Genome Editing Tools and Their Implications in Agriculture

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Received March 13, 2024
Accepted April 19, 2024
Electronic access April 30, 2024

The new era of crop and agricultural innovation has been made possible by developments in genetic modification, as trends have arisen in plant-based diets and other dietary forms. Today, genetic modification in agriculture has become widely established as its ability to create crops with desired traits, such as increased resistance to disease, temperature, and drought, is well known. Although genetic modification in various forms has occurred for thousands of years, the twentieth century has brought about extensive improvements to the agricultural field as modern genome editing has revolutionized the speed at which beneficial changes in crops can be made. As the population grows, the demand for increased yields and nutrition created by modified crops has grown. Modern genome editing tools include Zinc Finger Nucleases (ZFNs), Transcription Activator-like Effector Nucleases (TALENs), and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas systems. These technologies have many applications in agriculture. However, as these technological advancements continue to develop, questions of their ethicality arise. The main ethical issues that have risen include potential environmental threats, the elimination of species, reduced gene flow and biodiversity, and the transformation of or change in the human genome. This literature review aims to detail the applications and ethical issues of genome editing, emphasizing CRISPR-Cas systems in agriculture.

Keywords: Agriculture, CRISPR in agriculture, genetic modification in agriculture, ZFNs, TALENs, ethics of CRISPR, CRISPR applications

Introduction

Genetic modification, which can be defined as altering the genetic makeup or DNA of an organism, dates to the dawn of agriculture. Barrows et al. state that "For millennia, humans have modified plant genes in order to develop crops best suited for food"[1]. Approximately 12,000 years ago, humans began to cultivate crops, sparking the shift from nomadic lifestyles involving hunting and gathering food to permanent settlements revolving around the cultivation of crops. During what was known as the First Agricultural Revolution or Neolithic Revolution, genetic modification emerged in forms different from today. Researchers in agricultural biotechnology claim, "the earliest efforts, far predating Gregor Mendel's 19th-century discoveries on trait inheritance, involved the selective breeding of desirable characteristics, but the recombination of DNA in offspring was random"1. While the genotype is the set of genetic material in an organism and is often expressed through a pair of alleles, the phenotype is the set of observable traits in an organism. Over generations, random mutations occur in the genotype of an organism, allowing for the expression of different phenotypes. While most mutations to an organism's genome are harmful, some are beneficial. For example, they can provide a sweeter taste in crops. Over generations, beneficial traits such as size, color, and taste are chosen to be bred to increase the number of crops with those same characteristics. This traditional crop modification is a form of artificial selection. In comparison to natural selection, in which beneficial traits are chosen by the environment, as those with advantageous features have better chances at surviving and reproducing, artificial selection allows humans to hijack the process of random mutations as we specifically select those that we consider to be favorable.

Although artificial selection continues in agriculture today, technological advancements made by significant figures, such as Charles Darwin, Gregor Mendel, and Norman Borlaug, have led to significant improvements in crop production. Darwin's work involved the Theory of Evolution, bringing to light the idea of beneficial traits naturally being passed on over generations due to their advantage in survival and reproduction. Known as the father of genetics, Mendel established the law of dominance, the law of segregation, and the law of independent assortment, paving the way for understanding heredity and the genome. Norman Borlaug, the father of the Green Revolution, developed variations of wheat that allowed for resistance to disease and various growing conditions. The genetically modified wheat created food surpluses around the world, initiating an exponential-like growth pattern in the population. In other words, changes and new knowledge in the field of genetic modification have resulted in consequential impacts on the human population, as shown through the Agricultural Revolutions and Green Revolutions.

Recently, however, there have been many more contemporary discoveries in the field of genetic modification as genome editing

has become more widespread. This study aims to illustrate these recent developments and inform how these impact modern sources of food. Since 1927, it has been found that exposure to X-rays can cause genetic mutation. Humans induced mutation in the DNA of crops through the use of irradiation. These forms included UV light, X-rays, gamma rays, alpha rays, beta rays, and more[2]. While most differences resulted in negative results, beneficial traits arose, making this process valuable[2]. In other words, a form of accelerated artificial selection was created as mutations were still randomized in crops but at an increased rate compared to natural mutagenesis.

Following the use of induced mutation through irradiation, more advanced forms were developed. These forms include Zinc Finger Nucleases and Transcription Activator-like Effector Nucleases. While both traditional and accelerated artificial selection rely on the identification and propagation of beneficial traits produced via random mutation, modern genetic modification relies on the use of molecular tools to induce changes in the genome that are hypothesized to produce beneficial traits based on knowledge of plant biology and gene function. This directed mutation is capable of generating beneficial traits in a fraction of the time required for traditional methods. Thus, modern techniques allow for a rational and directed means of genetic modification as they do not involve waiting for random mutations, revolutionizing present-day genetic modification.

While increasing technologies in genetic modification have brought about significant improvements to crop yield and similar benefits, they also yield ethical concerns. These ethical concerns include significant environmental impacts, the loss of biodiversity, the inhumane treatment of animals, and risks to health. Thus, not only does this study aim to discuss the many new technologies in genetic modification and their ability to be applied to agriculture, but the study also aims to illustrate arguments regarding the ethics behind genetic modification in agriculture as there is little knowledge behind how technologies alter crops and their potential adverse effects.

This study includes data from locations around the world. There were little to no limitations in performing the study as there were plenty of sources to gather information from. While most sources are from the year 2020 and on, some sources are older and thus may contain different information. Additionally, there may be little bias in the papers gathered for research as the papers hold supportive views of genetic modification in agriculture.

Results

Zinc Finger Nucleases and Transcription Activator-like Effector Nucleases

During the late 20th century, a new form of genetic modification was developed: Zinc Finger Nucleases (ZFNs). Utilized in various settings to create double-stranded breaks within the genome of a wide range of organisms, including both eukaryotes and prokaryotes, ZFNs allow for the elimination or editing of specific genes. With a variety of applications in the agricultural field, Zinc Finger Nucleases became widely popular and heavily used during the early 21st century.

Zinc Finger Nucleases are composed of DNA-binding domains called zinc fingers, a nuclease named FokI, and a peptide link between the zinc finger domain and the nuclease[3]. While the nuclease initiates the genome editing event, the zinc fingers target the nuclease to a specific location on the genome that is intended to be edited. As ZFNs interact with the double-stranded DNA, the zinc fingers scan the base pairs of the DNA, with each finger recognizing three base pairs. Larger subunits of Zinc Finger Nucleases are formed by combining multiple fingers to increase specificity. As there is the repetition of the same sequence plenty throughout the enormous genome of an organism, the combination of fingers is critical to recognizing the specific region to cut as the recognition of base pairs signals the region for the FokI nuclease to create a break in the DNA. Researchers from the Department of Pathology at the University of Maryland School of Medicine explain, "With each finger recognizing three base pairs (bp), a three-finger subunit of ZFN binds to 9 base pairs on the DNA. Typically, two ZFN subunits containing 6 to 12 zinc fingers (or 3 to 6 zinc finger pairs), respectively, bind to between 18 and 36 nucleotides"[3]. Thus, as the quantity of zinc fingers in a subunit of a ZFN increases, so does the specificity of the ZFN. With a high level of specificity, ZFNs can successfully be used to accurately target specific regions of DNA to alter the genome, as the linking of multiple zinc fingers results in a larger DNA recognition site, making ZFNs such a useful tool in genome editing.

In addition, the FokI endonuclease creates a cut at specific cleavage domains as it "induces a DNA double strand break as a catalytic dimer"[3]. As the enzyme creates a homodimer, the joining of two identical molecules, ZFNs require two subunits on both sides of the double stranded DNA sequence. The use of ZFN subunits on both sides of DNA further increases the ability of the genome editing tool to identify unique sequences. The dimerization that occurs allows for the cleavage of the DNA strands. Thus, ZFNs follow a modular assembly method as "the independent DNA binding and cleavage domains can be optimized in isolation for effective DNA cleavage against custom-designed target sites"[4]. The modular structure of ZFNs, which allows for repeat domains, furthers the accuracy and precision of the system in genome editing.

Compared to ZFNs, Transcription Activator-like Effector Nucleases (TALENs) follow a similar structure. Developed in 2010, TALENs, too, consist of separate DNA binding and endonuclease domains, the latter often being the same Fok1 nuclease utilized in ZFNs. The DNA binding domain of a TALEN consists of repeats of amino acids linked in series, following a modular

design structure like ZFNs. While the main amino acid chains of repeats have approximately 33 to 35 amino acids, the last repeat is composed of only 20 amino acids[4]. The last repeat is thus known as a half-repeat. At the 12th and 13th amino acid in the repeat, there are residues known as Repeat Variable Di-Residues (RVDs) that are responsible for the DNA-binding specificity of TALENs, and the "number and order of RVD in each repeat determine the nucleotide-binding specificity"[4]. While each repeat of amino acids recognized only one nucleotide, TALENs are considered to have a high level of specificity through their modular structure[4]. The combination of chains of amino acids increases the number of nucleotides that can be recognized, allowing TALENs to bind with greater specificity than that of ZFNs[5]. Thus, TALENs are often chosen in the modification of the plant genome due to their advantageous specificity. Figure 1 presents basic models of ZFN and TALEN structures.

Challenges

While both methods of genome editing allow for great opportunities in the agricultural field due to their ability to easily create targeted double-stranded breaks in DNA, neither method of genome editing comes without drawbacks. "A major complication with the engineered nuclease is the binding of the nuclease to unintended genomic sites that share sequence homology with the on-target site"[7]. These off-target effects of genome editing can result in detrimental impacts to the genome as unintended genomic modifications may result in the impairment of the function of the gene. Changes to the phenotype will often result, and there may be negative or undesirable traits. However, as Jansing and her fellow researchers in the genome editing field claim, "to further minimize off-target events and associated cellular toxicity the FokI nuclease dimerization interface has been engineered to force heterodimer formation, and this variant is routinely used in ZFNs and TALENs"[5]. To summarize, Zinc Finger Nucleases and Transcription Activator-like Effector Nucleases are both highly effective methods of genome editing as they create specific double-stranded breaks in DNA to allow for their modification. ZFNs and TALENs opened the field of agriculture to the applications of genetic modification, as their methods have greatly contributed to crop improvement.

CRISPR-Cas Systems

Like ZFNs and TALENs, CRISPR-Cas systems work to create double-stranded breaks in DNA. CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats) are segments in DNA that act as bacterial defense systems. The transformation of the genome editing field has occurred through the pairing of CRISPR DNA segments with Cas proteins. Again, when comparing the different methods of genome editing, CRISPR-Cas systems also follow a separately bound structure as the Cas protein works to cleave the DNA while RNA works to identify the specific regions in DNA to cut. In CRISPR-Cas systems, a "CRISPR guide RNA is engineered to base-pair with a target site in a chromosome," and the "Cas endonuclease, bound to the guide RNA, then cleaves both strands of DNA at a site targeted by the guide RNA"[8].

Focusing on the guide RNA or gRNA, researchers can create specific gRNAs to target regions of the DNA that they desire to modify. Providing a general overview, when the Cas protein recognizes a point known as the protospacer adjacent motif (PAM), the target sequence is checked. While there is the repetition of many bases throughout a genome, the target sequence, regularly 20 base pairs long, indicates where the Cas protein should cut downstream of the PAM site.

CRISPR-Cas systems have been divided into two classes based on the Cas protein, with Class 1 systems having complexes of 4 to 7 proteins and Class 2 systems having only one Cas protein[9]. While there are a variety of Cas proteins, the widely utilized protein in genome editing is the Cas9 protein. The bi-lobed Cas9 protein derives from Streptococcus pyogenes and is a member of Class 2[10]. With the bi-lobed protein consisting of a large recognition (REC) and small nuclease (NUC) lobe, the NUC lobe contains a "protospacer-adjacent motif (PAM)-interacting domain (PI) and two cleavage domains known as the RuvC and HNH domains"[10]. While the RuvC and HNH domains work to cleave DNA, the REC lobe helps to activate Cas proteins[10]. Putting together the parts of CRISPR-Cas systems, the pairing of gRNA and the Cas protein creates a CRISPR-Cas complex, which binds to the DNA and creates a cleavage, as presented in Figure 2.

Non-Homologous End Joining and Homology-Directed Repair

Following the creation of a double-stranded break in DNA by ZFNs, TALENs, and CRISPR-Cas systems, several processes can occur. Although there are other pathways to repair double-stranded breaks in DNA, such as Single Strand Annealing, Non-Homologous End Joining (NHEJ) and Homology Directed Repair (HDR) are the main processes involved in genome editing.

In NHEJ, following the signaling of a break in DNA, a heterodimer that consists of proteins Ku70 and Ku80 binds to the ends of the DNA strands where the break has occurred. Next, a large protein called DNA-PKCS binds to the ends to promote the signaling of other end-joining components and proteins. These steps allow for the creation of a bridge between the broken strands, and DNA ligase then repairs and joins together the fragments of DNA[12]. In other words, the process of non-homologous end joining essentially fuses the broken strands together without the use of a DNA template. Thus, the process often results in errors, leading to the development of errors and

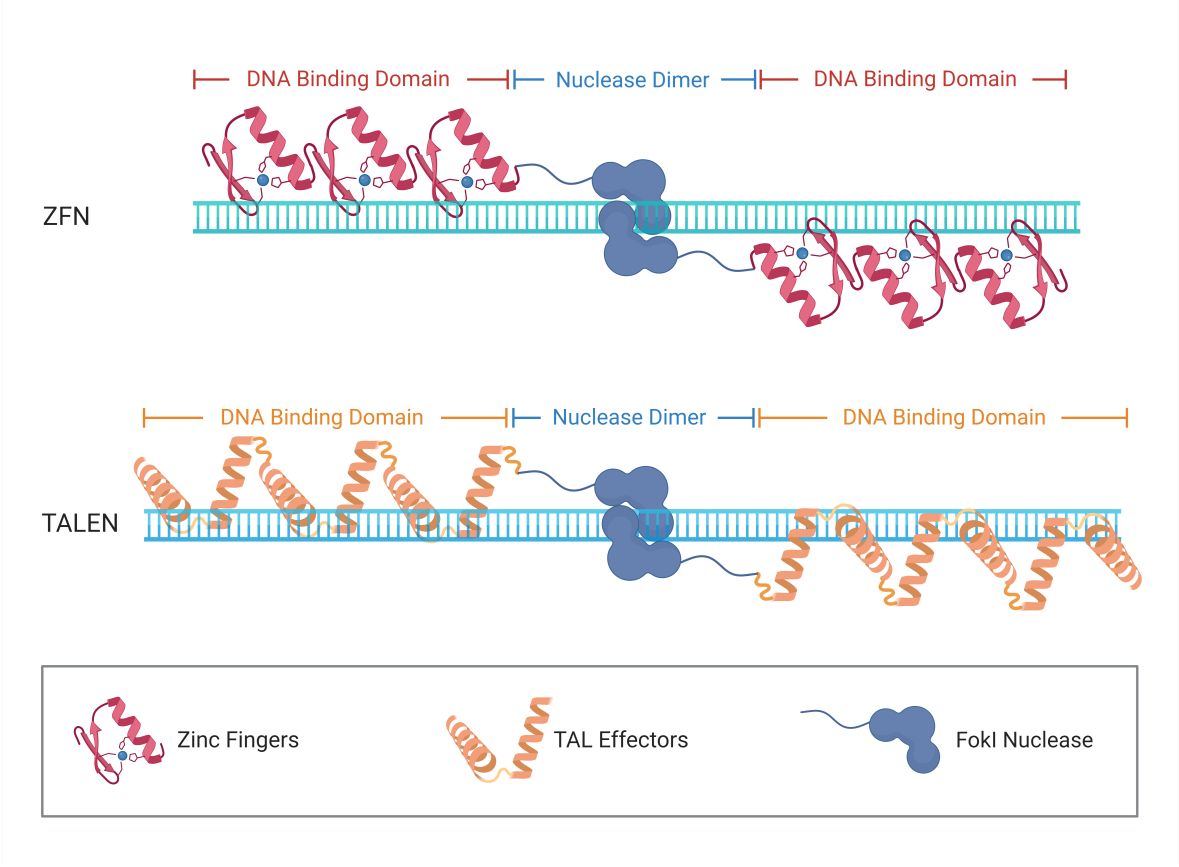

Fig. 1 Illustration of genome editing tools Zinc Finger Nucleases and Transcription Activator-like Effector Nucleases[6]

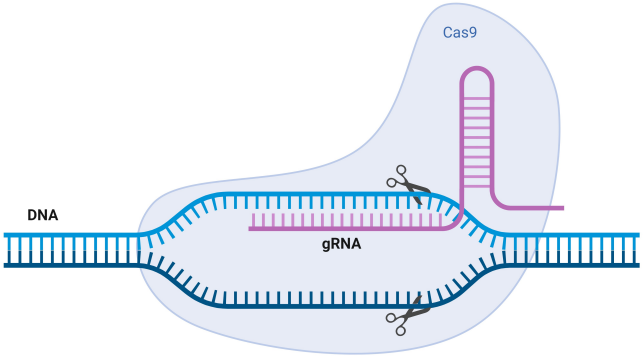

Fig. 2 This figure illustrates a CRISPR-Cas system with labeled parts, including the Cas9 protein, DNA, and gRNA[11].

the incorrect repair of the genome[12]. Often, insertions and deletions (indels) occur in the newly combined DNA strand due to the activity of DNA polymerases and nucleases.

Alternatively, HDR relies on the use of a DNA template to repair broken DNA. HDR utilizes a copy of DNA or template strand normally from a sister chromatid. Complex structures and steps follow, creating an overhand and displacement loop (D-loop). Enzymes then work to repair the section of DNA[13]. In other words, HDR results in the correct repair of a double-stranded break in DNA when the template from the sister chromatid is undamaged. However, when utilizing genome editing techniques, foreign DNA templates can also be utilized to insert genes into an organism. With the same steps occurring, the repair of the DNA occurs but instead with a region of foreign DNA. Thus, the process of HDR can result in the correction of the gene or the creation of a transgene. Refer to Figure 3.

Comparing NHEJ and HDR, both processes are utilized in genome editing as they serve important roles. While the error-prone nature of NHEJ has been harnessed to knock out genes, HDR is often utilized to make more precise and controlled modifications to the genome, including the insertion of transgenes and the creation of transgenic organisms. Thus, these processes are significant in the editing of the genome following the use of ZFNs, TALENs, and CRISPR-Cas systems.

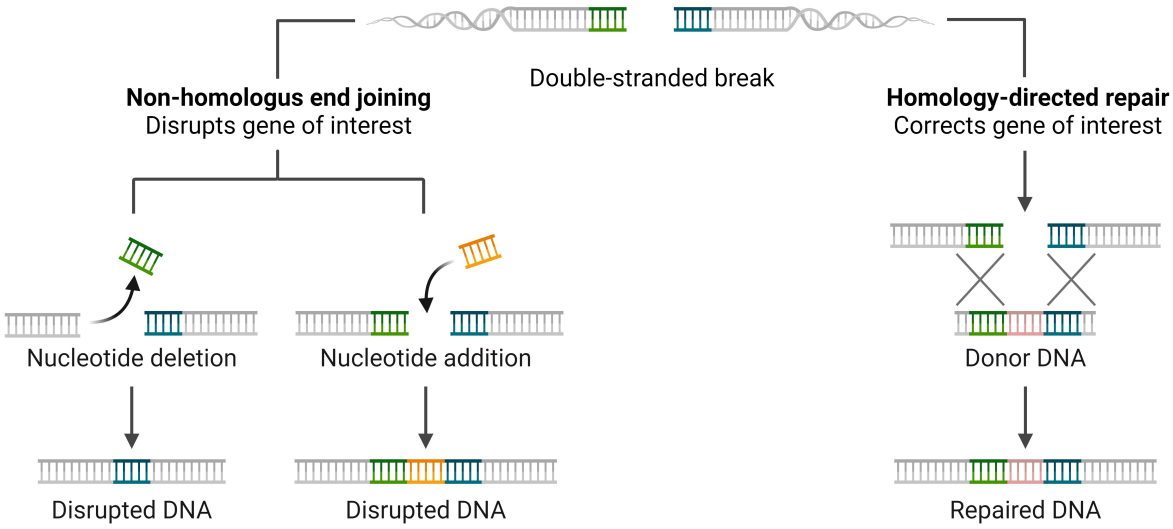

Fig. 3 Following a double-stranded break in DNA, the figure illustrates NHEJ and HDR as NHEJ results in indels while HDR results in repaired DNA. However, it is important to note that the donor DNA depicted under HDR can be of the same organism or foreign [14].

Methods of Delivery

While ZFNs, TALENs, and CRISPRs differ and have their own unique advantages in genome editing, their methods of delivery are often similar. As gene editing tools cannot cross the plasma membrane and nuclear membrane to access the genome, different forms of delivery are utilized. Biological, chemical, and physical methods are the three main types of delivery [15]. Biological methods rely on natural biological materials, including viral proteins, peptides, and cellular receptor membranes such as virus-like particles and cell-penetrating peptides [15]. On the other hand, chemical methods use artificially synthesized materials like polymers, lipids, and metals [15]. These forms include liposomes, gold-nanoparticles, and lipid nanoparticles [15]. While some physical methods, including electroporation and sonoporation, "rely on the physical energy of electricity or ultrasound to deliver the genes into cells," other forms involve microinjection [15]. While there are a variety of methods, not all can be applied to plant cells in agriculture. Examining one from each major category, common methods in plants include viral vectors, lipid nanoparticles, and electroporation.

Starting with viral vectors, there are many forms of viruses that are commonly used in delivering genome editing systems as they allow for the carrying of genetic material. Examples of these viral vectors in plants include Geminiviruses and the Tobacco rattle virus. Although there are more commonly used viruses such as adenoviruses and adeno-associated viruses, they apply less to plants and more towards genome editing in animals. Geminiviruses have proven to be highly effective for plant genome editing for several reasons. First, they are able to infect a wide range of host plant species [16]. Their method of replication is also highly efficient as it requires only one protein to replicate, and they can replicate through various methods, such as homologous recombination [16]. Comparing Geminiviruses to the Tobacco rattle virus (TRV), TRV has proven to be efficient as it too can infect a wide range of plants and can be easily introduced to plants through Agrobacterium, a method in which transfer DNA from the Ti plasmid region of the bacteria is integrated into the plant genome [17]. Not only does TRV have a relatively small genome, but its RNA genome also does not integrate with the plant genome, increasing TRV's efficiency and effectiveness [16]. Thus, different viral vectors can be significant in introducing genome editing tools into plants as they can be efficient and versatile.

LNPs are also common tools used in delivering genome editing systems. LNPs are divided into four major categories based on their primary lipid components: ionizable cationic, polyethylene glycol (PEG), zwitterionic phospholipids, and cholesterol [18]. Each form of LNP has its own advantages. With ionizable cationic lipids, their effectiveness comes from the fact that it is a "pH-dependent cationizable lipid, which is neutral at the delivery stage (neutral pH) but becomes cationic at acidic pHs, such as endosomal entry" [15]. Thus, the charge change allows for the dissociation of particles and disruption of the membrane [15]. PEG lipids "improve LNP stability, regulate particle size, decrease immunogenicity and increase circulation time" [18]. Moreover, zwitterionic phospholipids improve stability and delivery efficiency, while cholesterol lipids promote membrane fusion and increase particle stability [18].

Lastly, electroporation, "a process in which brief electrical

pulses create transient pores in the plasma membrane that allow nucleic acids to enter the cellular cytoplasm," has become one of the most popular methods of delivery[19]. Electroporation, which has a wide range of applications in a variety of cells and organisms, including plants, creates pores in membranes, with the permeability allowing for effective delivery[19]. To conclude, there are many methods of delivering genome editing tools into cells that each have their own benefits and uses.

Applications of CRISPR-Cas Systems in Agriculture

The ease, efficiency, and versatility of CRISPR-Cas systems have made them the preferred genome editing tool and have permitted a wide range of applications in agriculture. As previously discussed, the phenotypes of plants are influenced by their genotype. Thus, as CRISPR-Cas systems can edit the genotype of plants, they can be used to manipulate plants to have desired phenotypes and traits that are beneficial. Major beneficial traits that can result from the use of CRISPR-Cas systems include disease resistance, herbicide resistance, and flowering time. The altering of flowering time can lead to improved growth with varying conditions of temperature, sunlight, and precipitation. Improvements have been demonstrated in staple crops like rice, maize, and soybean.

Rice

Rice is a popular food throughout cultures around the world, and it remains one of the most important crops that act as a food source. The improvement of this staple crop would thus have a tremendous global impact. For example, the OsProDH gene in rice encodes for a mitochondrial enzyme named proline dehydrogenase, which is responsible for the degradation of proline in rice[20]. Researchers used CRISPR/Cas9 to overexpress and knock out the OsProDH gene in rice[20]. As proline protects plants from stress, the gene knockout allowed the rice to have higher thermotolerance[20]. The improvement of thermotolerance, the ability of plants to survive in high or extreme temperatures, revealed the significance of gene editing. Due to changes brought about by widespread climate change and global warming, the modified rice crops may be able to better adapt to and survive in extreme temperatures.

Another improvement in rice crops through the use of CRISPR-Cas systems is the creation of salt-tolerant rice. As rice is important in many diets and nutrition, salinity levels have a large impact. High levels of salt can decrease agricultural productivity and the growth of rice crops through osmotic stress and the disruption of ionic homeostasis[21]. The OsNAC45 gene is responsible for regulating abscisic acid signal responses in rice, and gene modification can result in increased salt tolerance[20]. A study conducted aimed to analyze the influence of the OsNAC45 gene on salt tolerance in rice. Through CRISPR-Cas editing Cas9 protein, the researchers were able to create transgenic knockouts of OsNAC45 in rice plants in addition to the overexpression of the gene. The study concluded that OsNAC45 is responsible for the regulation of the ABA (abscisic acid) pathway and is required for salt tolerance[21]. Other studies have also demonstrated further advancements in rice, including greater resistance to blight, increased nutrition, increased sensory quality, and improved yields[22]. For example, through the modification of the SWEET genes in rice, which the bacterial blight pathogen exploits, researchers could improve the resistance to blight[22]. One study investigated specifically the OsSWEET11 and OsSWEET14 genes in reducing bacterial blight. The researchers utilized CRISPR-Cas9 systems to target the knockout of these genes, finding that the genes influence the plant's susceptibility to bacterial blight[23]. Similarly, others identified that the OsSWEET13 gene was targeted by a species of bacterial blight: Xanthomonas oryzae pv. Oryzae. The study worked to create a null mutation of the OsSWEET13 gene, suggesting that genome editing in the SWEET susceptibility genes can provide greater resistance to bacterial blight[24]. Blight is a plant disease that results in the browning, yellowing, and death of plant tissue, and decreases in susceptibility can produce increased yields.

Another advancement in agriculture through gene editing is the increase in essential nutrients. Examining one study closely, CRISPR-based genome editing was utilized to insert PSY genes originating from maize[25]. The insertion of these genes increased the amount of Vitamin A or Carotenoids in the rice species Kitaake. Carotenoid-enriched staple crops are known as golden crops. Through CRISPR-Cas systems, golden crops can be applied to a variety of crops, supporting the health and nourishment of many, as deficiencies in Vitamin A can result in xerophthalmia, night and pediatric blindness, and an increased risk of death[25]. Thus, there have been many enhancements and developments in rice. However, there are many more possibilities in not only rice but also a wide range of crops.

Soybean

Like in rice, CRISPR-Cas-based gene modification has proven to be highly impactful in soybean plants. For example, the flowering of a soybean plant can be modified to optimize available sunlight at higher latitudes, resulting in increased reproductive success. The flowering of soybeans was modified through the induction of a mutation in the E1 gene. The scientists found that "the truncation of the E1 protein prevented the inhibition of the GmFT2a/5a gene, increase its expression, and led to an earlier flowering time under long-day (LD) conditions"[20]. The targeted mutation created a photo-insensitive soybean variant, contributing to soybean growth in higher latitudes.

Another study involving the modification of the same gene found that it was possible to regulate the flowering times and

yield of soybean through various methods of knocking out genes[20]. Through the upregulation and downregulation of the gene, the scientists were able to change the flowering time to either support longer or shorter day conditions and thus create more applications for growing soybean as they could be easily adapted to a wide range of conditions. In another study, "triple knockouts of GmF3H1, GmF3H2, and GmFNSII-1 were effectively performed using a multiplex CRISPR/Cas9 system in soybean and resulted in an increase in isoflavone content within the plants that at the same time conferred enhanced resistance to the soybean mosaic virus (SMV)[20]. The increase in isoflavone content has beneficial aspects to human health through consumption as it can be applied to alternative therapy in hormonal disorders, cancers, cardiovascular diseases, and more[26].

Maize

On top of significant crops like rice and soybean, there has been great success in the genome editing of maize. Two examples include the gene knockout of ZmPHYC1 and overexpression of ZmPHYC2 to result in altering of flowering time and plant height[20]. Maize has also been improved through the modification of the ARGos8 gene, resulting in improved grain yield under the conditions of drought[22].

To summarize, although genetic modification has occurred in agriculture for thousands of years, CRISPR-Cas systems have allowed for significant improvements. Due to the greatly shortened time it takes for changes to occur to the plant genome, the agricultural field has been revolutionized as crops are now able to survive harsh conditions or be improved in nutrition, yield, and more through changes induced by CRISPR-Cas genome editing.

The Ethical Issues of CRISPR-Cas Systems in Agriculture

While there are clear applications and benefits to utilizing CRISPR-Cas systems in agriculture, the editing of the plant genome results in questions of its ethicality, as many raise the point that CRISPR-Cas systems may pose threats to both environmental and human health.

The Extinction of Pest Species

Regarding the issues of ethics that arise when debating the potential environmental threats of genome editing, some present the following question: is it ethical to deliberately drive a pest species to extinction?[8] From one standpoint, the use of genome editing tools to drive a pest species to extinction can be considered unethical, as it could largely have unintended consequences. These consequences include a loss of biodiversity and the disruption of an ecosystem. As it may be unknown what effects the extinction of a species could create, the removal of a pest species may result in the imbalance of an ecosystem as they may serve important ecological roles. Thus, their removal could create trophic cascades. In addition, this process could be considered unethical as editing the genome of animals to reduce the impacts on crops raises questions.

On the other hand, it is argued that utilizing CRISPR-Cas systems to eliminate a pest species in agriculture can be highly beneficial. As pests harm growing crops, their elimination may serve to keep the ecosystem in check and reduce crop losses. The reduction of crop losses can assist in food production and thus decrease the use of other harmful methods of growing crops, such as the use of pesticides and herbicides. Thus, there are positives and negatives associated with the elimination of pest species and the indirect impacts they have on crops and plants.

Environmental Risk and Degradation

Continuing with the ethicality of genetic modification in agriculture, the concern for environmental risk and degradation has arisen[27]. As previously detailed, gene modification can bring about the extinction of species, resulting in harm to ecosystems and their structures. The genetic modification of crops can bring about further environmental risks, such as the development of invasive species. Concerns are held about the potential for modified crops to become invasive, outcompeting native species and further disrupting ecosystems. Competition created by genetically modified crops can lead to decreases in gene flow and biodiversity due to the loss of cultivation of traditional crop varieties[28]. However, Weale argues that much consideration goes into the implementation of genetic modification in crops. A precautionary approach pairing the development of genetically modified crops and the testing of the crops in laboratories can predict the effects of modified crops on an ecosystem prior to their use in agriculture[27]. Thus, regulations can aid in the proper use of CRISPR-Cas systems in agriculture to reduce and minimize negative environmental consequences.

Effects on Human Health

Another question regarding the ethical issue of the use of CRISPR-Cas systems in agriculture arises when considering the influence of genome editing in plants on human health[27]. As utilizing genome editing tools can result in off-target effects, there is concern regarding its potential impacts on human health if crops with off-target modifications to the genome are consumed. In addition, there are concerns with the consumption of genetically modified crops as there is the idea that consuming genetically modified crops results in the transformation or change in the human genome regardless of whether the changes were intended or off target. Another concern was that genetically modified food could contain potential allergens, which could be another threat to health. Other arguments include the unknown and potentially dangerous long-term effects of plants modified

by CRISPR-Cas systems.

However, from another perspective, proponents argue that significant tests have been conducted to reveal potential effects on human health. The introduction of modern genome editing into agriculture has led to rigorous risk assessments. Food passes through regulatory and legislative procedures to receive authorization for public and environmental safety across different countries[28]. Examples of organizations that assess the risk of genetically modified crops include the United States Environmental Protection Agency and the United States Department of Agriculture. These assessments have worked to address uncertainties in the consumption of genetically modified crops and foods and have helped to ensure the safety of human consumption. While concerns about altering the genes of crops are understandable, some concerns ignore the long history of genetic modification in crops by artificial selection as modification has far predated the use of recent molecular tools. Although CRISPR-Cas systems differ from artificial selection, today's forms of genome editing speed up the process but can result in the same outcome. While the results of gene editing and artificial selection can be similar, there are further applications and opportunities for modern tools. For example, the creation of transgenes cannot happen in nature. In addition, supporters of genome editing argue that it can result in widespread crop yield improvement and nutritional improvement, providing food surpluses throughout the world. These improvements help to address malnutrition and hunger. Thus, while there are different perspectives regarding the ethicality of using CRISPR-Cas systems in agriculture, both sides can be understood and considered.

Discussion

Reflecting on the limitations of the study, there were two main influencing factors. First, some sources contained bias as they strongly supported the use of genome editing in agriculture. The negatives of crop modification may not have been as well expressed as the positives. The other main limitation was limited access to sources. While there is a large abundance of research in genome editing, there was restricted or paid access to some journals, articles, and more. However, these limitations did not play a large role in the overall production of the paper, and this study nearly fully met the research objective of analyzing the development of genetic modification technology, their applications in agriculture, and ethical concerns with their use. While this literature review targets ethical concerns and presents various sides and perspectives to different arguments, further studies could be performed to thoroughly analyze the long-term impacts of the use of genome editing tools in agriculture. Due to the recency of the technology, little is known about potential harm to health over a long-term period and the consequences for the environment. Additionally, this review serves as a foundation for further research as there are constantly new developments in genomic editing. Thus, there soon may be new technology and methods to alter the genome of crops to bring about targeted traits and benefits.

In this review, CRISPR-Cas systems were more thoroughly analyzed due to their advantages in ease of design, efficiency, precision, and versatility compared to ZFNs and TALENs. For example, CRISPR-Cas systems are mainly composed of the Cas protein and guide RNA. On the other hand, ZFNs are composed of the Fok1 enzyme, peptide links, and large subunits of zinc fingers formed through the combination of multiple zinc fingers. Similarly, TALENs are made up of the Fok1 enzyme and consist of many amino acid repeats. Thus, CRISPR-Cas systems are capable of targeting specific DNA sequences with fewer components. Today, there are many problems that have contributed to the necessity of utilizing genome editing tools in agriculture. For example, as climate change occurs, the changes in temperature, precipitation, and more can be compensated for by utilizing genome editing in plants, as the editing of genes can result in increased tolerance to drought, salt, temperature, and other conditions. Furthermore, as the population increases at a significant rate, the need for increased nutritional value and yield becomes highlighted. Improved nutritional value and yields also play an essential role in the growing trend of plant-based meats and such dietary alternatives. Genome editing tools, including ZFNs, TALENs, and CRISPR-Cas systems, have revolutionized the field of agriculture, and they have and will continue to play a large role in addressing these concerns. Of these, CRISPR-Cas systems serve the most prominent role, as their ease of use and efficiency provide advantages over ZFNs and TALENs. Furthermore, their ability to identify and target specific sequences within various genomes reveals their wide range of applications in agriculture, as shown through rice, soybean, maize, and other crops. The increase in the use of CRISPR-Cas gene modification has thus increased ethical questions as societal changes have been brought about by modern technology.

Methods

In order to conduct this literature review, research databases including Google Scholar, Pub Med, the National Institutes of Health, and Journal Storage (JSTOR) were utilized. Examples of keywords and search terms included applications of CRISPR-Cas systems in agriculture and agricultural genetic modification. Filters and exclusion criteria were set primarily based on the recency of the papers. Sources considered in this study ranged from 2009 to 2024. Additional criteria for gathering data and information included checking the credibility of the publisher and ensuring that papers were peer-reviewed. Thus, relevant, peer-reviewed, and credible articles and papers published in scientific journals were selected for review. Keywords for searching included agriculture, CRISPR in agriculture, genetic modification

in agriculture, ZFNs, TALENs, ethics of CRISPR, CRISPR applications, and examples of sources included experiments on genetic modification in specific crops and studies on genome editing methods.

Additionally, the extraction of data from sources involved analyzing key findings and definitions. Through a combination of thematic analysis and narrative synthesis to establish similarities and connections across articles, key findings and examples were put together to form a cohesive paper. Overall, data was extracted from the sources to provide a comprehensible overview of genetic modification in agriculture, specifically in CRISPR-Cas systems.

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