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CRISPR Cas9 Genome Editing System for Global Food Security

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Genome editing in agriculture is expected to accelerate the second green evolution by ushering in a new era of precision plant breeding. This technology is regarded as one of the most groundbreaking plants breeding approaches, providing alternatives to the stringent rules of "genetically modified organisms" (GMOs). Conventional plant breeding methods have traditionally improved several crops. It is, however, labor- and time-consuming, and GM technology presents environmental and health issues. As a result, better breeding procedures are critical. As a result, clustered, regularly interspaced short palindromic repeats (CRISPR)-associated (Cas) nucleases may provide us with more opportunities to speed up the exact process of plant breeding to produce plants with desirable traits. Molecular breeders are now widely using these approaches to introduce diversity and alter the DNA of crops to generate favorable features that will end world hunger.

Introduction

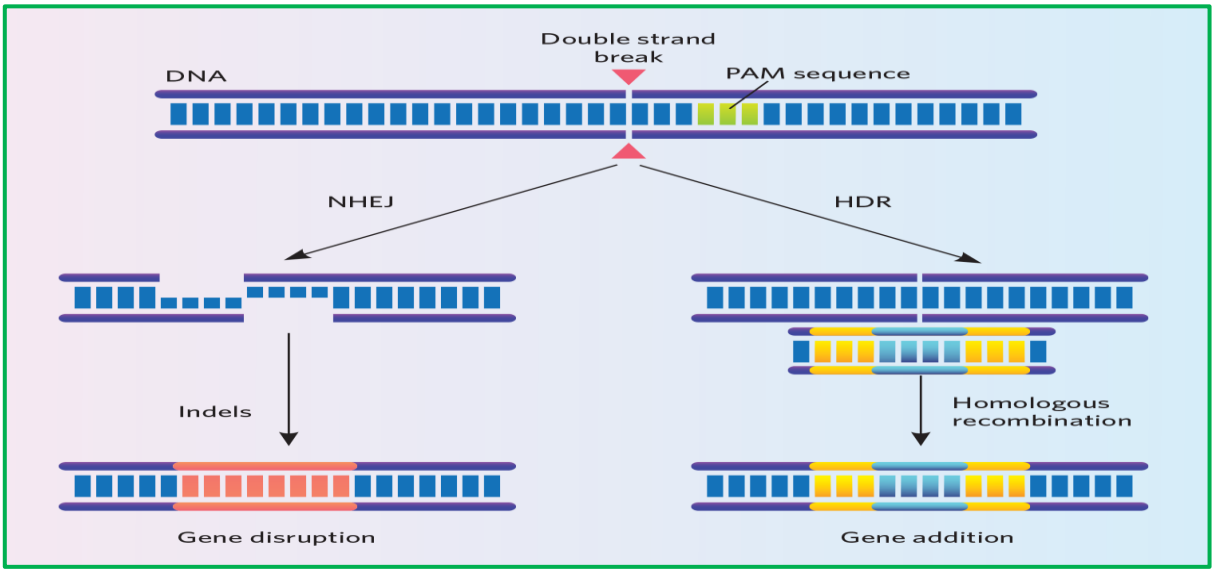
The rising world population, which will reach 10 billion by 2050, is humanity's most serious challenge. We must increase global food production by 60-100% to secure food and nutrition security (FAO state 2016). However, climate change, natural disasters, agricultural land loss, and biotic and abiotic stress contribute to a drop in agricultural production. As a result, we must develop cutting-edge technology to promote crop development in the production elements. Genetic engineering, transgenics, and mutagenesis have become increasingly popular over the last two to three decades for crop improvement, understanding gene function, and learning about plant biology fundamentals. However, the use of genome editing has increased in recent years as well. Plants and animals can now be precisely altered with site-specific nucleases. The Cas9protein nuclease from the Streptococcus pyogenes bacteria is used in the CRISPR gene-editing method, which stands for clustered regularly interspaced short palindromic repeats. Cas9is essentially an enzyme that uses CRISPR sequences to recognize and break specific DNA strands that are complementary to the CRISPR sequence. The CRISPR/Cas9system allows organisms' prokaryotic and eukaryotic genomes to be altered. CRISPR-Cas9is a novel technology that will enable geneticists, molecular biologists, and researchers to efficiently edit particular areas of the genomes of diverse organisms to build a better and more developed genome that may improve the individual creature. It is presently the most simple, sophisticated, accurate, and adaptive genetic alteration approach, ushering in a new study era. This approach can target single or multiple DNA spots.

History of CRISPR

The CRISPR-Cas system was initially identified as an adaptive immune response that protects prokaryotes from bacteriophages (viral infections). In 1987, Yoshizumi Ishino, a researcher at Osaka University, observed this trait in E. coli for the first time. They function

by changing the nucleic acids of invading bacteriophages. In 2012, a group of scientists led by George Church, Jennifer Doudna, Emmanuelle Charpentier, and Feng Zhang modified specific genome regions using CRISPR-Cas9. As a result, they were all awarded the Nobel Prize in 2020. Until now, enzymatically inactive Cas9, a variant of the CRISPR-Cas9nuclease utilized for targeted epigenome editing, has been employed (dCas9). Epigenome alteration can either activate or repress transcription. From bacterial immunity to human designer babies, it all began in China. In numerous ways, the invention of CRISPR CAS9has revolutionized genome editing.

Mechanism of CRISPR Cas9



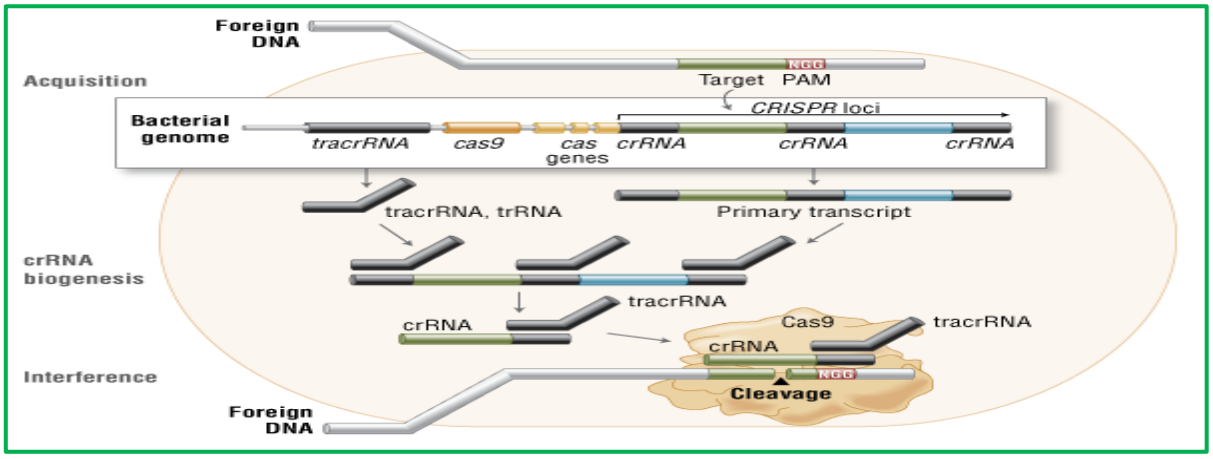
Main Components

- crRNA: This is an RNA that contains the guide Rna that locates the correct section of host DNA along with a region that binds to tracer RNA.
- Tracer RNA: This binds to crRNA and forms an active complex.
- sgRNA: It is a complex of RNA that consist of trcerRNA and some crRNA.
- Cas9: It is a protein whose active form can modify DNA. Many variations exist with a different function.
- CRISPR(cr) RNA + trans-activating (tra)crRNA combined = single guided (sg)RNA

CRISPR Cas9 word is two main words: CRISPR and Cas9. In contrast, CRISPR is a specific type of RNA, and Cas9is a nuclease protein; it is a kind of nucleotide protein with Crispr Cas9multiple genes can be targeted simultaneously, referred to as genome editing. The nucleases create a double-strand break at the desired location, which is repaired through NHEJ or HDR. CRISPR allows performing targeted alteration in the genome. It is an RNA-mediated powerful genomic editing tool that works with targeted DNA against the defense mechanisms of phage viruses. CRISPR is a bacterial DNA sequence family. The sequences contain DNA fragments from viruses that have infected the bacteria. The bacteria utilize these snippets to recognize and destroy DNA to protect themselves against future attacks by similar viruses. In a palindromic repeat, the nucleotide sequence is the same in both directions. Short lengths of spacer DNA from earlier exposures to foreign DNA are interspersed between each repeat (e.g., a virus or plasmid). CRISPR sequences are surrounded by small clusters of cas (CRISPR-associated system) genes. The CRISPR/Cas system is a prokaryotic immune system that confers resistance to foreign genetic elements, such as those present within plasmids and phages, providing a form of acquired immunity. To

supply acquired immunity against bacteriophage infection and plasmid transfer, many bacteria and archaea have evolved sophisticated RNA-guided adaptive immune systems encoded by CRISPR loci and the concomitant CRISPR-associated (Cas9) genes. During the immunization process following exposure to invading genetic elements from phage or plasmids, short fragments of foreign DNA are integrated into the CRISPR repeat-spacer array within the host chromosome as new spacers Genome edit. This system is only based on a fixed sequence of identifying and cutting alien DNA or RNA. CRISPR Cas9 mechanism can be divided into three major categories, are

1. Adaptation or spacer acquisition
2. crRNA Biogenesis
3. target interference



In the acquisition phase, foreign DNA is incorporated into the bacterial genome at the CRISPR loci. CRISPR loci is then transcribed and processed into crRNA during crRNA biogenesis. During interference, Cas9endonuclease complexed with a crRNA and separate tracrRNA cleaves foreign DNA containing a 20-nucleotide crRNA complementary sequence adjacent to the PAM sequence (Source: NEB expressions Issue I, 2014)

A comparison between, ZFN, TALEN, and CRISPR Cas9

Particular	ZFN	TALEN	CRISPR Cas9
Construction	Protein engineering for every single target	Protein engineering for every single target	20 bp nucleotides for sgRNA
Targeting	Protein-DNA interaction, less predictable	Protein- DNA interaction, less predictable	DNA RNA interaction is highly predictable
Delivery	Two ZFN are required around the target	Two TALEN are necessary around the target	Sg RNA complementary to the target sequence with CAS9protein
Multiplexing	Challenging	Challenging	Highly feasible
Feasibility of library construction and genome-wide screen	Technical challenging	Technical challenging	Highly feasible
Affordability	Resource-intensive and time-consuming	Affordable but time-consuming	Highly affordable

Source: Eid *et al.* (2016)

CRISPR Cas9 in crop improvement

As the genome sequences of an increasing number of crops become available and genome editing technology progress, it is now conceivable to modify any crop's genome for desirable features. CRISPR Cas9is a more precise genome editing technology than TALEN, ZFN, and Mega nuclease, allowing molecular researchers to change genomes more precisely. Previously, a few strains of bacteria were utilized to produce Cas9enzymes. However, numerous bacterial species are currently the source of this enzyme. It has been utilized in various crops for various qualities. CRISPR technology has been used to minimize the effects of biotic and abiotic stress on numerous kinds of cereal and other legume crops. Rice's OsERF922 (ethylene responsive factor) gene was targeted using a non-homologous end-joining technique that is effective and resistant to blast disease (Wang F. et al., 2016). Another gene, OsSWEET13, was targeted for disease resistance to bacterial blight.

Table. Application of CRISPR Cas9 genome editing technology in different crops

Crop	Method	Disrupted gene gene	Stress /trait	Reference
Biotic stress				
A. thaliana/ N. benthamiana	NHEJ	dsDNA of virus (A7, B7, and C3 regions)	Beet severe curly top virus resistance	Ji et al., 2015
A. thaliana	NHEJ	eIF(iso)4E	Turnip mosaic virus (TuMV) resistance	Pyott et al., 2016
N. benthamiana	NHEJ	BeYDV	Bean yellow dwarf virus (BeYDV) resistance	Baltes et al., 2015
N. benthamiana	NHEJ	ORFs and the IR sequence sDNA of virus	Tomato yellow leaf curl virus (TYLCV) and Merremia mosaic virus (MeMV)	Ali et al., 2015
Rice	NHEJ	OsERF922 (ethylene responsive factor)	Blast Resistance	Wang F. et al., 2016
Rice (IR24)	NHEJ	OsSWEET13	Bacterial blight disease resistance	Zhou et al., 2015
Bread wheat	NHEJ	TaMLO-A1, TaMLO-B1, and TaMLOD1	Powdery mildew resistance	Wang et al., 2014
Cucumber	NHEJ	eIF4E (eukaryotic translation initiation factor 4E)	Cucumber vein yellowing virus (CVYV), Zucchini yellow mosaic virus (ZYMV), and Papaya ring spot mosaic virus type-W (PRSV-W)	Chandrasekaran et al., 2016
Abiotic Stress				
Maize	HDR	ARGOS8	Increased grain yield under drought stress	Shi et al., 2017
Tomato	NHEJ	SlMAPK3	Drought tolerance	Wang et al., 2017
A. thaliana	NHEJ	UGT79B2	Susceptibility to cold, salt, and drought stresses A	

A. thaliana	HDR	MIR169a	Drought tolerance	Zhao et al., 2016
A. thaliana	NHEJ	OST2 (OPEN STOMATA 2) (AHA1)	Increased stomatal closure in response to abscisic acid (ABA),	Osakabe et al., 2016
Rice	NHEJ	OsPRX2	Potassium deficiency tolerance	Mao et al., 2018
Wheat	HDR	TaVIT2	Fe content	Connorton et al., 2017
Soybean	NHEJ	GmPDS11 and GmPDS18	Carotenoid biosynthesis	Du et al., 2016
Tomato	NHEJ	Rin	Fruit ripening	Ito et al., 2015
Potato	HDR	ALS1	Herbicide resistance	Butler et al., 2016
Cassava	NHEJ	MePDS	Carotenoid biosynthesis	Odipio et al., 2017
Cowpea	NHEJ	SYMRK	Cowpea SYMRK mutants	Duanmu & Fan et. al . 2019

Source: Jagannathan D *et al*. (2018).

This approach is not confined to cereal crops; it is also used to control viruses such as the banana streak virus in numerous other vegetable and fruit crops, such as bananas. MLO7 resistance to powdery mildew disease in grapes, NPR3 cacao resistance to Phytophthora tropicalis and Papaya alEPIC8 resistance Phytophthora palmivora resistance, LOB1 promoter in citrus the ability to resist citrus canker, Apple DIP1, 2, and 4 resistances to the fire blight disease, CEN4 for kiwifruit, and CEN rapid terminal flowering and fruit development. These features are for the management of quality, biotic and abiotic stressors.

Conclusion

This is an innovative and groundbreaking plant breeding technology for agricultural enhancement. In contrast to conventional methods, it can integrate the required gene with outstanding precision and speed to improve an agronomic trait. This technology is essential in numerous ways. Climate change is the most crucial problem affecting agriculture and other scientific fields. Using climate-smart gene insertion, we may employ this method to overcome this problem. Numerous adjustments have been made to the accompanying technology to increase the efficacy of the target. The FDA has authorized the cultivation of CRISPR-Cas9-modified mushrooms in the United States, where the cultivation of GMO crops is prohibited, and extensive precautions are required. It is the first species to acquire such authorization from the United States government. Following this, CRISPR-edited soybean and linseed received certification and advance. They will concentrate on fruit production. It will help farmers increase their yield, allowing them to realize their vision of a world without hunger. Now that the constraints to genome editing have been overcome, plant breeding may undergo a revolution. Using this technology, a great deal more can be accomplished. Modern agriculture proves that research and technology can work together to boost food yield and quality.

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