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Review

GENOME EDITING : A MAGICAL TECHNIQUE

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Abstract

This article includes different technologies associated with editing of gene. DNA can be altered using CRISPR/Cas9, TALEN and ZFN for improvement of crops and treatment of genetic disorders. This article provides details about different genes associated for rice improvement. Genome editing technique uses different methods of gene transfer for inserting a gene of interest into desired plant.

Keywords: CRISPR/Cas9, Genome editing, Yield & quality, TALEN, ZFN & DSBs

Introduction

Genome editing (also called as Gene editing) is a group of technologies that give scientists the ability to change an organism's DNA. These technologies allow genetic material to be added, removed or altered at particular locations in the genome. Genome editing technologies involves the usage of engineered site-specific nucleases (SSNs) to modify specific genes at desired locations in the genome. The SSNs such as zinc finger nucleases (ZFNs), transcriptional activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR)-associated endonuclease Cas9 (CRISPR/Cas9) make a double-stranded break (DSB) in the target DNA which is repaired by cell's own natural repair mechanism of homologous recombination or non-homologous recombination [1]. ZFNs are fusion proteins composed three to four base pair long sequences. The TALEN fusion proteins are designed to bind to specific DNA sequences that flank a target site. CRISPR/Cas9 discovered in 2012 by American scientist Jennifer Doudna, French scientist Emmanuelle Charpentier and colleagues.

Mechanism of Genome Editing

Genome editing technology generates site specific double stranded breaks (DSBs) in the targeted genomic sequence using programmable sequence- specific nucleases (SSNs). Three types of SSNs are used to introduce DSBs at selected sites: Zinc- finger nucleases, Transcription activator – like effector nucleases, CRISPR/ Cas 9. The DSBs are mainly repaired via two pathways: Non- Homologous end joining (NHE) & Homologous recombination (HR). In NHE, two broken ends are produced insertions & / or deletions in the target site. When homologous donor sequence is present at the DSBs, HR may be used & desired gene modifications occurs [2]. Overall, SSN induced DSBs are repaired more frequently by the NHE pathway than by the HR pathway [3].

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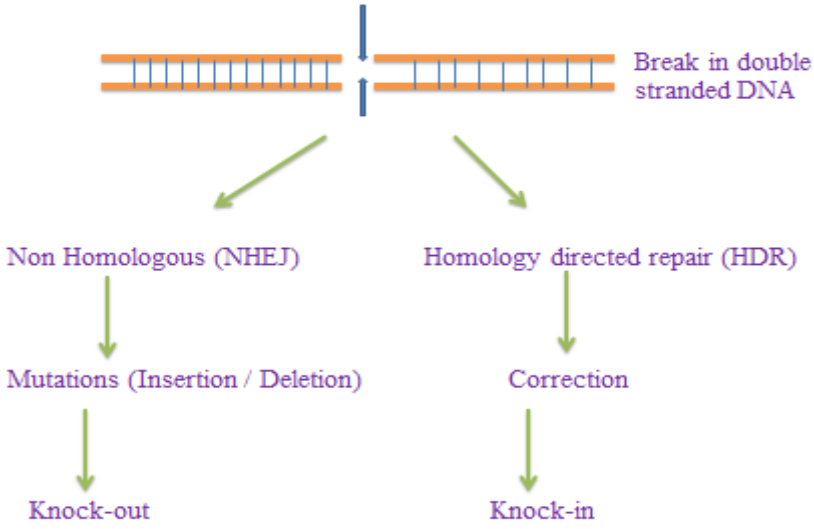

Fig: 1- Mechanism of Genome editing

Techniques of Genome Editing

TALEN

Transcription activator-like effector nuclease (TALEN) is a kind of proteins that works as bacterial effector and it allows for analyzing the development of DNA binding domain and such domains are formed without the incorporation of nuclease. These DNA binding domains are synthesized in such a way to obtain desirable results which are fused to a specific nuclease which later cleaves the DNA in that non-specific sequence order. The targetable nucleases are used to generate a double-strand break in double-stranded DNA into the targeted sites.

CRISPR / Cas 9

CRISPR/ Cas system provides a defense against foreign plasmids or viral DNA elements in bacteria & archea. They are divided into six types based on the variety of Cas genes & nature of the interference complex. Three components- mature crRNA, tracrRNA & Cas 9 are required for splitting the invading elements in type II CRISPR/ Cas systems. To clarify this, a dual tracrRNA: crRNA was designed as a single guide RNA (sgRNA) to direct the production of DSBs by Cas 9 in-vitro.

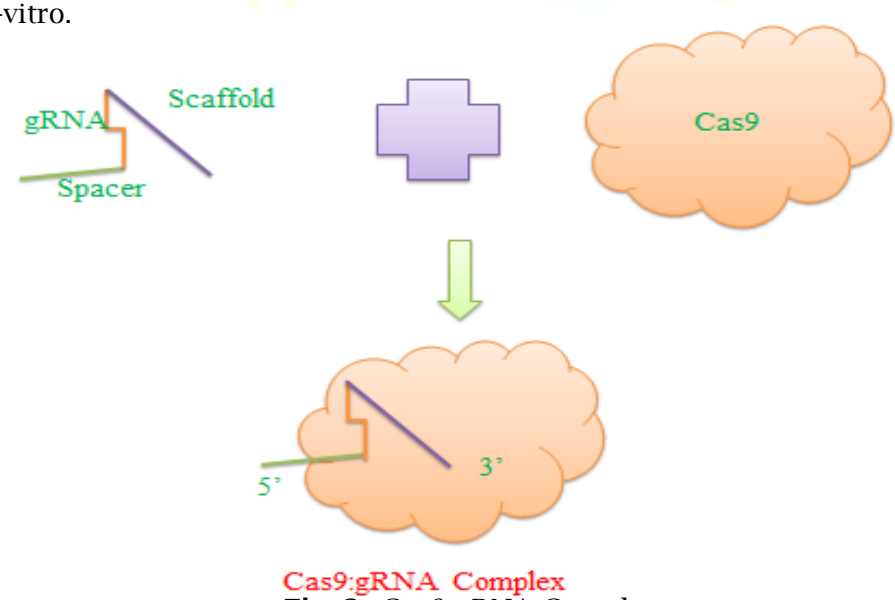

Fig: 2- *Cas9:gRNA Complex*

ZFN

Zinc fingers nuclease are those DNA binding domains which are generally identified by three base pair of DNAs. Change in the residue which can be either a minor change around the alpha helix present in the domain can result in the change of the specificity of the DNA binding sites.

Base Editing

It is a precise genome editing approach. It generates targeted point mutations without DSBs, foreign donor templates or HR. Current base editors usually contain a sgRNA & a catalytically impaired Cas 9 nuclease [Dead Cas9 (dCas9) or Cas9 Nickase (nCas9)] fused with single stranded DNA deaminase. Based on different kind of deaminase, there are two major group of DNA based editors: Cytidine base editors (CBEs) & Adenine base editors (ABEs). With CBEs, Cytidine deaminase is used to convert cytidine(C) to Uridine (U) within the editing window, creating a mismatched base pair with Guanine (G) on the opposite strand. However, the U intermediate is mutagenic; most organism have evolved an uracil base excision repair (BER) pathway to excise U from genomic DNA with Uracil DNA N-glycosylase. Therefore, Uracil glycosylase inhibitor protein (UGI) is used to impede uracil excision, increasing C to T editing efficiency of CBEs. CBE applied to wheat, Arabidopsis, rice, tomato etc.

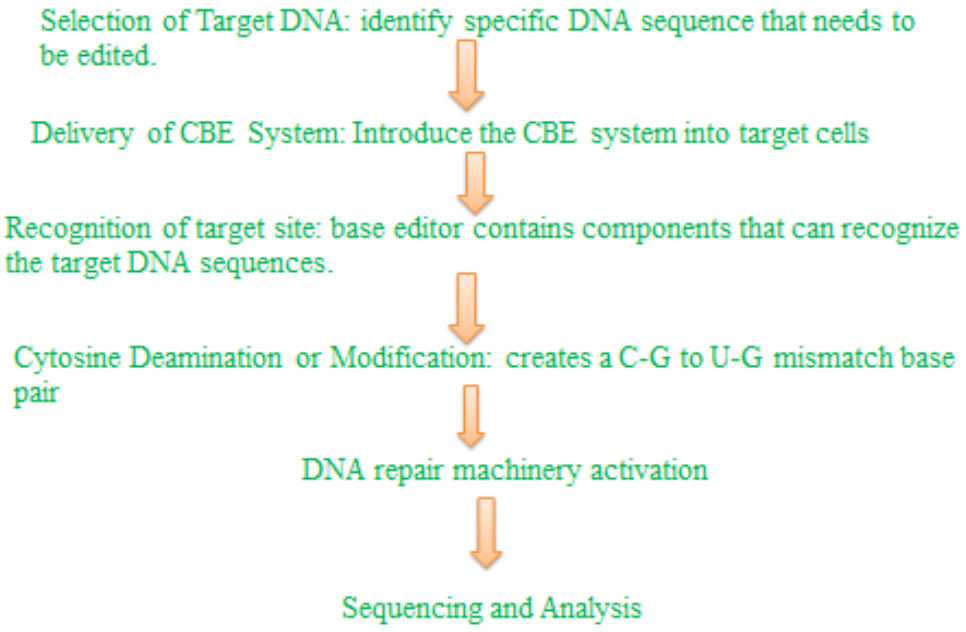

Fig: 3*- Mechanism of CBE- Mediated C to T base editing*

Theoretically, dCas9/ nCas9 fused with adenosine deaminase yields ABE. There is no natural deaminase that deaminates Adenine in DNA. By extensive protein engineering of E. coli tRNA adenine deaminase (Tad A) researchers produced a deaminase variant [Tad A*] that can deaminate adenine [A] in DNA. ABE applied to wheat, rice, potato, Arabidopsis etc. [4&5]. Base editors (CBEs & ABEs) can efficiently mediate all four transition mutations (C—T, A—G, T—C, and G—A) at targeted loci; this will undoubtedly facilitate research and breeding in plants.

Primer Editing

Prime editing borrowed from CRISPR, is another precise genome editing method that can generate all 12 types of base substitutions, targeted small insertions, deletions, and combinations of these editing results in deletions, and

combinations of these editing results in the target site. The prime editor mainly consists of a catalytically impaired Cas9 fused with an engineered reverse-transcriptase and a prime editing guide RNA (pegRNA). The latter contains a prime binding site at the 3' end of the sgRNA and a reverse transcriptase template, specifying the target site and also encoding the desired sequence edit.

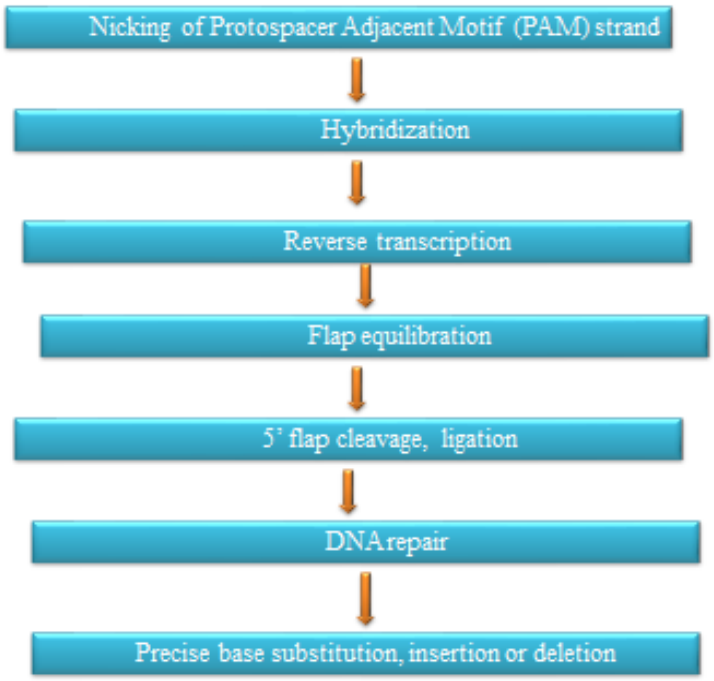

Fig: 5- *Mechanism of primer editing*

Table: 1- Genome-editing technologies

Technology	Description	Mechanism
CRISPR-Cas9	Revolutionary and widely used genome editing tool	RNA-guided DNA cleavage
TALENs	Transcription Activator-Like Effector Nucleases	DNA binding proteins linked to nucleases
ZFNs	Zinc Finger Nucleases	DNA binding proteins fused to FokI nuclease
Base Editors	Enables precise single-base changes without creating DSBs	Catalytically defective Cas9 or Cas12a
Prime Editing	Efficiently insert, delete, or replace target DNA sequences	Cas9 nickase and reverse transcriptase
CRISPR-Cas12a (Cpf1)	Alternative to Cas9 for genome editing	DNA cleavage using crRNA and trans-activating CRISPR RNA (tracrRNA)

Table 2: Gene Transfer Methods in Genome Editing [6]

Gene Transfer Method	Description
Viral Vectors	Utilizes viral vectors to deliver genome editing components (e.g., Cas9 and guide RNA) into target cells
	Common viral vectors include adenoviruses, lentiviruses, and adeno-associated viruses (AAVs)
	Can integrate into the host genome (lentiviruses) or remain episomal (AAVs)
Non-Viral Methods	Utilizes non-viral methods to deliver genome editing components, such as plasmids, nanoparticles, liposomes, or electroporation
	Does not integrate into the host genome
Electroporation	Uses an electrical pulse to create temporary pores in the cell membrane, allowing genome editing components to enter the cell
	Suitable for delivering plasmids or mRNA
Nanoparticles	Utilizes nanoparticles, such as gold nanoparticles or lipid nanoparticles, to deliver genome editing components
	Can carry plasmids, RNA, or proteins
Lipofection	Employs lipid-based carriers to deliver genome editing components into cells
	Suitable for delivering plasmids or RNA
CRISPR Ribonucleoproteins	Directly delivers pre-assembled CRISPR-Cas9 ribonucleoprotein complexes into cells
	Guide RNA and Cas9 protein are combined before delivery, ensuring rapid genome editing
Transfection	Uses chemical or physical methods to introduce genome editing components into cells
	Suitable for delivering plasmids, RNA, or proteins

Table 3: List of genes targeted by genome editing tools for rice improvement [7]

S.NO.	Applications perspectives	Targeted gene	Gene-editing strategy	Molecular Function
1.	Yield and quality improvement	GW2 & GW5	CRISPR/Cas9	Improvement of grain weight
		CCD7	CRISPR/Cas9	Increased tiller number
		LOX3	CRISPR/Cas9	Enhanced storage tolerance
2.	Biotic stress tolerance	OsSWEET13	TALEN	Enhanced resistance to bacterial blight.

3.	Abiotic stress tolerance	BEL Os ALS Os SAPK2	CRISPR/Cas9 TALEN CRISPR/Cas9	Herbicide resistance Herbicide resistance Drought tolerance
4.	Nutritional improvement	OsNRAMPS SBEIIb	CRISPR/Cas9 CRISPR/Cas9	Low cadmium content Generation of high amylose rice
5.	Nitrogen use efficiency	NRT1.1B	Base editing	Enhance nitrogen use efficiency

Conclusion

Keeping the above in view, it can be concluded that gene editing technology provide a great platform for researchers to develop a Herbicide-resistance, Disease-resistance varieties of different crops. It is also useful for improving the grain quality and yield of wheat and rice. With the help of this technology, researchers can develop new genomes that can be used in the treatment of different kinds of diseases such as cancer, sickle-cell anemia etc. The process is executed by cutting the DNA sequence with specific enzymes and thus this phenomenon is referred to as nuclei engineering.

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