CRISPR-Cas Technology for Plant Breeding

by

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Abstract

Clustered regularly interspaced short palindromic repeats, or CRISPR, is a relatively new gene-editing technology that has the possibility of making a large impact to plant breeding. This paper will introduce how the CRIPSR system works, and will give a few examples of how the technology is currently being used in plant breeding. It will also look at the current regulatory systems and how that might affect the use of CRISPR technology in our food system. Finally, this paper will look at consumer acceptance of GMOs; how GMOs have been perceived in the past, and what learnings we can take as we advance CRISPR into new plant biotechnologies.

Introduction

Plant breeding has a long history, going back to the beginning of human civilization, where seeds were saved from plants that had the best traits or best yield (Brown, 2015). Plant breeding has been used for years to produce varieties that perform better than the previous generations. Over time, plant breeding has become more advanced as scientific technologies have been improved; from the random saving of seeds for the next generation to using technologies such as CRISPR to make precise edits in the plant genome.

Advancements have been made in traits such as yield, drought resistance, and pest resistance. These advancements benefit society, whether it be by providing more food because yields have increased, or being able to provide more affordable food because famers are able to use less pesticides or water to grow their crops, and therefore are able to pass that savings onto the consumer.

New genome editing technologies are being used in many different fields, including medicine, plant breeding, and animal breeding. In this paper, the focus will be on the role of CRISPR-Cas technology in plant breeding. CRISPR-Cas technology may greatly benefit plant breeding and completely change the way plant breeding is currently being conducted. With CRISPR-Cas technology, changes to the plant genome can be done in a nucleotide-specific manner; either modifying, inserting or knocking out a gene (Bortesi, 2015). This is an exciting technology for the plant breeding field, and has the potential to bring new products to the market in a much quicker and cheaper way, possibly by avoiding the lengthy gene deregulation process required for genetically modified crops accomplished through plant transformation.

CRISPR-Cas 9 Overview

Clustered regularly interspaced short palindromic repeats (CRISPR) are DNA sequences, approximately 25-50 nucleotides long, separated by short sequences called spacers (Marraffini, 2010), that are being used as part of a relatively new gene-editing technology. CRISPR systems were first detected in *Escherichia coli* in 1987, but the actual function of CRISPRs were not discovered until the mid-2000s (Ishino, 2018).

In 1993, CRISPRs were observed in archaea, and then in genomes of multiple bacteria and archaea. In the early 2000s, it was discovered that the spacer regions of CRISPRs were similar in various organisms. Through comparison of CRISPR regions from various organisms, four common characteristics were discovered. First, they are in intergenic regions. Second, they contain multiple short repeats with little variation. Third, the repeats are interspersed with nonconserved sequences. Fourth, a common leader sequence is located on one side of the repeat cluster (Ishino, 2018).

In the mid-2000s, three independent studies found that the spacer regions were homologous to sequences of bacteriophages, prophages, and plasmids, and that phages and plasmids failed to infect those strains that had the homologous spacer sequences. They proposed that CRISPR sequences provide a defense system (Ishino, 2018).

CRISPRs were first characterized as an adaptive immune system by Barrangou et al. (2007). The function of CRISPR as an adaptive immune system was first established in *Streptococcus thermophilus*. Insertion of the phage sequence into the CRISPR region of *S. thermophilus* resulted in a phage-resistant strain. When a bacterium survives a virus attack, the cell integrates part of the viral DNA into its own genome, which was coined as the CRISPR sequence (Bartkowski, 2018), providing a "genetic memory" of the infectious virus (Marraffini, 2010), an example of which is presented in the top portion of Figure 1. The next time the bacterium is attacked by the virus, an RNA copy of the viral DNA is made which serves as a targeting mechanism. The RNA copy binds to an enzyme, called Cas9, and the system then cuts the viral DNA at a specific sequence, which was integrated into the bacterial genome during prior infection. This eliminates any further multiplication of the virus (Bartkowski, 2018). The bottom portion of Figure 1 illustrates this mechanism. CRISPRs can be reprogrammed so that the CRISPR-Cas system can recognize DNA molecules that haven't been encountered before (Marraffini, 2010). It was discovered that the CRISPR-Cas RNA (crRNA) complex can cleave target DNA *in vitro* (Ishino, 2018).

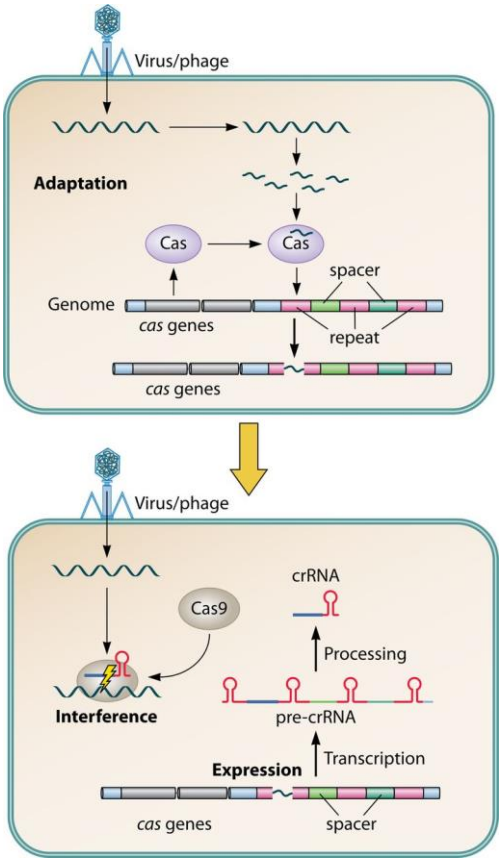


Figure 1. The process of CRISPR as an acquired immune system. The top image shows the invading DNA that is recognized by the Cas protein, and then incorporated into the spacer regions. The bottom image shows pre-crRNA generated through transcription and then processed into crRNA. Foreign DNA is then captured and cleaved. (Source: Ishino, 2018).

Comparative analyses of CRISPR regions amongst multiple organisms revealed four conserved genes are regularly present next to the CRISPR regions. These are called CRISPR-associated genes (Cas genes) (Ishino, 2018). These Cas genes fall into two classes of CRISPR-Cas systems, Class 1 and Class 2. Class 1 systems work with multi-subunit effector complexes that are made up of 4-7 Cas proteins, which are common in bacteria and archaea, and represent about 90% of all identified CRISPR-Cas loci. Class 2 systems use a single multidomain effector

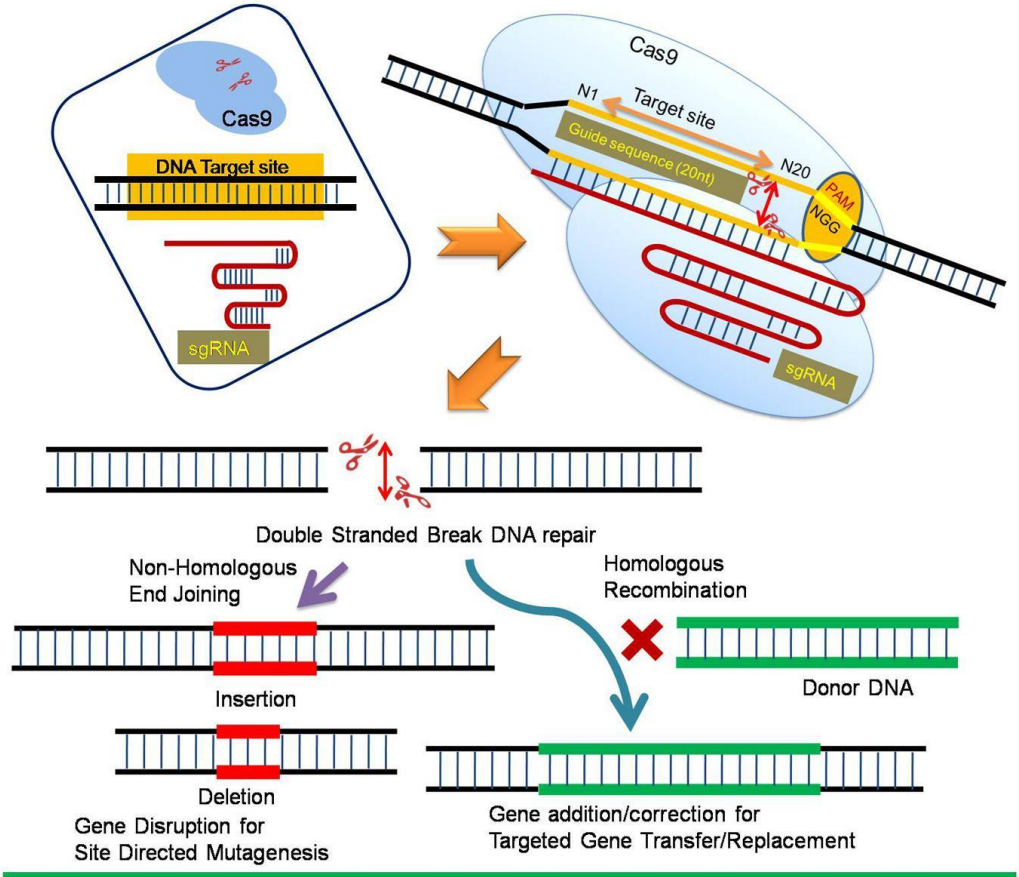
protein, are found only in bacteria, and represent about 10% of all identified CRISPR-Cas loci. Within Class 1 systems, there are three types: type I, type III, and type IV. Within Class 2 systems, there are three types: type II, type V, and type VI. The type II system includes the Cas9 protein (Ishino, 2018).

CRISPR-Cas technology for genome editing is based on this naturally occurring immunity mechanism in bacteria (Bartkowski, 2018). The *S. thermophilus* CRISPR-Cas system has been applied to gene editing in human nerve and mouse kidney cells (Ishino, 2018). The CRISPR-Cas 9 system, the most common CRISPR-Cas mechanism, is used in gene editing. It is adapted from *Streptococcus pyogenes* (Ferreira et al., 2018). Today, the type II signature protein Cas9 is the one that is the most popular and the most used effector protein (Brandt, 2019). Cas9 can be guided to a specific DNA target sequence adjacent to a protospacer-adjacent motif (PAM), a short DNA sequence of usually 2-6 base pairs located just after the DNA region that is being targeted by the CRISPR system (Belhaj, 2013).

Two non-coding RNAs guide the CRISPR-Cas9 complex: CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA). In 2012, the synthetic single guide RNA (sgRNA) was created by fusing crRNA with tracrRNA, which functions similar to the crRNA/tracrRNA complex. Because of this advancement, the CRISPR-Cas9 system was brought down to two components — Cas9 and sgRNA. The sgRNA guide sequence is located at the 5’ end, and specifies the DNA target (Belhaj, 2013). When it reaches the target, the Cas9 recognizes the PAM sequence (5’-NGG-3’) and cleaves the DNA sequence at three nucleotides upstream of the PAM, creating a double strand break in the DNA molecule (Ferreira et al., 2018). The N in the NGG sequence can be any nucleotide. Based on these observations, RNA-guided engineered nucleases (RGENs) have been invented to modify target sequence in a sequence-specific manner

for precise genetic modifications. RGENs are comprised of the Cas9 nuclease and an engineered single guide RNA (sgRNA) which has 20 nucleotides at the 5' end that directs the Cas9 nuclease to the target site (Khatodia, 2016).

Other types of Cas nucleases can recognize different PAM sequences providing researchers the flexibility of using a different Cas protein. However, Cas9 is still the most commonly used endonuclease (Synthego, 2019). The ability to reprogram CRISPR-Cas endonuclease specificity using sgRNAs has been of great advantage for genome editing applications. By modifying the guide sequence, sgRNAs with different target specificities can be created (Belhaj, 2013).



Schematic representation of Cas9/sgRNA system of CRISPR technology

Figure 2. A schematic representation of the CRISPR-Cas9 system. Cas9 is guided to the target site by the sgRNA. The target site is located at 5' of the PAM sequence. Once at the target site, there is a double stranded break in the DNA and the DNA is then repaired by either non-homologous end joining or homologous recombination process (Khatodia, 2016).

Figure 2 shows a general schematic of the CRISPR-Cas9 system. The sgRNA guides the Cas9 to the target site, eventually leading to a double-stranded break. Double-stranded breaks (DSBs) created by CRIPSR-Cas can be repaired by either homologous directed repair (HDR) or non-homologous end-joining (NHEJ) (Chen, 2019). HDR occurs when there is a corresponding homologous template (Su, 2016). The repair template for HDR can be a sister chromatid, an exogenous DNA, or a single-strand DNA containing the desired sequence that will be incorporated into the break site (Chang, 2017). HDR can introduce precise point mutations like nucleotide substitutions or HDR can insert desired sequences through recombination of the target locus with exogenously supplied DNA templates (Khatodia, 2016).

Non-homologous end-joining (NHEJ) does not need a homologous template (Su, et al., 2016). DNA double-stranded breaks are largely repaired by non-homologous end-joining process (Chang, et al., 2017). Usually, NHEJ causes random insertions or deletions, which can result in frameshift mutations in a coding region of a gene. The frameshift mutations can create a gene knockout (Bortesi, 2015). While NHEJ is very efficient and useful for large scale knockouts, it is not a precise as HDR-mediated genome editing.

CRISPR-Cas technology is precise; changes can be as simple as one base pair being removed or replaced. Therefore, the genetic changes in the descendants are indistinguishable from point mutations that could occur naturally (Gross, 2016).

Off-target activity is one of the concerns of using CRISPR-Cas. Off target activity is when a non-targeted sequence is recognized, instead of or in addition to the target sequence. Mismatches between the DNA target and the guide sgRNA sequence within the last 8-10 base pairs of the target sequence are not tolerated by Cas 9; whereas mismatches towards the 5' end of the target sequence are better tolerated by the enzyme in performing its endonuclease cleavage function (Belhaj, 2013). The whole genome sequencing in plants has shown that negligible mutations occur at off-target sites. In plants, off-target mutations induced by CRISPR-Cas9 is not of a major concern because background mutations can be purged via segregation in the selfing-generations.

One way to reduce off target activity could be to lower the level of Cas9 and/or sgRNA expression. Use of truncated sgRNAs has also shown to alleviate the problem. Shorter sgRNAs are more sensitive to nucleotide mismatches and could reduce off-target mutations (Belhaj, 2015). The best way to reduce off target activity is to select the right target sites, based on sequence information. The best target sites would be the ones that have minimum predicted off targets (Hahn, 2019).

Before 2013, the two most prominent genome editing tools for site-specific double strand DNA breaks were zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENS). Both are artificial fusion proteins, created by fusing an engineered DNA-binding domain to the nonspecific nuclease domain of a restriction enzyme (Bortesi, 2015). These tools were not widely adopted due to complicated design and laborious and time-consuming assembly of specific DNA binding proteins for a target gene sequence. In addition to simplicity in design, CRISPR-Cas, can be multiplexed to target several genes in parallel, and is now accessible to many plant science laboratories (Rogowsky, 2017).

Impact of CRISPR Cas Technology on Plant Breeding

CRISPR-Cas can be a useful tool in plant breeding. However, to apply CRISPR-Cas technology to improve a trait one must have the knowledge of the genes that govern the trait (Rogowsky, 2017). Use of CRISPR-Cas in plant breeding looks to be more promising than in animal breeding. In plant breeding, small edits in the genomes can lead to large improvements in efficiency because of the specific targeting mechanism of CRISPR-Cas. CRISPR-Cas in plant breeding can also affect disease resistance, and nutritional value (Gross, 2016). The lengthy recurrent backcrossing process can now be replaced with the rapid CRISPR-Cas technology (Rogowsky, 2017). Backcrossing is time consuming and can take easily seven years for at least four rounds of backcrossing to transfer the desired trait from the donor parent to the recurrent parent. More importantly, the developed CRISPR-Cas-mediated products are free from any possible linkage drag. Linkages of undesirable genes with the trait-gene of interest many times compromise the yield potential of cultivars developed through backcrossing. With CRISPR-Cas, the traits can be immediately incorporated requiring significantly less time than traditional backcross breeding and products are free from any linkage drag.

The first food edited with CRISPR-Cas and cleared by the United States Department of Agriculture (USDA) is the common white button mushroom (*Agaricus bisporus*). The technology was used to reduce browning of the mushroom. CRISPR-Cas allowed knocking out just one of six genes encoding polyphenol oxidases (Gross, 2016). By deleting a few base pairs of one of the six polyphenol oxidase genes, the enzyme activity was reduced by 30%. As a result, oxidation of phenols was reduced significantly and so was the browning (Waltz, 2016).

Another product of CRISPR-Cas technology is waxy corn. Waxy corn, genetically modified using CRISPR-Cas 9 by Corteva, contains 100% amylopectin, used in processed foods, adhesives, and high gloss paper. Using the CRISPR-Cas9 gene-editing tool, the Corteva team knocked out the endogenous waxy gene *Wx1*, which encodes the endosperm's granule-bound starch synthase that synthesizes amylose from ADP-glucose pyrophosphorylase (Waltz, 2016).

Some examples of traits that have been approached with CRISPR-Cas technology are listed below:

- Resistance to powdery mildew in wheat (Wang et al., 2014)
- Resistance to 3 potyviruses in cucumber (Chandrasekaran et al., 2016)
- Resistance to Botrytis in grape (Wang et al., 2017)
- Canker resistance in citrus (Peng et al., 2017)
- Dwarfism in barley (Lawrenson et al., 2015)
- Oil enriched in oleic acid in soybean (Haun et al., 2014)
- Starch quality (Andersson et al., 2017), phosphate transport (Zhou et al., 2017), and auxin transport in potato (Wang et al., 2015)
- Non-browning mushrooms (Gross, 2016)
- Waxy corn (Waltz, 2016)

Regulation of GMOs and CRISPR technology

GMOs, genetically modified organisms, are defined as organisms (plants, animals, or microorganisms) in which the DNA has been altered in such a way that it would not occur naturally by mating and natural recombination. They can be developed by transferring selected

individual genes from one organism into another (WHO, 2014). Regulation of genetically modified crops is conducted by three agencies in the United States: the United States Department of Agriculture (USDA), the Environmental Protection Agency (EPA), and the Food and Drug Administration (FDA).

Regulation of GMOs available in the Library of Congress website is presented below:

> *"GMOs are regulated in the United States under the Coordinated Framework for Regulation of Biotechnology, published in 1986, pursuant to previously existing statutory authority regulating conventional products, with a focus on the nature of the products rather than the process in which they are produced.*
>
> *Plant GMOs are regulated by the US Department of Agriculture's Animal and Plant Health Inspection Service under the Plant Protection Act. GMOs in food, drugs, and biological products are regulated by the Food and Drug Administration under the Federal Food, Drug, and Cosmetic Act and the Public Health Service Act. GMO, pesticides and microorganisms are regulated by the Environmental Protection Agency pursuant to the Federal Insecticide, Fungicide and Rodenticide Act and the Toxic Substances Control Act. The form of regulation varies depending on the type of GMO involved" (Acosta, 2014).*

Under the current regulations, it takes on average $136 million (Figure 3) and 13.1 years (Table 1) for a GMO to go from discovery to commercialization (McDougall, 2011). These charts do not include deregulation of stacked transgenes. It is expected that CRISPR-Cas edited genotypes are most unlikely to go through the same rigor of deregulation process as for the GM-

cultivars. (USDA, 2018). An added advantage of CRISPR-Cas in plant breeding is the feasibility of multiplex sgRNAs for editing two or more target genes (Belhaj, 2015). This can save time in creating the products for multiple traits as well as going through the regulation process.

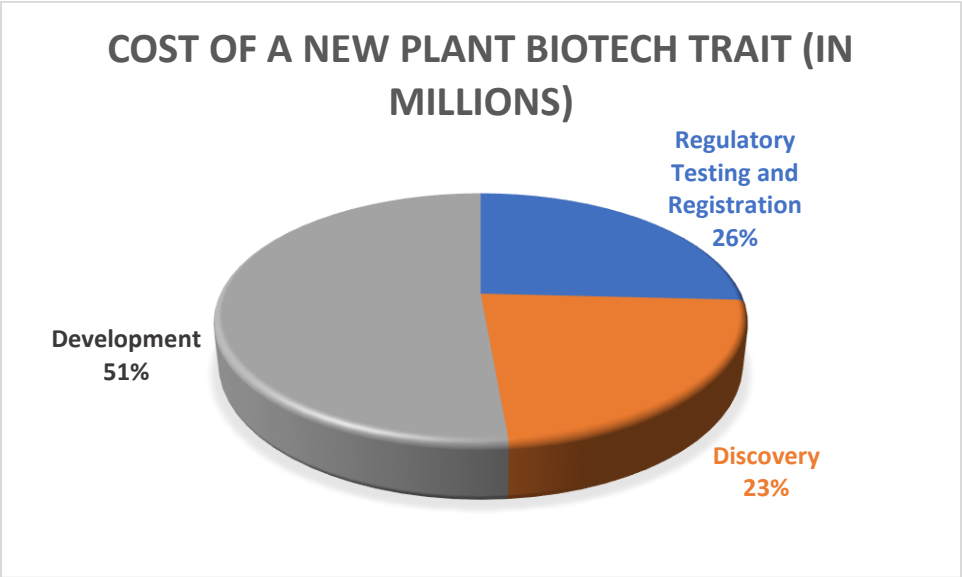


Figure 3. The overall cost of a new plant biotechnology trait, introduced between 2008 and 2012. This is based on data from six largest biotechnology crop developers of seed industries. The chart represents the cost of discovering a trait, development of a product for production, and regulatory testing for deregulation and registration (*Source:* McDougall, 2011).

Table 1. The overall time taken from discovery to commercialization of a new plant biotechnology trait, introduced between 2008 and 2012. This is based on data from six largest biotechnology crop developers of seed industries.

	Canola	Corn	Cotton	Soybean	All crops
Number of Years from Discovery to Commercialization	11.7	12	12.7	16.3	13.1

The table does not include time taken to obtain regulatory approval for traits governed by stacked transgenes, which are incorporated in a large portion of most crops today (*Source*: McDougall, 2011).

Regulations of GMO around the world can be either process-based or product-based. Process-based GMOs are subjected to regulatory reviews based on the scientific assessment of the processes used to create the GMOs and the risks to human health and the environment. Product-based GMO regulations is based on the final product rather than on the processes used to generate the GMOs (Araki, 2015). European Union countries regulate GMO based on the process (Zetterberg, 2017); whereas, in the U.S. GMO is regulated based on the products (Acosta, 2014).

The first CRISPR-Cas edited crops could be cultivated and sold without any oversight or deregulation process under its biotechnology regulations division of the United States Department of Agriculture (USDA) because the products are not different from the ones developed through traditional plant breeding. In the USDA press release of March 28, 2018, the United States Secretary of Agriculture Sonny Perdue stated that USDA does not regulate, nor do they have plans to regulate plants that could otherwise have been developed through traditional plant breeding techniques, as long as they are not plant pests or were developed using plant pests (USDA, 2018). This is an important development for the new CRISPR-Cas technologies because it will facilitate rapid release of novel crop cultivars without the hassle of expensive and time-consuming regulatory processes applied to GMOs developed through plant transformation.

The first example of a CRISPR-Cas edited product is the mushroom, *Agaricus bisporus*. The USDA concluded that the CRISPR-Cas edited mushroom required no state regulation in the U.S. because no foreign DNA has been introduced into the organism (Gross, 2016). As stated earlier, the non-browning type mushroom was created by knocking out a gene encoding polyphenol oxidase that is responsible for browning. There was no trace of any foreign DNA in the CRISPR-Cas edited mushroom; therefore, the modified mushroom was considered a GM

product indistinguishable from those obtained through conventional mutation breeding or use of an allele generated through spontaneous mutation (Bartkowski, 2018).

In the past, when the U.S. government was developing its regulation for GMOs, viral or bacterial genes were used in developing genetically modified plants (Waltz, 2016). Those GMOs can be distinguished from products that occur naturally, which is why GMOs are regulated under the current U.S. regulation process.

CRISPR-Cas edited waxy corn is expected to be marketed in 2020. Neil Gutterson, the Senior Vice President and Chief Technology Officer of Corteva Agriscience, reported that his team started to work on developing the new waxy corn trait in early 2015. “One observation or lesson we have with our first product is that the reduced time to market is significant,” he added. It takes less than five years for a CRISPR-Cas edited corn, as opposed to eight years for a hybrid corn, to reach farmers. The USDA treated the CRISPR-Cas edited waxy corn the same way as it treats a similar product developed through conventional plant breeding approaches because it does not add any foreign genes (Bomgardner, 2017). If the United States continues to allow CRISPR-Cas edited crops to be cultivated and sold without any USDA oversight, this would reduce the cost of development of a product by millions of dollars (Waltz, 2016). The ease of use of the technology and no need of any deregulatory process are expected to facilitate development of CRISPR-Cas mediated genome editing by even smaller companies and developing countries, which is not the case for the current transgenic products, popularly known as GMOs (Gross, 2016).

GMOs and Consumer Acceptance

Google Trends shows that google searches for the term “GMO” from 2004 peaked in May of 2013 [Reference or Figure]. Since their introduction to the commercial market in the 1990s, genetically modified (GM) foods have received quite a bit of opposition from the public because of concerns about possible environmental and health risks. The majority of scientists, however, are favorable towards GM foods (McPhetres, 2019). In 2016, the National Academy of Sciences formed a Committee on Genetically Engineered Crops. They studied over 900 publications, attended meetings and webinars, and gathered public comments. Their research found no convincing evidence for negative health or environmental effects of GM foods (Gould, 2016).

While the research shows no convincing evidence, the general public’s understanding of GMOs or the science behind GMO production is very low. Most consumers receive their information on GMO food products from the media, the internet or other news sources, which are unlikely reliable than scientific publications (Wunderlich, 2015). In a survey taken by Oklahoma State University in 2015, 82% of those surveyed support mandatory labels on GMOs, and 80% said they support mandatory labels on foods “containing DNA” (Lusk, 2015). The majority of items bought in a grocery store “contain DNA”; the majority of the public seems to be unaware of this scientific fact.

McPhetres et al. (2019) reported that a lack of science knowledge for GM technology is a strong predictor of GM food skepticism. McPhetres et al. (2019) also showed that if people learned about the science behind GM technology, they tend to develop positive attitudes towards GM foods, a greater willingness to accept GM foods, and lowered their perceptions that GM foods bear health risk (McPhetres, 2019).

Discussion

CRIPSR-Cas is a relatively new technology that allows precise editing of DNA sequences. This paper focuses on the invention of CRISPR and development of CRISPR-Cas technology that has been used in designing crop cultivars. It discusses no requirement of a regulatory process for the products generated by the CRISPR-Cas technology in the United States. CRISPR-Cas is an exciting new tool for plant breeders because it allows the plant breeding process to be precise and requires much less time as compared to that in the traditional plant breeding in generating a new trait. Whereas in the past, a plant breeder may take over seven years to add a new trait through back crossing, CRISPR-Cas can create new alleles of a gene or knockout a gene in a matter of 2-3 years to genetically improve a trait. Such products are also completely free from any possible linkage-drags of undesirable genes located next to the target gene, which is usually happened during cultivar development through backcrossing of the desirable target allele into an established cultivar. A possible pitfall of the technology are off-target mutations, which can be purged from the final product through selfing and segregation.

The regulation of GMO products depends on the countries' laws and how the product is viewed; if it regulated based on the nature of the product or the process used to create the product. In the United States, it is currently being regulated based on the nature of the product. The products developed through CRISPR-Cas technology are indistinguishable from products developed through induced or spontaneous mutations, and is distinct from GMOs generated through introduction of foreign genes through plant transformation. Therefore, the crop cultivars developed using CRISPR-Cas technology are considered no different from the products developed through traditional plant breeding approaches and do not require regulation.

While the lack of regulation of products developed by CRISPR-Cas technology is a large factor for successful application of the technology in crop improvement, it is equally important to have consumer acceptance of the CRISPR-Cas products. Without consumer acceptance, CRISPR-Cas edited products face the same challenges as GM products have. With GM foods, most consumers are uneducated on the science, and there is a lot of skepticism regarding genetically engineered foods. In the beginning, when transgenic technology was used, there was a lack of efforts in providing awareness of the technologies among the consumers. Scientists and farmers thought consumers would accept GM products because they provide so many benefits. Not knowing enough about the technology and because of misconceptions about GMO propagated in the media, the consumers developed negative attitude towards GM products. As a result, consumers buy certified non-GM products or organic products, avoiding GMOs. They are concerned about their health and well-being; they negatively view GM foods. Only 52% of consumers aware how GMOs are created or developed (Crawford, 2015). In a 2015 Pew Research Center study, 57% of U.S. consumers considered eating GM foods "generally unsafe." Eleven percent of scientists associated with the American Association for the Advancement of Science consider the same (Hofbauer, 2016). Table 2 presents the consumer knowledge of GMOs collected through four surveys.

Table 2. Consumer surveys on knowledge of GMOs.

Sample Size	Main findings
n=1201	48% know that GMOs were available in supermarkets
	31% believe that they have most likely consumed a GMO product
n=600	Limited self-rated knowledge about GMOs
	48% know very little about GMOs
	16% know nothing at all about GMOs
	30% know a fair amount about GMOs
	5% know a great deal about GMOs
n=1148	43% know GMO products are sold in supermarkets
	26% believe that they have most likely consumed a GMO product
	54% know very little or nothig about GMOs
	25% have never heard of GMOs
n=491	59% knw that GMO soybeans are sold in US supermarkets
	56% mistakenly believe that GMO tomatoes are sold
	55% mistakenly believe that GMO wheat is sold
	50% mistakenly believe that GMO chicken is sold

Source: U.S. consumers (Wunderlich, 2015).

It is also hard for consumers to have the correct information when there are misleading labels. For example, the "Non-GMO Project" label is also used on foods of many crops for which no known GMOs are available. As for example, a non-GMO-labelled cereal made from 100% wheat although there is no GM wheat grown anywhere commercially in the world. Similarly, "Non-GMO Project" certified avocado oil with no GM avocado grown anywhere (Wager, 2016). These misleading labels add to the confusion of GMOs for the general consumer. Most consumers will take the label as is, and assume there is a GMO version of the product. Even salt is sometimes labelled as non-GMO.

Scientists, by educating on science of GMO, can change public' opinions. Take the example of Bill Nye, GMO opponent. For over 10 years, he had strong concerns over GM crops. In 2015, he publicly announced that he changed his mind on GMO after visiting Monsanto scientists. Opponents of GMO denounced him and said that propaganda scientists brainwashed him into changing his position. But, Bill Nye wrote in his book "Unstoppable: Harnessing Science to Change the World" that he accepted the evidence and became aware of the

unscientific nature of the anti-GMO movement (Pomeroy, 2016). It is clear that it will be an uphill battle to gain wide acceptance of GMOs, but if more people can be educated on the science, the benefits, and the safety of the products, there could be a wider acceptance of the technology.

I think it will be best to educate the consumer on CRISPR-Cas technology even before the products are made available to the market to avoid any negative impression of the safe GM products using this technology. Otherwise as in GMO, generated through transgenic technologies, it will be hard to gain the public trust on this new CRISPR-Cas edited GM products. Transparency and education are the ways to avoid any misperception of CRISPR-Cas technology and gain trust of consumers for making this novel high precision genome-editing technology a successful one, urgently needed for a second generation of green revolution!

Conclusions

CRISPR-Cas technology has allowed scientists to make sequence-specific changes to a plant genome. These changes are indistinguishable from changes that could occur naturally through spontaneous or induced mutation. Because of this principle, current regulations for CRISPR-Cas products in the United States are different from those for GMO. The USDA does not have any plans to regulate CRISPR-Cas edited crops, which means it will be affordable to develop CRISPR-Cas products by both small and large seed companies; and we expect to witness a wider participation of scientists for improving crops. If the USDA continues not to regulate CRISPR-Cas edited crops in the United States, the major deciding factor for using CRISPR-Cas technology will be the acceptance of CRISPR-Cas edited foods by consumers.

Educating the consumers for science of the new technology will be the key in convincing the consumers that the developed new cultivars through this technology is not different from the ones developed through traditional mutation breeding approach. Scientists have learned a great lesson while working on transgenic technology on how important it is to educate the public on the science of plant transformation and transgenic plants to gain their trust. This experience is expected to make the CRISPR scientists more proactive in educating the public regarding the science behind this genome editing technology.

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