CURRENT STATUS OF GENOME EDITING IN PLANTS AND ITS APPLICATION: A REVIEW.

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SUMMARY

In today's world, almost one billion people suffer from chronic malnourishment, while at the same time our agricultural systems are degrading, exacerbated by the loss of biodiversity and the increasing uncertainties of climate change. With the global population projected to exceed 9 billion by 2050, contemporary agriculture will face enormous challenges, requiring crops with higher yields and of improved quality, and needing fewer inputs. Although conventional breeding is currently the most widely used approach in crop improvement, it is labour intensive and it usually takes several years to progress from the early stages of screening phenotypes and genotypes to the first crosses into commercial varieties. Genetically modified (GM) crops that have beneficial traits are produced by the transfer of genes or gene elements of known function into elite crop varieties. Despite the promise that GM crops hold for global food security, their use is affected by largely unsubstantiated health and environmental safety concerns. Thus, the introduction of genome editing into modern breeding programs have facilitated rapid and precise crop improvement. Genome editing could be used to edit the genome of any organism. It is the manipulation of the specific gene loci to gain genome modifications, such as insertions, deletions or point mutations. Editing genomes with the Bacterial Immune System Technology (BIST) has emerged as a powerful technology for genome editing and is now widely used in research to explore gene function. This technology has been increasingly applied to the study and treatment of crop pests and diseases and human diseases. The technology of genome editing involves cuts at specific DNA sequences with enzymes called engineering nucleases.

Keywords: Genome editing, ZFN, TALEN, CRISPR-Cas 9, Meganucleases.

The recent advances in genetics analysis and genetic manipulation through Genome editing have shown the greatest potential for rapid and efficient editing of genomes in plant species (Yang *et al*., 2016; Xuan *et al*., 2017). Genome editing has become a powerful tool for functional characterization of plant genes and genetic improvement of agricultural crops (Yang *et al*., 2016; Xuan *et al*., 2017). The impact of innovations in high-throughput DNA sequencing and in genome editing have been felt broadly, from work on model organisms, to evolutionary studies, to improvement of food organisms, to medical applications (Carroll, 2017). Twentieth-century advances in plant and animal breeding and agricultural practices did much to help meet the increasing food, fiber, feed, and fuel needs of ever-increasing world population (CAST, 2018). As population growth continues through this century, those needs continue to increase while the amount of land and water available for production decreases. In addition, climate change is impacting land and water availability further and altering the incidence of droughts, floods, and other severe weather events, as well as the distribution and prevalence of diseases and pests. Meeting the increasing needs of the world population in the face of these challenges, sustainably, is a daunting yet essential task (CAST, 2018).

Continued successes in crop and livestock improvement will be critical. Resistance to pests and diseases, tolerance to adverse environmental conditions, and improved nutritional quality will be essential. In addition, adapting plants to increase their efficacy for environmental remediation and improving animals for use as models for human disease will be important. Meeting the needs of the increasing world population will also depend on social and engineering innovations, including changes to improve food distribution, decrease socio-economic disparities, and mitigate barriers to trade and moderate political and market dependencies. The power of genome editing however, suggests that with conducive social and regulatory conditions in place, it can substantially increase the positive impacts of plant and animal breeding on human welfare and sustainability (CAST, 2018).

BACKGROUND OF GENOME EDITING

Genome editing, or genome engineering, is a type of genetic engineering in which deoxyribonucleic acid (DNA) is inserted, deleted, modified or replaced in the genome of a living organism. Unlike early genetic engineering techniques that randomly insert genetic material into a host genome, genome editing targets the insertions to site specific locations. Genome editing could be used to edit the genome of any organism. It is the manipulation of the specific gene loci to gain genome modifications, such as insertions, deletions or point mutations (Cong *et al*., 2013). Genome editing is the alteration of a targeted DNA sequence, achieved by cutting the DNA molecule at a selected point, which activates the cell's own repair system and thus results in small deletions or insertions (Akbari, 2015). This is commonly used to inactivate a target gene or target sequence. When, at the same time, exogenous DNA is introduced, this can support the repair at the target site and enable a predetermined exchange of single or multiple nucleotides (targeted mutagenesis), for example to replicate or rectify a naturally occurring mutation. In this eventuality, the genome-edited organism would be indistinguishable in this specific place of the genome from an organism in which the mutation occurred naturally. The same method can also be used to insert or exchange fragments of foreign DNA at a predetermined site in the genome, generally then resulting in an organism carrying a transgene. Genome editing can also be defined as a collection of advanced molecular biology techniques that facilitate precise, efficient, and targeted modifications at genomic loci (Chen and Gao, 2013; Gao, 2015). Advances in recent years have made genome editing applicable in many contexts and for many purposes, including plant and animal improvement (CAST, 2018). The common methods for such editing use engineered nucleases, or "molecular scissors". These nucleases create site-specific Double-Strand Breaks (DSBs) at desired locations in the genome. The induced double-strand breaks are repaired through non-homologous end joining (NHEJ) or homologous recombination (HR), resulting in targeted mutations ('edits') (Barrangon, 2015).

Basic molecular biology principle behind gene editing

The principle behind genome editing involves the manipulation of nucleic acid in the laboratory. This is based on their physical and chemical properties, which in turn are reflected in their biological function. Naturally, DNA is a stable molecule, the stability is often provided by the robust repetitive phosphate-sugar backbone in each DNA strand.in which the phosphate links the 5' position of one sugar to the 3' position of the next. The link between all the atoms i.e. phosphorus, oxygen and carbon are all covalent bond (Jerry and Malcolm, 2008). These are divided into endonucleases and exonucleases, the former attack internal site in a DNA strand, while the later nibbles away at the ends. It was a discovery of restriction endonucleases (or restriction enzyme), which cut DNA strand at a specific positions, coupled with DNA ligases, which can join two double-stranded DNA molecules together, that opened up the possibility of recombinant DNA technology ('genetic engineering') (Jerry and Malcolm, 2008).

Double strand break repair

Genome editing relies on the concept of DNA Double Stranded Break (DSB) repair mechanics. There are two major pathways process that repair DSB; non-homologous end joining (NHEJ) and homology directed repair (HDR) (Barrangon, 2015). NHEJ uses a variety of enzymes to directly join the DNA ends while the more accurate HDR uses a homologous sequence as a template for regeneration of missing DNA sequences at the break point. This can be exploited by creating a vector with the desired genetic elements within a sequence that is homologous to the flanking sequences of a DSB. This will result in the desired change being inserted at the site of the DSB. While HDR based gene editing is similar to the homologous recombination based gene targeting, the rate of recombination is increased by at least three orders of magnitude (Barrangon, 2015).

Why genome editing?

In today's world, almost one billion people suffer from chronic malnourishment, while at the same time our agricultural systems are degrading, exacerbated by the loss of biodiversity and the increasing uncertainties of climate change (Foley *et al*., 2011). With the global population

projected to exceed 9 billion by 2050, contemporary agriculture will face enormous challenges, requiring crops with higher yields and of improved quality, and needing fewer inputs (Tilman *et al*., 2017). Although conventional breeding is currently the most widely used approach in crop improvement, it is labour intensive and it usually takes several years to progress from the early stages of screening phenotypes and genotypes to the first crosses into commercial varieties.

Genetically modified (GM) crops that have beneficial traits are produced by the transfer of genes (transgenes) or gene elements of known function into elite crop varieties. Despite the promise that GM crops hold for global food security, their use is affected by largely unsubstantiated health and environmental safety concerns. Government regulatory frameworks that aim to safeguard human and environmental biosafety have led to significant cost barriers to the rapid widespread adoption of new GM traits (Prado *et al*., 2014). As a result, the advantages of GM traits have been restricted to a small number of cultivated crops.

The risks involved in altering genomes through the use of genome-editing technology are significantly lower than those associated with GM crops. This is because most edits alter only a few nucleotides, producing changes that are not unlike those found throughout naturally occurring populations. Once the genomic-editing agents have segregated out, there is no way to distinguish between a 'naturally occurring' mutation and a gene edit. Thus, the introduction of genome editing into modern breeding programs have facilitated rapid and precise crop improvement (Zhang *et al*., 2018).

USE OF ENGINEERED NUCLEASES

The key to genome editing is creating a DSB at a specific point within the genome. Commonly used restriction enzymes are effective at cutting DNA, but generally recognize and cut at multiple sites. To overcome this challenge and create site specific DSB, four distinct classes of nucleases have been discovered and bioengineered to date. These are: the Zinc finger nucleases (ZFNs),

transcription activator like effector nucleases (TALEN), the clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) system and Meganucleases.

Zinc-finger nuclease (ZFNs)

Zinc-finger nuclease (ZFNs) stands for the DNA binding part of ZFNs it is made of zinc-finger proteins, which bind to about three DNA bases. Different patterns of zinc finger proteins bind to altered sequences of DNA, although this is hard to predict without testing them first. The nuclease part of ZFNs is normally a Fok1 nuclease, which cuts the DNA. Two FokI molecules come together to make a cut in the DNA, so a pair of ZFNs are made, one binding to each strand (Bibikova *et al*., 2003; Carroll, 2011).

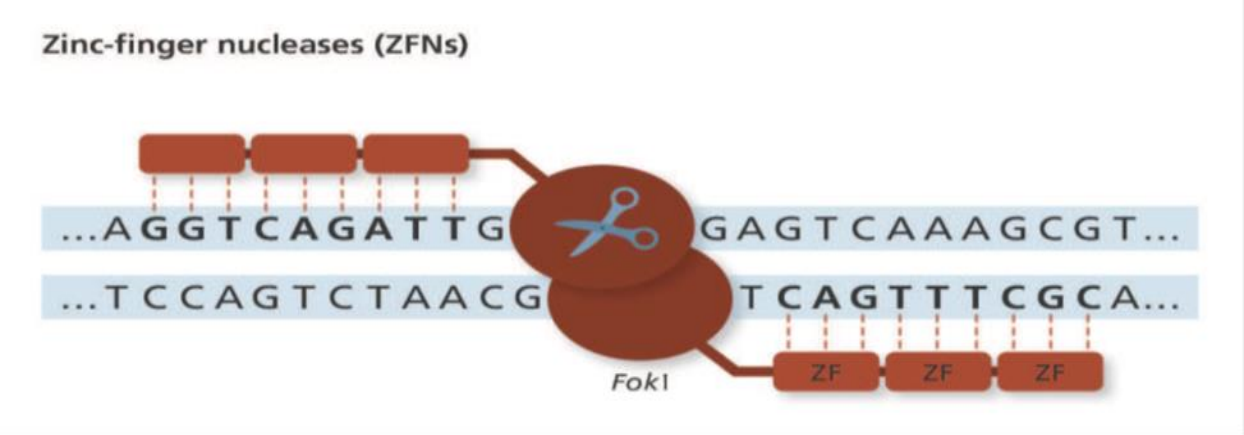

Figure 1: Illustration showing the components of zinc-finger nucleases (ZFNs).
Source: Genome Research limited (www.yourgenome.org/facts/what-is-genome0editing. Accessed January, 2020).

Transcription activator like effector nucleases (TALEN)

Transcription activator-like effector nucleases (TALENs) are specific DNA binding proteins that feature an array of 33 or 34-amino acid repeats. TALENs are artificial restriction enzymes designed by fusing the DNA cutting domain of a nuclease to TALE domains, which can be tailored to specifically recognize a unique DNA sequence. These fusion proteins serve as readily targetable

"DNA scissors" for gene editing applications that enable to perform targeted genome modifications such as sequence insertion, deletion, repair and replacement in living cells (Gaj *et al*., 2013).

The DNA binding domains, which can be designed to bind any desired DNA sequence, comes from TAL effectors, DNA-binding proteins excreted by plant pathogenic *Xanthomanos app*. TAL effectors consists of repeated domains, each of which contains a highly considered sequence of 34 amino acids, and recognize a single DNA nucleotide within the target site. The nuclease can create double strand breaks at the target site that can be repaired by error-prone non-homologous end-joining (NHEJ), resulting in gene disruptions through the introduction of small insertions or deletions. Each repeat is conserved, with the exception of the so-called repeat variable di-residues (RVDs) at amino acid positions 12 and 13. The RVDs determine the DNA sequence to which the TALE will bind. This simple one-to-one correspondence between the TALE repeats and the corresponding DNA sequence makes the process of assembling repeat arrays to recognize novel DNA sequences straight forward. These TALENs can be fused to the catalytic domain from a DNA nuclease, FokI, to generate a transcription activator like effector nuclease (TALEN). The resultant TALEN constructs combine specificity and activity, effectively generating engineered sequence-specific nucleases that bind and cleave DNA sequences only at pre-selected sites. The TALEN target recognition system is based on an easy-to-predict code. TAL nucleases are specific to their target due in part to the length of their 30+ base pairs binding site. TALEN can be performed within a 6 base pairs range of any single nucleotide in the entire genome (Perez-Quintero *et al*., 2013).

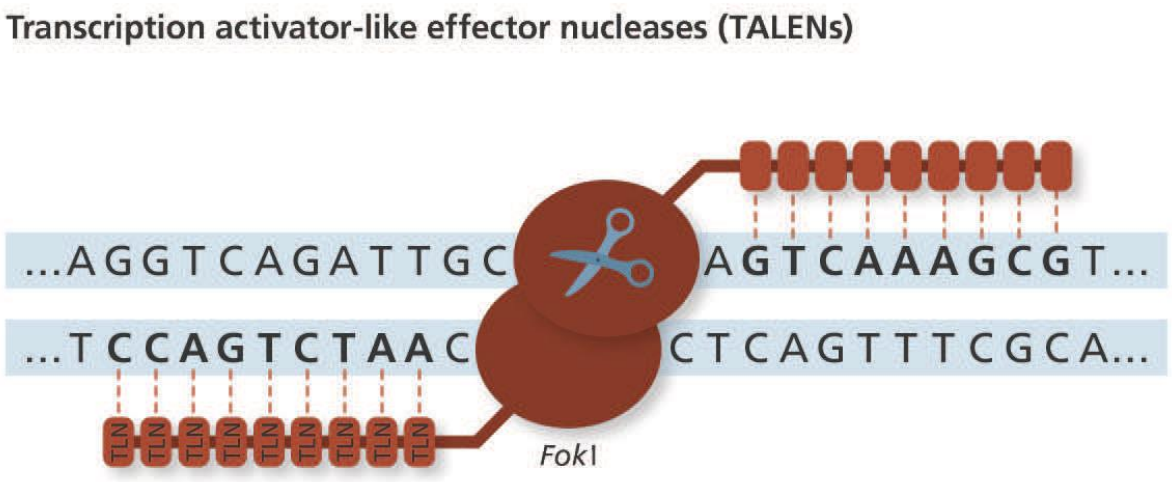

Figure 2: Illustration showing the components of Transcription activator-like effector nucleases (TALENs).

Source: Genome Research limited (www.yourgenome.org/facts/what-is-genome0editing. Accessed January, 2020).

CRISPR-Cas9 System

The gene editing process includes the generation of a double stranded break (DSB) at the targeted DNA sequence. This DSB subsequently triggers two competing DNA repair systems which are homology-directed repair (HDR) or non-homologous end-joining (NHE). NHEJ process can give rise to insertion or deletion (termed INDELs) in mammalian cells, either of which could change the protein coding sequences. In contrast, HDR involves homologous recombination with a donor DNA sequence to then introduce precise DNA mutations or the insertion of specific sequences in the targeted locus, such as the insertion of the DNA sequence encoding Green Fluorescence Protein (GFP). The application of the CRISPR/Cas9 approach only requires designing the guide RNA (gRNA) sequence complementary to any desired target region to direct the Cas9 nuclease to this site (Lin *et al*., 2014).

The specificity of the CRISPR/Cas9 system is produced by the involvement of two essential components which are the Cas9 nuclease and the required gRNA. The gRNA determines the specificity for a target DNA sequence through base-pair mediated binding to complementary DNA sequence. The binding of the gRNA then co-localizes Cas9 at the same site, which leads to cuts in the DNA backbone and the generation of DSB at the site (Hsu *et al*., 2014).

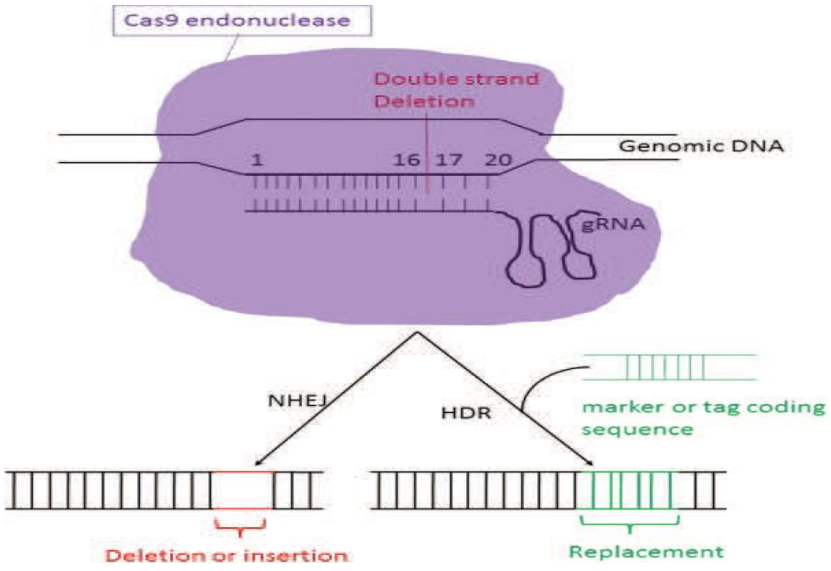

Figure 3: CRISPR working mechanism.

Source: Genome Research limited (www.yourgenome.org/facts/what-is-genome0editing. Accessed January, 2020).

Guide RNA hybridizes with 20bp genomic DNA sequence and directs Cas9 endonuclease (colored in pink) to generate a double strand break which is usually located between 16 and 17bp regions in the target sequence. Subsequently, DNA mutagenesis is generated from DNA repair process, through either the (NHEJ) or the (HDR) mechanism. The final mutation could include insertion or deletion with several base pairs of DNA sequences (NHEJ pathway), or replacement with a particular DNA sequence used as a marker for further study (encoding for a fluorescence protein,

tag protein, antibiotics, or the recognition sequence for a restriction enzyme digestion) (Doench *et al*., 2016).

Meganucleases

Meganucleases discovered in the late 1980s, are enzymes in the endonuclease family which are characterized by their capacity to recognize and cut large DNA sequences (from 14 to 40 base pairs) (23). The most widespread and best known meganucleases are the proteins in the LAGLIDADG family, which owe their name to a conserved amino acid sequence. Meganucleases found commonly in microbial species, have the unique property of having very long recognition sequences (>14bp) thus making them naturally very specific (Smith *et al*., 2006; De-Souza, 2012). However, there is virtually no chance of finding the exact meganuclease required to act on a chosen specific DNA sequence. To overcome this challenge, mutagenesis and high throughput screening methods have been used to create meganuclease variants that recognize unique sequences (Seligman *et al*., 2002; Smith *et al*., 2006). Others have been able to fuse various meganucleases and create hybrid enzymes that recognize a new sequence (Chevalier *et al*., 2012). Yet others have attempted to alter the DNA interacting amino acids of the meganuclease to design sequence specific meganucelases in a method named rationally designed meganuclease (De-Souza, 2012). Another approach involves using computer models to try to predict as accurately as possible the activity of the modified meganucleases and the specificity of the recognized nucleic sequence (Ashworth *et al*., 2010). A large bank containing several tens of thousands of protein units has been created. These units can be combined to obtain chimeric meganucleases that recognize the target site, thereby providing research and development tools that meet a wide range of needs (fundamental research, health, agriculture, industry, energy, etc.) These include the industrial scale production of two meganucleases able to cleave the human XPC gene; mutations in this gene result in *Xeroderma pigmentosum*, a severe monogenic disorder that predisposes the patients to skin cancer and burns whenever their skin is exposed to UV rays (Redondo *et al*., 2008). Meganucleases have the benefit of causing less toxicity in cells than methods such as Zinc finger nuclease (ZFN), likely because of more stringent DNA sequence recognition (De-Souza, 2012). However, the

construction of sequence-specific enzymes for all possible sequences is costly and time consuming, as one is not benefiting from combinatorial possibilities that methods such as ZFNs and TALEN-based fusions utilize.

APPLICATION OF GENOMIC EDITING

Genome editing using Meganuclease, ZFNs, and TALEN provides a new strategy for genetic manipulation in plants and are likely to assist in the engineering of desired plant traits by modifying endogenous genes. For instance, site specific gene addition in major crop species can be used for 'trait stacking' whereby several desired traits are physically linked to ensure their co-segregation during the breeding processes. Progress in such cases has been recently reported in *Arabidopsis thaliana* and *Zea mays*. In *Arabidopsis thaliana*, using ZFN-assisted gene targeting, two herbicide-resistant genes (tobacco acetolactate synthase SuRA and SuRB) were introduced to SuR loci with as high as 2 % transformed cells with mutations. In Zea mays, disruption of the target locus were achieved by ZFN induced DSBs and the resulting NHEJ. ZFN was also used to drive herbicide tolerance gene expression cassette (PAT) into the targeted endogenous locus IPK1 in this case. Such genome modification observed in the regenerated plants has been shown to be inheritable and was transmitted to the next generation.

Modifications have been made on extremely wide variety; the production of plants that are resistant to insect attack is one of the applications that have received a lot of publicity. Gene from the bacterium *Bacillus thuringiensis* coding for an insecticidal toxin which is highly poisonous to certain group of insects are inserted into crop plant. The plant becomes resistant to insect attack after the expression of this gene by a plant (e.g. cotton) and thus substantially reduces the need for spraying the crop with insecticide.

Resistance to herbicides (principally glyphosate) is another widely published application. This is often confused with insecticide resistance, but the cases are quite different. This enables the farmer

to spray the crop with a broad spectrum herbicide to eliminate weeds when the gene for herbicide resistance is expressed in the crop plant.

The introduction of genes that convey tolerance to environmental factors such as salt or cold, taste or colour, or influence of texture and also modifications to improve the nutritional content of plant e.g. the development of "Golden-Rice" which accumulates provitamin A due to the integration of genes from various sources, can make a major contribution to alleviating vitamin A deficiency.

A potentially successful example of the application of genome editing techniques in crop improvement can be found in banana, where scientists used CRISPR/Cas9 editing to inactivate the endogenous banana streak virus in the B genome of banana (*Musa* spp.) to overcome a major challenge in banana breeding.

In addition, TALEN-based genome engineering has been extensively tested and optimized for use in plants by a U.S. food ingredient company, Calyxt, to improve the quality of soybean oil products and to increase the storage potential of potatoes.

Using the CRISPR/Cas9 technology, Jiang et al. (Jiang *et al*., 2017) have obtained "a biotech" oil from *Camelina sativa* seeds with an improved fatty acid composition, which makes it more beneficial to human health, more resistant to oxidation, and more appropriate for the production of certain commercial chemicals including biofuels (Jiang *et al*., 2017).

Recently, the research effort of Institute Agricultural Research (IAR) has reportedly yielded positive results. The results of scientific effort applying modern techniques of genetic engineering also called genetic modification has led to the development of cowpea variety that can protect itself against the destructive efforts of insects. This Pod Borer Resistant Cowpea (PBR - Cowpea) is genetically modified for Lepidopteran insect pest (*Maruca vitrata*). This makes it possible for farmers to grow cowpea in their fields with very minimal insecticidal sprays.

Wheat genotypes resistant to powdery mildew disease were obtained by TALEN and CRISPR/Cas9-mediated genome editing on mildew resistance locus O (MLO) (Wang *et al*., 2014). Genome editing technologies have also been used to produce plants resistant to bacterial leaf blight, caused by *Xanthomonas oryzae* pv. *oryzae* (Li *et al*., 2012).

CONCLUSION

Although much progress has been made in genome editing technology in the last few years, some problems remain to be solved: off-target effects, influence of chromatin structure, side effects on nearby genes, mechanisms underlying the different effects of different RNAs on mutation efficiency, and methods for efficient delivery in polyploidy plants. Despite these challenges, with the tremendous enthusiasm of the research community, gene editing technologies as represented will improve rapidly. This simple, affordable, and elegant genetic scalpel is expected to be widely applied to enhance the agricultural performance of most crops in the near future. With the progress already made in the development of genome-editing tools and the development of new breakthroughs, genome editing promises to play a key role in speeding up crop breeding and in meeting the ever-increasing global demand for food. In this review, we have described the current applications of four standard genome-editing techniques for crop improvement and which also have great potential in agriculture. Given the availability of a variety of genome-editing tools with different application, it is important to consider the optimal system for a given species and purpose. Once appropriate genome editing tools have been selected, the target sequences are designed and introduced into the most suitable vectors, and the appropriate genetic carriers (DNA, RNA, or RNPs) for delivery are selected. After the genetic carriers has entered the target plant cells, the target sequences will be modified and edited and will ultimately give rise to edited plants.

REFERENCES

1. **Akbari O. S. 2015.** Safeguarding gene drive experiments in the laboratory. *Science* **349**, 927929.
2. **Ashworth J., Taylor G. K., Havranek J. J., Quadri S. A., Stoddard B. L. and Baker D. 2010.** "Computational reprogramming of homing endonuclease specificity at multiple adjacent base pairs". Nucleic Acids Research. **38** (16): 5601–8. doi:10.1093/nar/gkq283. PMC 2938204. PMID 20435674.
3. **Barrangou R. 2015**. The roles of CRISPR-Cas systems in adaptive immunity and beyond. Curr Opin Immunol. 32:36-41.
4. **Bibikova M., K., Beumer J. K., Trautman and Carroll D. 2003.** Enhancing gene targeting with designed zinc finger nucleases. *Science* 300:764, doi:10.1126/science.1079512.
5. **Carroll D. 2011.** Genome engineering with zinc-finger nucleases. *Genetics* 188:773–782, doi:10.1534/genetics.111.131433.
6. **Carroll D. 2017.** Genome editing: past, present, and future. *Yale Journal of Biology and Medicine* 90: pp.653-659.
7. **Cong L., Ran F. A., Cox D., Lin S., Barretto R., Habib N., Hsu P. D., Wu X., Jiang W., Marraffini L. A. and Zhang F. 2013.** Multiplex genome engineering using CRISPR/Cas systems. Science. 339(6121):819-23.
8. **Chen K. and Gao C. 2013**. Targeted genome modification technologies and their applications in crop improvements. *Plant Cell Rep*.**33**, 575–83.
9. **Chevalier B. S., Kortemme T., Chadsey M. S., Baker D., Monnat R. J. and Stoddard B. L. 2002.** "Design, activity, and structure of a highly specific artificial endonuclease". Molecular Cell. **10** (4): 895–905. doi:10.1016/S1097- 2765(02)00690-1. PMID 12419232.
10. **CAST. 2018.** Genome Editing in Agriculture: Methods, Applications, and Governance. *A paper in the series on The Need for Agricultural Innovation to Sustainably Feed the World by 2050:* ISSUE 60: 1-6.

11. **De-Souza N. 2012.** "Primer: genome editing with engineered nucleases". Nature Methods. **9** (1): 27. doi:10.1038/nmeth.1848. PMID 22312638.
12. **Doench J. G., Fusi N., Sullender M., Hegde M., Vaimberg E. W., Donovan K. F., Smith I., Tothova Z., Wilen C., Orchard R., Virgin H. W., Listgarten J. and Root D. E. 2016.** Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. Nat Biotechnol. 34(2):184-191.
13. **Foley J. A., Ramankutty N., Brauman K. A., Cassidy E. S., Gerber J. S. and Johnston M. 2011.** Solutions for a cultivated plants. *Nature* **42**, 478:337**.**
14. **Gao C. 2015.** Genome editing in crops: from bench to field. Natural Science Review **5**, 2:13.
15. **Gaj T., Gersbach C. A. and Barbas C. F. 2013.** "ZFN, TALEN, and CRISPR/Cas based methods for genome engineering". Trends in Biotechnology. 31 (7): 397–405. doi:10.1016/j.tibtech. 04.004. PMC 3694601. PMID 23664777.
16. **Hsu P. D., Lander E. and Zhang S. 2014.** Development and applications of CRISPRCas9 For genome engineering. Cell. 157(6):1262-78.
17. **Lin S., Staahl B. T., Alla R. K. and Doudna J. A. 2014.** Enhanced homology directed Human genome engineering by controlled timing of CRISPR/Cas9 delivery. Elife. 15,3: e04766.
18. **Prado J. R., Segers G., Voelker T., Carson D., Dobert R. and Phillips J. 2014.** Genetically engineered crops: from idea to product. Annual Review of Plant Biology 90, 65:769
19. **Pérez-Quintero A. L., Rodriguez-R L. M., Dereeper A., López C., Koebnik R., Szurek B. and Cunnac S. 2013.** "An improved method for TAL effectors DNA-binding sites prediction reveals functional convergence in TAL repertoires of *Xanthomonas oryzae* strains".**8**(7):e68464.Bibcode:2013PLoSO...868464P. doi:10.1371/journal.pone.0068464. PMC 3711819. PMID 23869221.
20. **Redondo P., Prieto J., Muñoz I. G., Alibés A., Stricher F., Serrano L., Cabaniols J.**

P., Daboussi F., Arnould S., Perez C., Duchateau P., Pâques F., Blanco F. J. and Montoya G. 2008. "Molecular basis of xeroderma pigmentosum group C DNA recognition by engineered meganucleases". Nature. 456 (7218): 107–11. Bibcode:2008Natur. 456..107R. doi:10.1038/nature07343. PMID 18987743.

21. **Seligman L. M., Chisholm K. M., Chevalier B. S., Chadsey M. S., Edwards S. T., Savage J. H. and Veillet A. L. 2002.** "Mutations altering the cleavage specificity of a homing endonuclease". Nucleic Acids Research. **30**(17): 3870–9. doi:10.1093/nar/gkf495. PMC 137417. PMID 12202772.
22. **Smith J., Grizot S., Arnould S., Duclert A., Epinat J. C., Chames P., Prieto J., Redondo P., Blanco F. J., Bravo J., Montoya G., Pâques F. and Duchateau P. 2006.** "A combinatorial approach to create artificial homing endonucleases cleaving chosen sequences". Nucleic Acids Research. 34 (22): e149. doi:10.1093/nar/gkl720. PMC 1702487. PMID 17130168.
23. **Stoddard B. L. 2005.** "Homing endonuclease structure and function". Quarterly Reviews of Biophysics. **38** (1): 49–95. doi:10.1017/s0033583505004063. PMID 16336743.
24. **Tilman D., Balzer C., Hill J. and Befort B. L. 2011.** Global food demand and the sustainable intensification of agriculture. Proceedings of *Natural Academic Science* U S A. **4**, 108:20260.
25. **Xuan L., Surui W., Jiao X., Chun S. and Jianhe W. 2017**. Application of CRISPR/Cas9 in plant biology. *Acta Pharmaceutica Sinica B;* 7(3):292–302.
26. **Yang X., Rongbin H., Kaitlin J. P., Gerald A. T. and Degao L. (2016**. Advances and perspectives on the use of CRISPR/Cas9 systems in plant genomics research. *Current Opinion in Plant Biology*, 30:70–77.
27. **Zhang, Y., Massel, K., Godwin, I. D. and Gao, C. 2018.** Applications and potentials of genome editing in crop improvement. *Genome Biology* 19:2-10
28. **Jiang W. Z., Henry I. M., Lynagh P. G., Comai L., Cahoon E. B. and Weeks D. P.**

2017. "Significant enhancement of fatty acid composition in seeds of the allohexaploid, Camelina sativa, using CRISPR/Cas9 gene editing," *Plant Biotechnology Journal*, vol. 15, no. 5, pp. 648–657.

29. **Wang Y., Cheng X. and Shan Q. 2014.** "Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew," *Nature Biotechnol*, vol. 32, pp. 947–952.
30. **Li T., Liu B., Spalding M. H., Weeks D. P. and Yang B. 2012**. "High-efficiency TALEN-based gene editing produces disease resistant rice," *Nature Biotechnology*, vol. 30, no. 5, pp. 390–392.
31. **Jeremy W. D. and Malcolm V. S. 2008.** From Genes to Genomes: Concepts and Applications of DNA Technology, Second edition, John Wiley and Sons, Ltd pp 394.