Agri Articles
(e-Magazine for Agricultural Articles)
Volume: 02, Issue: 03 (MAY-JUNE, 2022)
Available online at http://www.agriarticles.com
©Agri Articles, ISSN: 2582-9882

Perspectives on the Application of Genome Editing Tools in Crop Improvement Programme

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Abstract:

Genome editing has occurred since past to present through the process of natural occurring mutations and advent of specific chemical and physical mutagenic agents resulted in discovery of over ~2900 genetic changes in plants that have played benefited role in agriculture and mankind. So, It was a technological means to accelerate the occurrence of nucleotide changes that could have resulted naturally, given enough time. The innovations in genome editing tools such as ZFN, TALENs, CRISPR/Cas9 and ODM, have further advanced the understanding of mutagenesis such that mutations can be performed in a precise and directed manner for crop improvement programme.

Keywords: Genome editing, ZFNs, TALENs, CRISPR/Cas9, Crop Improvement

Introduction:

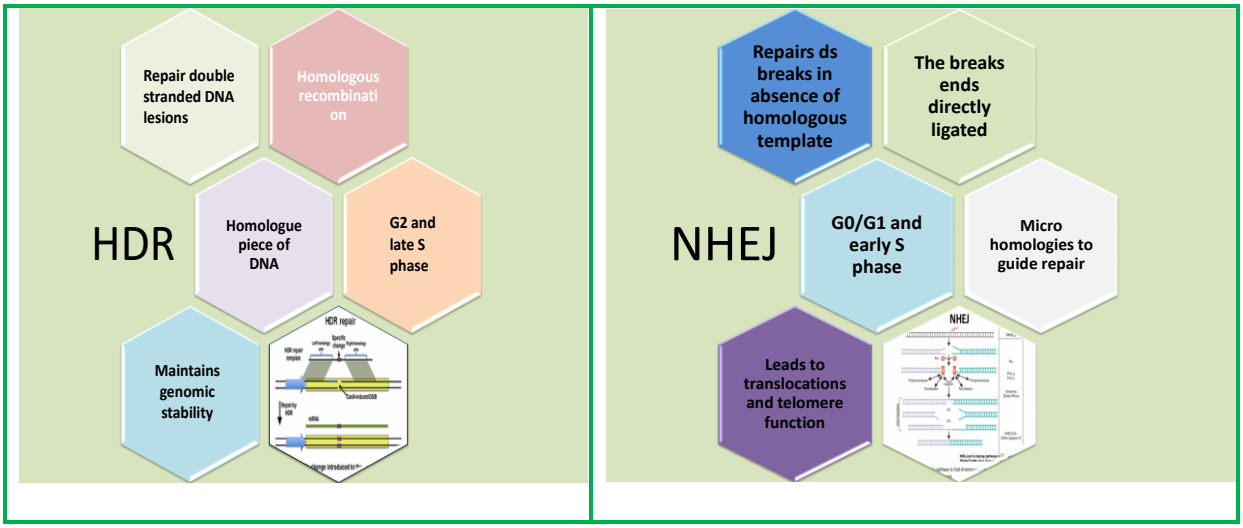
Genome editing is the targeted interventions at the molecular level of DNA, deliberately to alter the structural or functional characteristics of organisms. This includes the different technologies associated with the editing of the gene (Kamburova *et. al.* 2017).

Cleavage and rejoining of DNA molecule

First effort for Designing was done for " Artificial enzymes" as oligonucleotides (Knorre and Vlasov, 1985) and Designing of chimeric nucleases consisting of two units (1)cleavage of DNA (2) binding to sp. nucleotide sequences (Palpant and Dudzinski, 2013).

General Prinicples: Editing the genome using following techniques-

- HDR (Homology-directed repair)
- NHEJ (Nonhomologous end-joining)
- Early approaches: modifying genetic sequences using homologous recombination
- **ZFNs** (Zinc finger nucleases)
- **TALENs** (Transcription activator-like effector nucleases)
- **CRISPR/Cas9 systems** (Clustered Regularly Interspaced Short Palindromic Repeats - associated protein-9 nuclease (Cas9)
- **ODM (**Oligonucleotide directed mutagenesis)



ZINC FINGER NUCLEASES (ZFNs):

ZFNs recognizes an endogenous target site within the gene and consists of zinc finger motifs and domains **(**Porteus, M. H., and Carroll, D. 2005).

- Possess Cys2-His2 ZF domain
- Each domain consist of 30 amino acids which are folded upto ββα configuration
- Cys2-His2 ZF proteins bind to DNA by inserting α helix of the protein into the major goove and has ability to recognize 3 tandem nucleotides in the DNA
- ZFN monomer consist of 2 functional domains: ZF Cyss2-His2 domain at N-terminal region and non-specific FokI DNA clevage domain at the C-terminal region where as ZFN dimer consist of 4 ZF domains which forms unique sites in the genomes

First report: 1996- ZFs used for inactivation of endogenous genes in *Arabidopsis thaliana* and *Zea mays* has led to successful development of herbicide tolerant genotypes. Targeted modification of malate dehydrogenase (MDH) in plants have shown to increase yield (Kamburova *et. al.* 2017).

Overview of TALENs and ODM:

TALENs:

It is the DNA-binding domain (amino acids repeats) + FokI endonuclease where each amino acid recognizes one nucleotide of the target DNA sequence and FokI functions as a dimer (Wright *et. al*., 2014).

ODM: (Oligonucleotide directed mutagenesis)

It is done for targeted mutagenesis with 20 to 100 fold base long oligonucleotide and Based on mismatch between an artificial oligonucleotide and the DNA **(**Mohanta *et. al.*, 2017).

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR- Associated (Cas) System

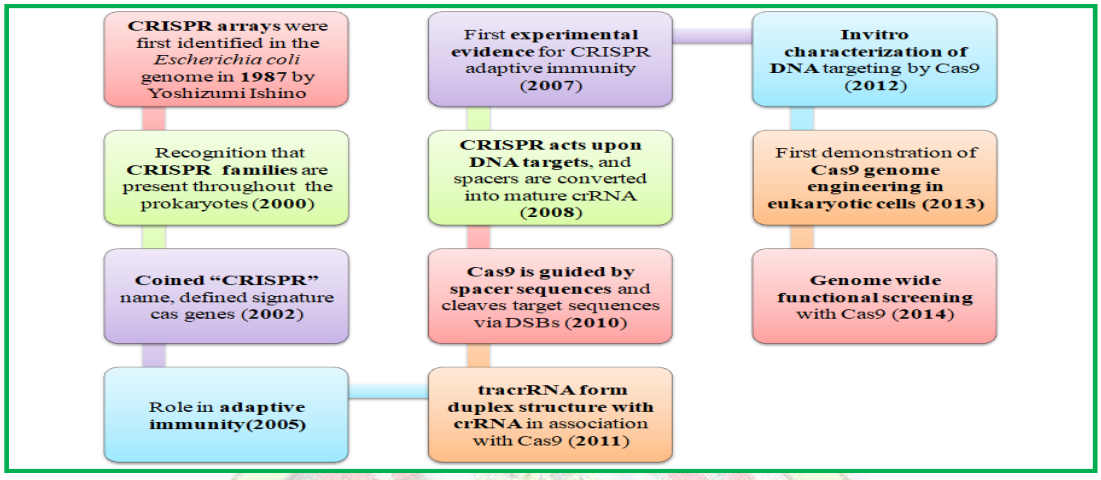
It is derived from a natural process found in bacteria to protect themselves from pathogens. It targets genes for editing and regulating. The CRISPR loci consist of several non-contiguous direct repeats separated by stretches of variable sequences (spacers) and lie often adjacent to *cas* genes. CRISPR repeats ia about 23-47 bp with Spacers: 21-72 bp. CRISPR/Cas provides adaptive, sequence based immunity against bacterial plasmids and phages **(**Makarova *et. al*., 2011, Zhu,*et. al*., 2020).

Popular system: CRISPR/Cas type –II-A system (*Streptococcus pyogens*) and composed of three genes CRISPR RNA (crRNA), trans-activating crRNA (tracrRNA) and Cas9 protein which created "CRISPR/Cas genome editor constructs.

Simplified system: Cas9 protein and single guide RNA involving CRISPRtracrRNA and short mature crRNA was created.

- **pRGE32 (10.1 kb):** Cas9, Ter, Amp, u3p, gRNA, UBIp

Chronology: **(**Pickar-Oliver, A., and Gersbach, C. A. (2019), Cong *et. al.* 2014)



CRISPR project involves simple steps in laboratory with fundamental plant transformation set up. So It is Simple, Efficient with Wide capabilities.

Table: Genome editing and applications

Type	Kingdom (Organism)	Components	Cleavage domain of proteins for processing step	Nature of target
Type I	Bacteria (*Escherichia coli*) Archaea (*Pseudomonas aeruginosa*)	Cas1, Cas2, Cas3, Cas5, Cas6, and Cas7	HD nuclease domains of Cas3	DNA
Type II	**Bacteria (*Streptococcus thermophilus*)**	**Cas1, Cas2, Cas9, and Cas4/Csn2**	**RuvC-like nuclease domain near the N terminus and HNH nuclease domain in the middle of Cas9**	**DNA**
Type III	Archaea (*Staphylococcus epidermis, Lactococcus lactis, Pyrococcus furiosus*)	Cas1, Cas2, Cas10, and Cas6	Catalytic triad of Cas6 protein and Csm/ Cmr complex	DNA/ RNA

Practical applications

Functional genomics :

- Creation of point mutations, insertion, deletions and correction or substitution
- Regulate gene activity
- Gene activating and repressing

Crop improvement:

- High yielding
- Resistant to biotic and abiotic stresses
- High nutritional value

These includes:

- Biotech oil – *Camelina sativa*
- Targeted mutagenesis of *SP5G* gene of tomato – plants with rapid flowering
- *OST2* gene – Arabidopsis that confer salt stress resistance (Osakabe *et. al*., 2016)
- Modulating gibberellin biosynthesis- dwarf fruit trees for increasing productivity (Gao, S., and Chu, C. 2020).)
- Inhibiting ethylene biosynthesis- varieties with extended shelf life
- Intereference against geminiviruses using transient transformation system such that *N. benthamiana*
- Deactivation of ethylene responsive factor to enahnce the immunomodulatory components in plants immune system
- knock out of *elF4E* gene in *Cucumis sativus* confers resistance to cucumber yellow vein yellowing virus and papaya ring spot virus
- Herbicide resistance plants by editing *ALS2* gene in maize (Li *et. al*. 2020).

Conclusion:

Genome editing tools have also been rapidly incorporated into agriculture and life science biotech research resulting in new opportunities to help feed a growing global population with maintaining food security. Genome editing is considered as efficient for its application in functional genomics and Crop yield improvement- targeted mutagenesis and associated breeding because of its attractive features like simplicity, high specificity and amenability.

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