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Breeding Designer Crops

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Introduction

Designer crops are the crops that are designed by the breeder through genetic manipulation of crop over native genotype of plant using advance techniques of molecular biology. As we all know that 21st century agriculture is continuously struggling enormous challenges, it can be due to the growing population of the country by 10 billion in coming 2050. Feeding this growing population will definitely require to increase productivity of crops. One common approach can be through the conventional breeding but it will not bridge the gap between current level of crop production and expectation levels in coming years to come in food processing. On other hand, the greatest challenge of today's scenario is the adverse impact of climate change in the form of increasing temperature, weather variability and invasive crops and pests on the crops that affect the crop productivity along with the agronomic traits. To overcome these challenges, The potential solution is utilization of new breeding genome editing technologies which is a tool provided by the nature and breeders make very wise use of the tools to apply in plant breeding Since, the genes encodes the particular trait in an organism. It allows very efficient trait management for the breeder and can make the plant more resistant innatively rather than externally applying chemicals which is currently also done in organic farming. Therefore, by manipulating the genetic makeup of crops it is possible to make designer crops that improves resilience to environmental stress and give plants an innate resistance to certain diseases.

Genome editing

Genome editing is making a change to an organism's DNA at a specific site and is advanced molecular biology techniques that facilitate precise, efficient, and targeted modifications at genomic loci.

Principle of genome editing:

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The basic principle of genome editing involves production of site specific double-strand DNA breaks(DSB) and the subsequent endogenous repair through error prone non homologous end joining (NHEJ) or the error free homology directed repair (HDR) pathways.(Puchta 2005)

Tool for Genome editing:

1. ZFN : Zinc Finger Nuclease
2. TALEN : Transcriptor Activator Like Effector Molecule
3. CRISPR-Cas9 :Clustered Regularly Interspaced Short Pallindromic Repeats

ZFN ,TALEN were earlier discovered two decades ago but the CRISPR-Cas9 is the newly discovered advanced tools revolutionizing in field of the plant breeding for enhancing crop productivity through desirable crop improvement.

ZFN : Zinc Finger Nuclease

Mechanism

- First identified as DNA binding motif in Transcription factor TFIIIA from African clawed frog (*Xenopus laevis*)
- Small protein structural motif coordinates with one or more zinc ions to stablize the fold
- Contain multiple finger like protrusions that make tandem contacts with their target molecule
- These are the hybrid restriction enzymes and consist of two parts N Terminal DNA binding domain and a non specific DNA cleavge domain at C terminal.
- Utilize fok 1 nuclease as DNA cleavge domain, fok1 naturally found in in *Flavobacterium okeanokoites.*(fig 1)
- Each individual ZFN repeat targets a three nucleotide binding at a site.

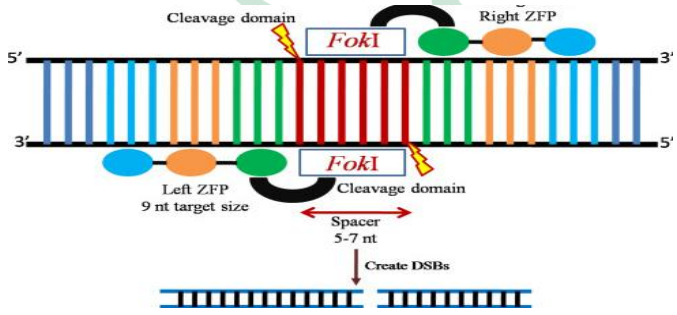

Fig 1: Mechanism of ZNF

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TALEN: Transcriptor Activator Like Effector Molecule

Mechanism

- Derived from *Xanthomonas oryzae* by Ulla Bonas(1989)
- TAL array: series of DNA binding domain assemble to recognize specific sequence
- Consist of 33-34 aminoacid sequences out of which 12th and 13th residue varies that determines the specificity of nucleotide binding.
- Each individual TALE repeat targets a single nucleotide, allowing for more flexible target design .

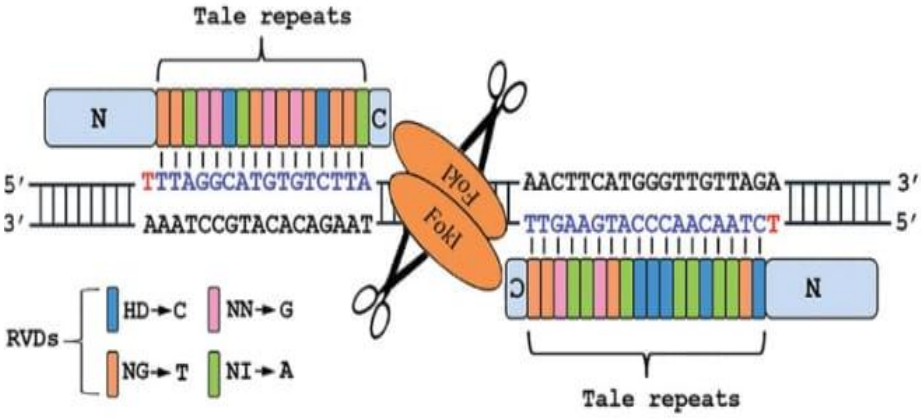
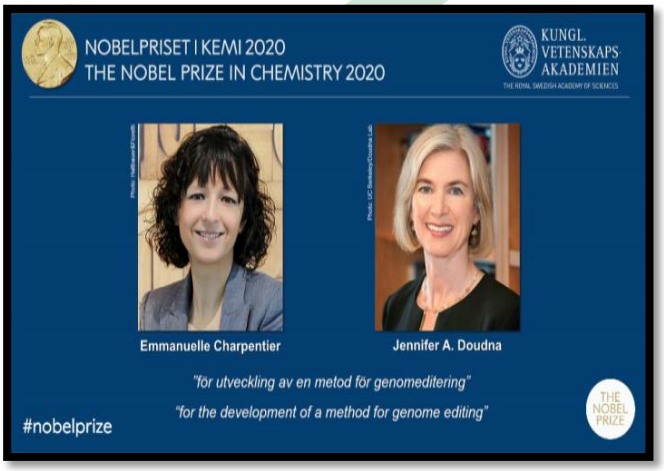


Fig 2: Mechanism of TALEN

CRISPR-Cas9 : Clustered Regularly Interspaced Short Palindromic Repeats

CRISPR Cas has been the most revolutionary of editing tools because of its simplicity and efficiency of editing. Its discoverers, Dr. Emmanuelle Charpentier and Dr.Jennifer Doudna, were awarded the Nobel Prize for Chemistry in 2020.



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Features

- An adaptive immune system in the bacteria to fight against foreign DNA i.e Bacteriophage DNA
- It consist of genomic locus 'Tandem Direct Repeats' sequence and protospacer.
- It involves two components – Cas9 and Guide RNA
- The guide RNA helps to direct the cas protein to a particular region of the genome and the cas protein then makes a cut in the DNA.
- After DNA is cut, normal cellular process repair the break resulting in different types of modifications depending on the absence or presence of a DNA template.

Mechanism

- CRISPR-Cas proteins are derived from the prokaryotic adaptive immune system and can target foreign DNA for cleavage using the CRISPR RNA (crRNA). Cas9 is obtained from Type II CRISPR-Cas systems and creates breaks in 20 nucleotides strand of DNA that is complementary to crRNA, whose maturity is dependent on the trans-activating RNA, tracrRNA.
- TracrRNA is the RNA that shares partial complementarity with crRNA and binds to the Cas9 endonuclease.
- Chimeric design of single-guide RNA (sgRNA) by fusing crRNA and tracrRNA, with multiplexing capability, and possible design considerations that can increase its on-target specificity. The sgRNA targets the Cas9 endonuclease to genomic sites complementary to its 5' end.
- The target DNA sequence needs to be followed in sequence by a protospacer adjacent sequence (PAM), typically the NGG sequence. The five nucleotides that are upstream of the PAM sequence constitute the seed region for target recognition.
- The two main component of CRISPR CAS9 are the wild-type Cas9 resulting in targeted gene knockout and the catalytically-inactive (non-cleaving) mutant dCas9 gene, with two silencing mutations of the RuvC1 and HNH nuclease domains resulting in targeted gene knockdown.
- The dCas9 mutant can also be tagged to various effector molecules, resulting in DNA labeling, transcriptional activation or repression, or chromatin immunoprecipitation (ChIP) (Fig-03).

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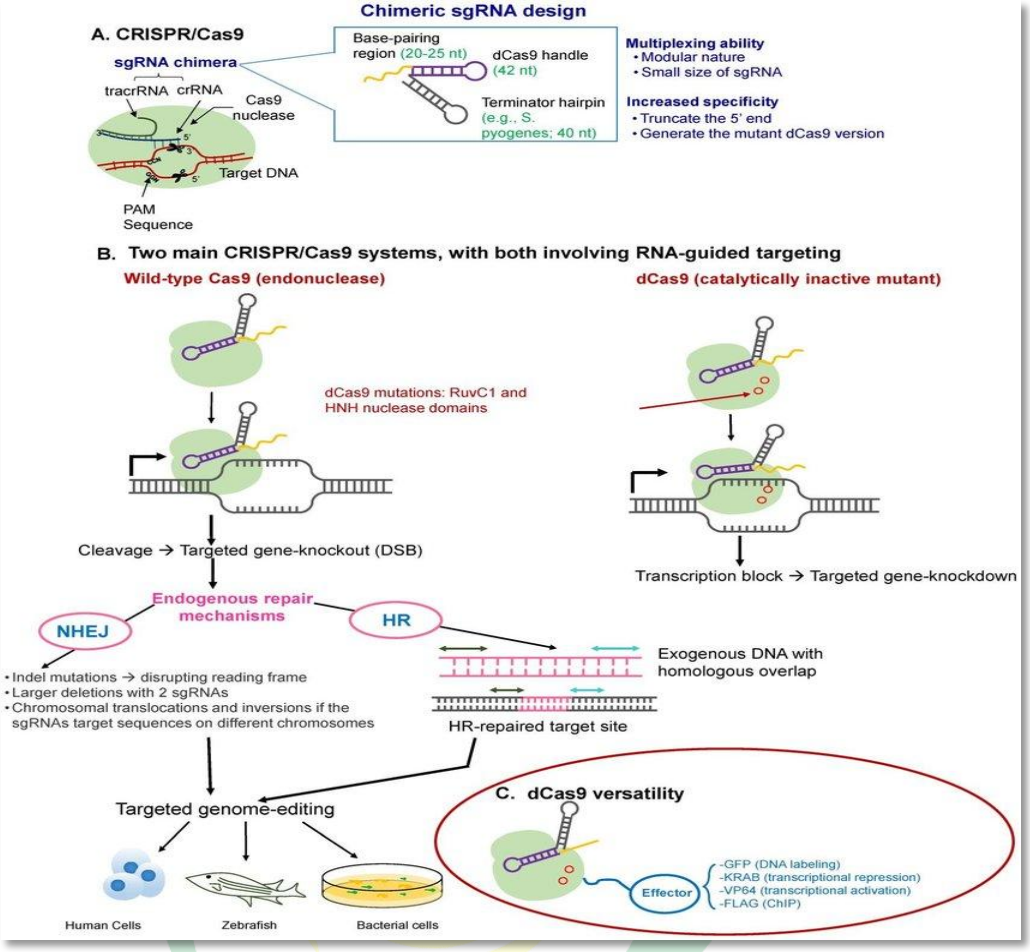

Fig3: Mechanism of CRISPR-Cas9

Applications of Genome editing

1. **Increasing abiotic stress tolerance** –eg. Use of CRISPR/Cas9 to knockout OsRR22, a gene associated with salt susceptibility in rice (Zhang A. *et al*., 2019). Another application is in drought and high temperature tolerance by targeting stomatal development in rice with reductions in stomatal densities had better yield in severe drought and were able to maintain lower temperatures despite no differences in yield. (Caine *et al*., 2019) (Fig-5)
2. **Disease Resistance**- In tomato, gene editing approaches have improved resistance to bacterial speck and tomato yellow leaf curlvirus (Tashkandi *et al*., 2018; Ortigosa *et al*., 2019). Editing of JAZ2 in tomato using CRISPR/Cas9 reduced infection by *Pseudomonas syringae* pv. tomato, the causal agent of bacterial speck, by reducing pathogen mediated stomatal opening.

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3. **Enhancing Nutrition** - In banana, gene editing using CRISPR/Cas9 to generate knockouts of genes for the biosynthesis of gibberellins has facilitated the development of a semi-dwarfed variety. This variety may be more resistant to lodging as a result of intense winds, typhoons, and storms, anticipated to increase in severity as a result of climate change (Shao *et al*., 2020)
4. **Biomass Production** -Rise in biomass produce and its quality can be directly proportional to rise in sugar and bioethanol production. Recent progress in sugarcane molecular marker technologies gives an exclusive tools for breeders to improve sugarcane for bioenergy production. For example, researchers have detected important molecular markers and QTLs linked with sugarcane biomass traits and they are being evaluated for sugarcane yield improvement (Hoarau *et al.*, 2002, Aitken *et al*., 2005 Bilal *et al*., 2015, Racedo *et al*.,2016)
5. **Quality Improvement** - The TALEN technique was used to disrupt the *BADH2* gene encoding for betaine aldehyde dehydrogenase (BADH), that gives γ-aminobutyric acid (GABA), instead of the main flavor compound, 2-acetyl-1-pyrroline (2-AP), from the same primary substrate of γ-aminobutyraldehyde (Shan *et al.*, 2015). Indian Basmati rice and Thai Jasmine are ricevarieties that are very popular among the consumers and have a higher market price than common rice are produced through genome editing technique. The speciality of these varieties are the improvement in their quality content and aroma. (Shan *et al*., 2015).

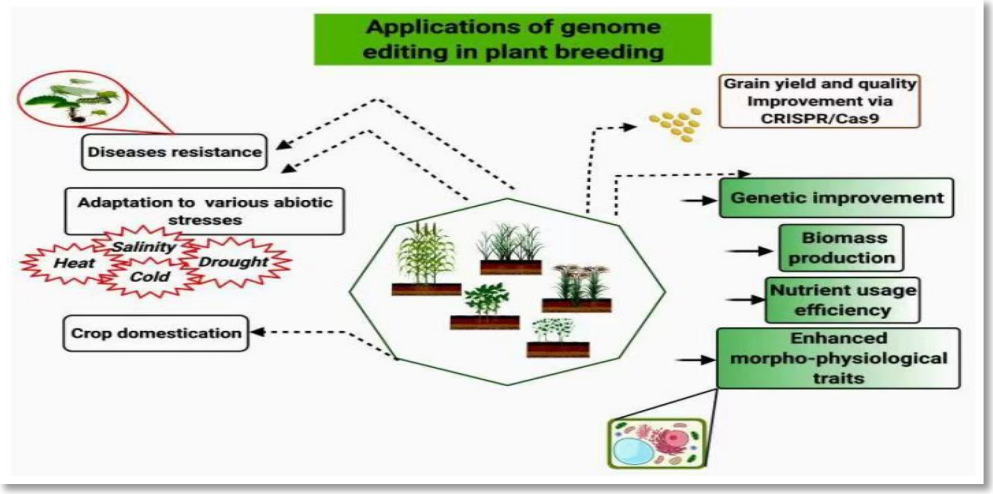

Fig 5. Applications of genome editing in plant breeding

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