IOWA SOYBEAN ASSOCIATION RESEARCH UPDATE

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CRISPR Technology:

This technology has been in the news a lot during the past year and has been touted for its potential to simplify and accelerate genetic modifications for applications in medicine, industry and agriculture. It might be fun for some to know that CRISPR stands for **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats, but knowing that factoid is really not helpful to most readers. The purpose of this article is to give a little bit of background on the technology and the potential utility – opportunities and pitfalls - for crop improvement and soybean production.

CRISPR in nature:

Like many biotechnology tools, CRISPR was invented by nature and then discovered, understood and developed for utilization by scientists. In nature, bacteria use CRISPR as part of a rather elegant defense system to protect themselves against invading viruses. The bacteria contain nucleic acids (RNA) and proteins that recognize and destroy an infecting virus' genetic material before it can be used to produce toxic proteins. While the system is rather complex, there are two key features that are important for our discussion here. First, the CRISPR-related bacterial nucleic acid (RNA) is complementary to and specifically recognizes certain virus RNA or DNA sequences. As such, the CRISPR-related RNA is able to attach itself to the invading 'foreign' virus nucleic acid. Second, when the CRISPR RNA attaches to the invading target nucleic acid, it 'guides' a CRISPR-related protein that also binds to the virus nucleic acid. Therefore, this RNA is called the guide RNA. The bacterial protein (Cas9 or similar) has specific enzymatic capabilities that enable it to cut the foreign nucleic acid and inactivate or destroy it. When a bacteria uses its CRISPR defense system, it blocks the virus infection and keeps itself alive and healthy.

CRISPR in biotechnology:

It is an over simplification, but acceptable for our purposes here, to say that "nucleic acids are nucleic acids" in their simplest form, whether they are viral, bacterial, plant or animal derived. In the world of biotechnology, scientists learned quite some time ago that naturally occurring gene modifying systems, like CRISPR, can be manipulated for our benefit. Once scientists studied and learned that the fundamental mechanisms of CRISPR involve nucleic acid sequence-dependent and site-specific recognition and cutting, they purified and characterized the CRISPR components and found that they could synthesize guide RNAs with sequences complementary to specific target nucleic acids in a plant or animal genome. When scientists introduce or express both the guide RNA and the protein enzyme (Cas9 or similar), they can program a system to cut nucleic acids, DNA in this case, at very specific and precise locations in order to modify that DNA in a desired way (Figure 1). Thus, CRISPR in biotechnology is essentially a tool to recognize and cut DNA at very specific locations for subsequent modification.

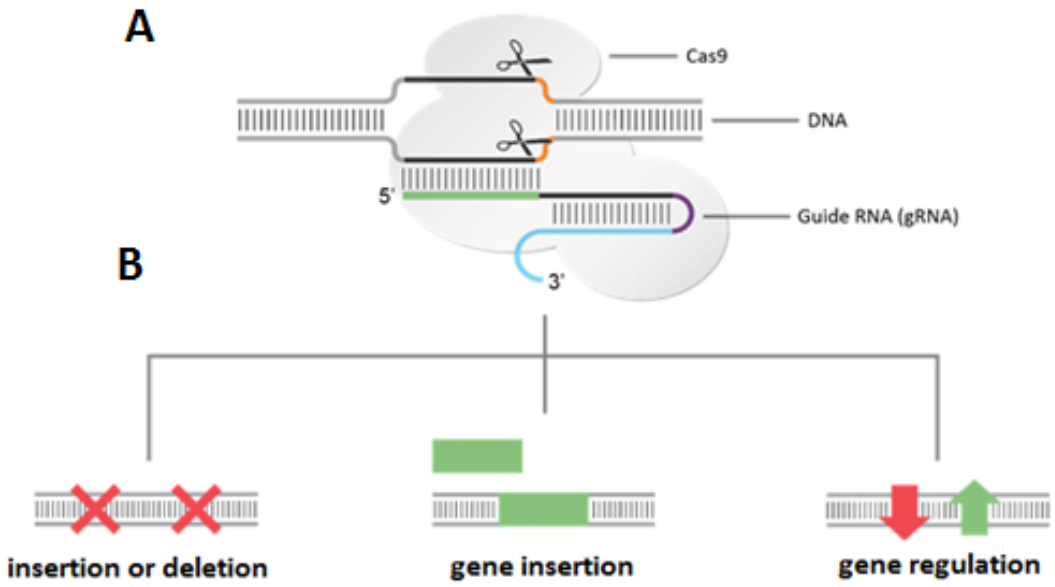

Figure 1A: CRISPR function for site-specific DNA modification. Cas9 is identified as the bacterial enzymatic protein that is guided to a specific site on the target DNA by the specific sequences in the guide RNA (shown in green). After the target DNA has been cut, other cellular processes work to repair the damage. **Figure 1B:** Small insertions or deletions (called *indels*) can be random or specific. If random, the mutation usually results in silencing or knocking-out (turning off) protein expression from that gene. If specific, the indel mutations may regulate gene expression (gene regulation) for more or less protein. Other specific indel mutations modify genes to produce proteins that have new and better functionality. Finally, if novel genes are provided they may be inserted (gene insertion) into the cut DNA during repair for the production of entirely new beneficial proteins.

Application of CRISPR to crop improvement:
Application of CRISPR as a biotechnology for crop improvement allows scientists to modify a target crop plant's genome (DNA) in very precise ways, provided scientists know the plant's genomic sequence and the genes that code for, or allow production of, specific proteins with known functions. When scientists know a gene's and a protein's function, they can interrupt, decrease, increase, modify, enhance or replace it for beneficial results (Figure 1). CRISPR may facilitate very efficient and site-specific gene editing for crop improvement.

It is important to understand that successful application of CRISPR for site-specific gene editing is predicated on years of complex and expensive genome sequencing along with gene discovery and functional characterization. Crop plant genome sequencing and characterization have been worked on for many years, mostly at universities and at some companies. If CRISPR technology had been known and characterized 10 or 20 years ago, it would not have been useful for crop improvement because scientist knew so little about crop plant genomes, their genes and the functions of the proteins that were produced from those genes. Scientists learn more about genomes, genes and proteins every day. Much of the basic research funded at universities by checkoff dollars, federal agency grants and

company investments make it possible to develop current and future knowledge and biotechnology tools for crop improvement.

When the crop plant's genes are sequenced and their functions understood, CRISPR can allow scientists to conduct site-specific gene modifications in several ways. The type of modification is dependent on the target DNA and natural cellular mechanisms for repairing damaged DNA.

Anytime that DNA is damaged or cut, the cell tries to reconnect it. When the CRISPR system cuts target DNA, the DNA may repair itself with a "deletion" or an "insertion". Generally, when a gene deletion or insertion occurs it is called a mutation because the DNA loses or acquires a very short piece of DNA. This usually results in a gene "knock-out" or gene "silencing", which simply means that the gene has been disrupted or destroyed so that it won't express a normal, functional protein. Occasionally, the deletion or insertion is non-lethal to the gene and it expresses a protein that may have a changed (improved or diminished) function. This may make the protein more or less functional and may be beneficial for crop improvement. In still other cases, the naturally occurring DNA repair system may insert a longer piece of DNA that has been introduced by the scientist for expression of a unique or new protein from a gene in a specific location in the genome for crop improvement. An example might be the site-specific insertion and expression of a protein for herbicide resistance from a genome location that is known to give optimum expression efficiency and levels, without negatively impacting other crop characteristics like yield or disease resistance.

Will crops modified by CRISPR be regulated?:
The short answer to this question is "it depends", or it may depend. Here's why. First, it will be up to the regulatory agencies to determine if any or how many of the CRISPR techniques and the derived plants will be reviewed and regulated. Second, it is important to know that, in most cases, plants have to be stably transformed (by the tools and techniques of biotechnology and genetic engineering) in order to get the guide RNA and the enzymatic cutter protein (Cas9 or similar) into crop plant cells for site-specific gene editing. If this is done, there will likely be regulatory review required. A lot of work is being undertaken to develop systems that will excise the CRISPR sequences from the plant genome after the specific modifications have been made. Other scientists are working with novel DNA delivery systems, like modified plant viruses, to introduce the CRISPR components to make the plant genetic changes but not to incorporate them into the plant's genome. In addition, the type of genetic modifications may determine the types and levels of regulatory review and approval. For example, and as discussed above and described in Figure 1, if the plant genetic material is simply modified by a small insertion or deletion to silence (knock out) a gene or mutate it in a way that will enhance its expression or function, there may not be as much regulatory review necessary prior to commercial development and release. If, however, the CRISPR techniques are used to specifically break the crop plant's genetic material and insert a new gene that will code for a novel protein, that process and the resulting plants will probably be carefully reviewed and regulated in ways that are similar to current biotech crops.

In Summary:
CRISPR is a potentially powerful and exciting new biotechnology tool that may be used for crop improvement by increasing efficiency, specificity and cost-effectiveness of site-specific gene modification (editing) and gene introduction. In soybeans, there have been some biological challenges

to adapting this technology but those are being addressed. Farmers should keep in mind that this technology, like all others, also has limitations. For example, CRISPR technology depends on a thorough understanding of the plant's DNA sequences and the proteins that are produced by those sequences. In addition, plants must be amenable to several genetic engineering processes, the expression of the CRISPR components and the resulting edited genomic sequences. Finally, review and regulation of CRISPR-derived crops will depend on the processes and products being submitted for commercialization.

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