes encode protein domains known to cut DNA.

Because bacteria were well-known to employ proteins evolved to cut the DNA of invading bacteriophages (viruses that infect bacteria) it was quickly hypothesized that CRISPR was somehow assimilating sequence from the genomes of these invading viruses into the spacers between the repeats and then using those spacer sequences and Cas proteins to cut DNA of infecting viruses. A research team at Danisco (acquired by DuPont in 2011) led by Philippe Horvath provided the first biological evidence that CRISPR-Cas constitutes an immunity system against viruses in bacteria. The research conducted by this team demonstrated that strains of the yogurt-making bacteria *Streptococcus thermophilus* that survived viral infection had incorporated sequences from the invading viral genome into their CRISPR loci. After that, those strains of *Streptococcus thermophilus* became resistant to subsequent infection by that virus (Barrangou et al., 2007). This breakthrough built upon previous pioneering work by Horvath and colleagues in the early 2000s that initially utilized CRISPR for bacterial identification, then for its ability to improve the resistance of starter culture strains against bacteriophage attack.

Over the years, numerous CRISPR-Cas systems with distinct characteristics have been identified. However, it was a particular series of discoveries in 2011 and 2012 that led one of these CRISPR-Cas systems to the forefront of the genome editing revolution. Through these discoveries, three components of this system were characterized as both necessary and sufficient to specifically recognize and cut DNA, the molecule that makes up genomes and encodes the instructions for life (Figure 2).

**Figure 2.** Illustration of a naturally-occurring CRISPR system. Viral DNA sequences are incorporated into bacterial CRISPR arrays, which then produce crRNAs that are complementary to the foreign DNA site, crRNAs hybridize to tracrRNAs and associate with the Cas9 protein. The combined complex recognizes and cuts foreign DNA that matches the sequence previously incorporated into the CRISPR array in bacteria.
The first component is a protein referred to as Cas9, a protein encoded by the Cas9 gene. In addition to harboring the mechanism responsible for cutting the DNA molecule it also recognizes a very short stretch of a nearby DNA sequence responsible for initiating the DNA recognition process. The second component is an RNA (a relative of DNA) originating from the CRISPR locus that contains the sequence specifying the DNA sequence to cut. This RNA is referred to as the CRISPR RNA (crRNA) and is responsible for binding to the target DNA. The third component is a second RNA (referred to as the tracrRNA) also originating from the CRISPR locus and serving as a link for associating the crRNA sequence with the Cas9 protein (Deltcheva et al., 2011).

In 2012, several scientific journals published reports that these three components were sufficient to recognize and cut DNA and that the crRNA could be altered to recognize virtually any DNA sequence, from any organism. To simplify the system and improve its efficacy, it was determined that the crRNA and tracrRNA could be combined into a single RNA (Figure 2). This easily programmable two-component RNA-guided Cas9 system is the most widely used CRISPR-Cas tool today across all branches of the life sciences (Jinek et al., 2012; Mali et al., 2013).

CRISPR-Cas Facilitated Crop Improvement

The CRISPR-Cas protein, Cas9, facilitates genome editing by functioning as precise and programmable molecular scissors that cut the DNA at a specific location. Following the cut of the target DNA sequence, the CRISPR-Cas system takes advantage of naturally-occurring cellular DNA repair mechanisms to delete genes, edit genes, or insert genes. Higher organisms, including plants, continuously encounter DNA breaks caused by external sources such as sunlight as well as internal processes such as those that release free radical molecules. To endure, these organisms have developed efficient mechanisms for repairing the multitude of DNA breaks that occur in each cell every day. DNA repairs can be generally classified two ways: 1) non-homologous end joining and 2) homology directed repair (Figure 3).

Non-homologous end-joining, abbreviated as NHEJ, is the dominant DNA repair pathway in plants. It does not use a DNA template for the repair process and instead functions by simply identifying two broken ends of DNA and pasting them back together. This DNA repair process can often result in the insertion or deletion of random DNA sequences at the repair site. If the broken DNA sequence represented a plant’s gene, the function of this gene becomes disrupted and it is “deleted”.

Homology-directed repair, often referred to as HDR, requires a second unbroken strand of DNA that harbors sequence that is identical to that flanking the broken DNA as well as the desired change (edit, a specific and targeted alteration of the sequence, or additional genetic material to insert). It uses that unbroken DNA strand as a template to repair the DNA.

Deleting Genes

Genes can be deleted by targeting Cas9 to cut the desired gene. Repair by the NHEJ pathway can be used either to disrupt the DNA sequence that codes for genes or, in the case of two cuts flanking the gene, the entire gene can be removed. An example of such application is Pioneer’s next generation of waxy corn hybrids.

Assisted breeding

CRISPR-Cas enables direct transfer of characteristics among members of the same species, for example different corn inbreds. HDR can be achieved in a destination corn inbred by using a repair template derived from the DNA sequence of interest from a different corn inbred. This direct and targeted transfer of a desired corn characteristic (for example, stress resistance), and only that characteristic, using CRISPR-Cas is both more efficient than traditional plant breeding methods and avoids introducing any additional genetics of the source inbred. Because Pioneer also has the expertise to move characteristics directly into elite inbreds, breeding precision associated with this process provides the capacity to introduce characteristics directly into the newest elite inbreds and with less negative impact (e.g. yield drag) from the introgression process (Figure 4).

Transgenic Traits

CRISPR-Cas facilitated HDR can also be used to introduce characteristics from non-native sources (i.e., transgenes). The ability of CRISPR-Cas to do this in a targeted manner provides a significant advantage over transgene insertion methods used to develop the transgenic products currently on the market. CRISPR-Cas can be also used to co-locate several transgenes together so that they segregate as a single breeding locus, thereby substantially simplifying trait introgression process.
Improved Waxy Corn Products

In spring of 2016, Pioneer announced its first commercial agricultural product developed through the application of CRISPR-Cas advanced breeding technology – the next generation of waxy corn hybrids (Pioneer news release, 2016). These hybrids are expected to be available to U.S. growers within five years, pending field trials and applicable regulatory reviews.

Originally characterized in the early 1900s, waxy corn kernels contain >97% amylepectin starch instead of the normal ~75% in no. 2 yellow dent corn and is milled for a number of everyday consumer food and non-food uses (Figure 5). In the United States, about a half-million acres of waxy corn are grown each year. Pioneer is the leading supplier of waxy corn hybrids globally.

Waxy corn is an ideal product for implementation of CRISPR-Cas advanced plant breeding due to the challenges and limitations associated with developing waxy hybrids through conventional breeding. Current waxy corn products contain a partial deletion of the waxy gene responsible for amyllose production in the endosperm. Deleting the waxy gene leads to the disruption of amyllose production so that almost the entire grain starch becomes composed of amylepectin. Pioneer routinely breeds this form of disrupted waxy gene into new elite corn inbreds; however, this process takes several years and carries with it non-elite genetics as baggage. As a result, waxy hybrids carry a yield penalty compared to the most recent elite varieties. Using CRISPR-Cas advanced plant breeding, the waxy gene can be caned deleted entirely and directly in most current elite inbreds. This direct deployment of the waxy characteristic reduces the time necessary to create waxy hybrids and is expected to eliminate the yield drag associated with the introgression of the characteristic through conventional breeding.

Agricultural Applications of CRISPR-Cas Advanced Breeding

Pioneer is establishing a CRISPR-Cas advanced breeding platform to develop seed products for greater environmental resiliency, productivity, and sustainability. CRISPR-Cas has numerous potential agricultural applications including improvements to yield, disease resistance, and drought tolerance, as well as improvements beneficial to the end user such as output characteristics and nutritional content.

In a recently published research paper, Pioneer scientists describe the first application of CRISPR-Cas to improve a corn plant’s own ability to withstand drought stress (Shi et al., 2016). CRISPR-Cas was used to target a gene identified for its innate ability to promote drought tolerance. Field trials of the resulting elite corn hybrids exhibited an average 5 bu/acre increase in grain yield under water-limited stress during flowering, and no decrease in yield under optimal water availability. Additional trials are currently being conducted to determine commercial potential under a variety of environments. Other Pioneer publications related to CRISPR-Cas advanced plant breeding include reports demonstrating the efficiency and flexibility of the CRISPR-Cas system in both corn (Svitashev et al., 2015; Svitashev et al., 2016) and soybean (Li et al., 2015).
CIMMYT Collaboration

In fall of 2016, Pioneer and the International Maize and Wheat Improvement Center (CIMMYT) entered into an agreement to jointly develop improved crops using CRISPR-Cas genome editing for characteristics that address the needs of smallholder farmers around the world. The first project will apply the CRISPR-Cas tool to address maize lethal necrosis disease in Sub-Saharan Africa. First observed in Kenya in 2011, the disease spread to neighboring countries in less than five years and reduces maize production by an average 3 percent in dry areas and 32 percent in wetter environments, with up to 90 percent grain loss on some farms. In Kenya, maize lethal necrosis affects nearly a quarter of the total maize production, with annual losses representing approximately $52 million (Mahuku et al., 2015). Pioneer welcomes the opportunity to collaborate with others to realize the full potential of the CRISPR-Cas advanced breeding technology.

References


1 DuPont Pioneer Research Scientist - Molecular Engineering
2 DuPont Pioneer Agronomy Information Manager

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