CRISPR Cas 9 Mediated Genome Editing for Crop Improvement

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Abstract:-

CRISPR CAS9 is one of the most powerful technology in recent times that edits entire genome in various species and it belongs to the family of DNA sequence which is found in the genome of prokaryotic organism like bacteria and Archaea. It is a technology for the targeted editing and regulation of genes that can be applied to a number of biological systems. And it has been implemented in plant development which is bringing drastic change in the crop development disciplines, literally how this technology brings evolution in entire genome of species by inducing mutation in it or by editing entire genome of species which provide food security and reach the goal of zero hunger in the world, social impact, and knock out technology knocking out the old GMO technology by slashing the ethical dilemmas of GMOs

Keywords:-knock out, Transactivating, mutagenesis, CRISPR CAS 9, insertion, locus, proto spacer

Introduction:-

The CRISPR discovery of clustered DNA repeats occurred independently in three different Arts of world. It was called from Osaka University by Yoshizumi Ishino and his colleagues in 1987. In 1993 researchers of *Mycobacterium tuberculosis* in the Netherlands published two articles about a cluster of interrupted direct repeats(DR) in that bacterium. CRISPR are sections of prokaryotic DNA that mediate an acquired immune response against invading plasmids and viruses. Cas9 was the simpler system from *Streptococcus pyogenes* that relies on the protein Cas9. It is a four component system that includes two small cr-RNA molecules and two trans-activating CRISPR RNA(tracrRNA). The Casgenes encode nucleases that are capable of cleaving DNA. The CRISPR array is then transcribed to from cr-RNA, Which forms a complex with Transactivating (tra) crRNA and Cas9. The protospacer section of the cr-RNA then binds to the invading DNA, which is cleaved by the Csa9. The CRISPR system has been adapted to create a technique capable of gene editing at specific locations in the genome. The first step of gene editing is to perform a Double Stranded Breaks(DSB) at the desired DNA sequences. This is achieved by designing RNA guided nucleases. In their simplest form, Cas9 is complexed with a guide RNA(g-RNA) which consist of a cr-RNA and tranr-RNA. The g-RNA contains a complementary sequence to the DNA sequence of interest and therefore guides Cas9 to the desired location. Cas9 then cleaves the DNA to create a DBS. DBS can be repaired by the endogenous Non-Homologous End-Joining(NHEJ) and Homologous-Directed Repair(NDR). CRISPR-Cas9 method of genome editing through NHEJ is imprecise but can reach efficiencies of 20%-60%, whereas gene editing efficiency by HDR is more precise but has only been shown to reach 0.5%-20%.

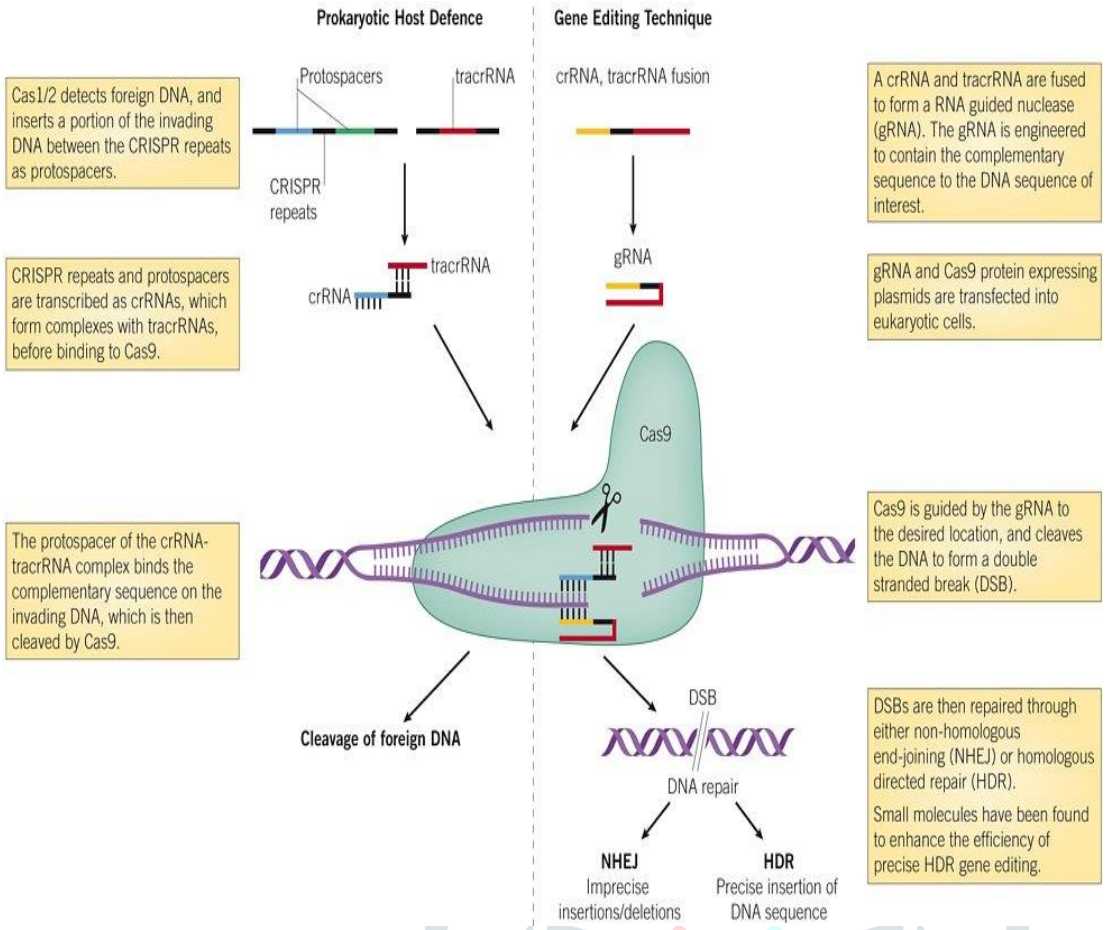

Fig 1: Schematic showing the CRISPR-Cas system as a prokaryotic host defence mechanism and as a gene editing technique. The CRISPR system mediates an acquired immune response against invading plasmids and viruses in prokaryotes, and has been adapted for the insertion of gene and DNA sequences in eukaryotes cells.

As HDR is more precise than NHEJ, improving the efficiency of HDR is vital for the use of the CRISPR-Cas9 system in many applications. Recent research has demonstrated that small molecules can enhance the efficiency of HDR - mediated gene editing. The β3 adrenergic receptor partial Agonist(a substance which initiated a physiological response when combined with the receptor) L-755507 (Fisher, et al., 1998) has been shown to enhance the efficiency of HDR -mediated GFP insertion and point mutation by 3-fold and 9-fold, respectively in pluripotent stem cell. The small molecules SCR7 (Chu, et al., 2015). Pyrazine has also been shown to enhance HDR efficiency, enhancing insertion of short DNA fragments by up to 19 folds. Small molecules that enhance HDR efficiency could be integral to the application of the CRISPR-Cas9 gene editing system.

CRISPR-Cas 9 In Plant Breeding:-

CRISPR- Cas 9 is a short palindromic DNA sequences which is repeated and regularly spaced. CRISPR molecule also resides with CRISPR associated genes, these will encode proteins that unwind DNA and make cuts in the DNA of interest which are known as helicases and nucleases (Barrangou, et al., 2007). Which consists of gene silencing, (HDR) Homology Directed Repair, transient gene silencing and transcriptional repression

There are three steps included for protecting bacteria from repeated attacks

These steps will follow a series of chronology which takes place one after one (fig.1&2).

At first the process of adaption takes place in which the infection of viral genetic material into the bacteria and after infection the viral DNA is processed and embedded in between the repeats called as spacers and these spacer's acts as genetic memory of past infections

At second step transcription of CRISPER locus and production and processing of CRISPR RNA from transcription process and this RNA contains copies of infecting viral DNA sequences in its spacers.

At third step viral DNAs or mobile genetic elements are identified by CRISPR RNA and directs the CRISPR associated proteins and these protiens have capability to cut and knock out the viral material.

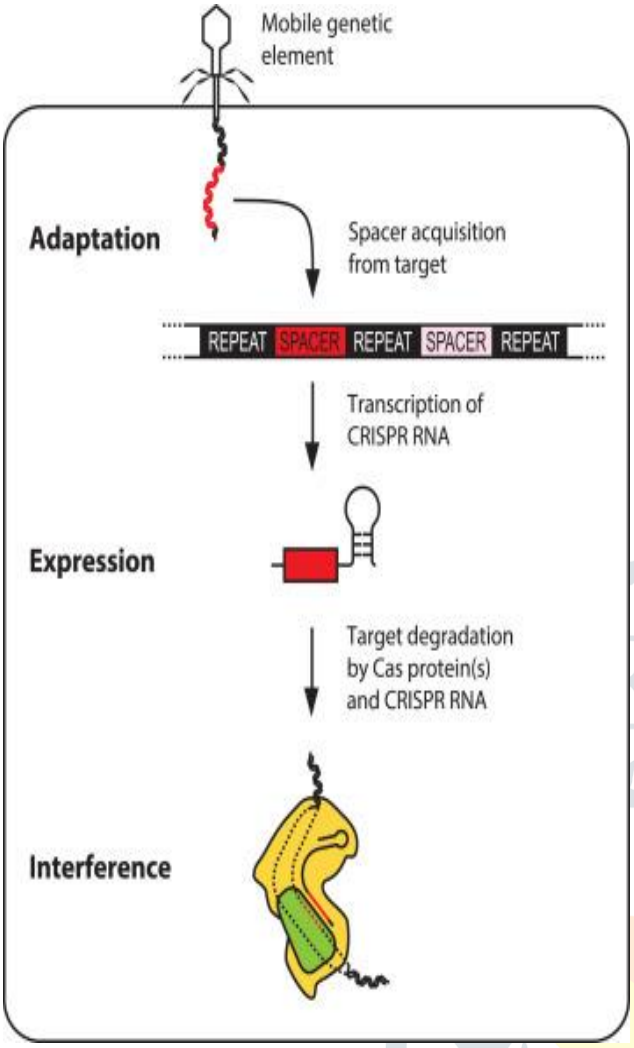

Fig:- 1. CRISPR Cas Immunity

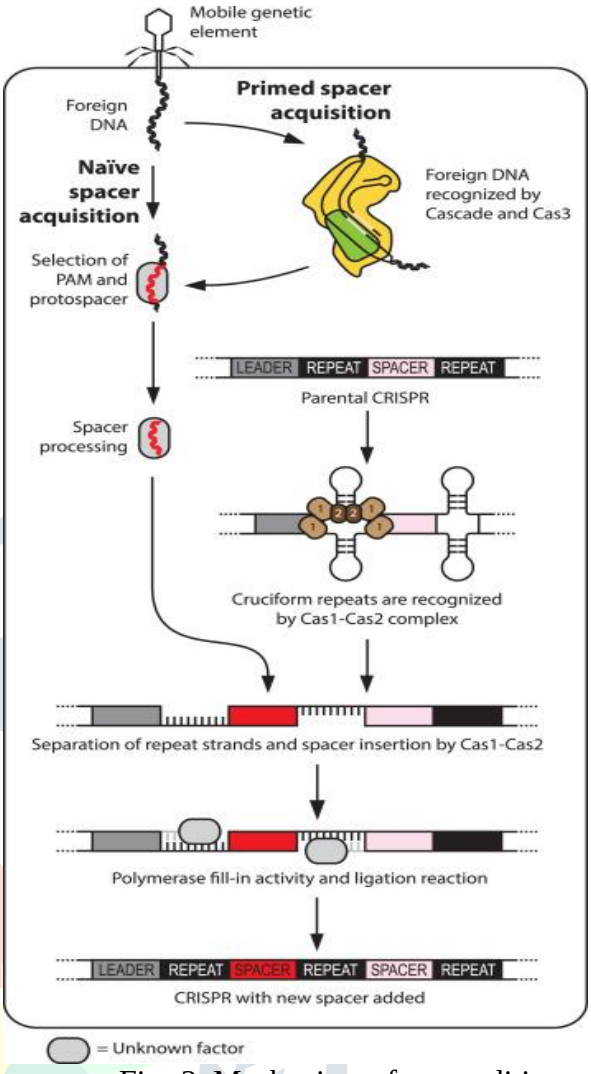

Fig:-2. Mechanism of gene editing

Applying CRISPR technology for precise Plant Breeding:-

It is the technology which can be applied as it simply destroys/demolishes or knock out undesirable traits at locus or DNA sequence (Chen, et al., 2019).

Increasing Yield:- as population of world is in boom causing threat to food security where at thrust this technology is here to improve yield but when we use mutation in yield it is an complicated trait of locus depends on many other correlated factors which obviously show minimal effect on yield such as grain number, grain size, in such cases the CRISPR Cas 9 is the only effective and sound producing technology of agriculture sciences in improving yield characters.

Improving quality:- In today's world not only quantity but also quality has grabbed a place in day to day life because of improved economic status of most of the developed countries population ,in such condition this technology can bring a dynamic development in quality improvement such as fragrance in rice starch content storage quality with commercial.

Biotic and abiotic stress resistance:- mostly diseases causing major losses in yield and quality for such biotic stress the CRISPR plays versatile role in knock out the Avr gene in sequence which is compatible to disease

CRISPR-CAS9 In Crop Improvement :-this technology has been used widely in all the species but wheat, rice, maize has shown a very good results in many ways the following concepts are some of the case studies in crop improvement of some economically important crops

RICE GENOME:- (Xie and Yanget al., 2013) of rice genome 1in 10 sites has an abundant potential to target trait of interest and it was continued by (Shan et al., 2013) demonstrated crisper technology in genomic modification of three rice gene, which control the plant in abiotic stress response they are

- (OsPDS) Pytoene desturase
- (OsBADH2) betaine aldehyde dehydrogenase
- OsMPK2) mitogen activated protein kinase

WHEAT GENOME:-It's an staple crop of world in cereals and the CRISPR technology was demonstrated in its protoplast successfully for resistance of mildew in locus "0" and to increase transgenic lines (Kim et al., 2018) in wheat protoplast for two abiotic stress related genes they are

- Some protiens in Wheat show dehydration response to abiotic effects such proteins are Dehydration Responsive Element Binding Protein 2 and in short it is called as (TaDREB2)
- Wheat ethylene responsive factor ERF shows certain response to freezing stress and *R.cerealis* in short it is known as 3(TaERF3)
- Lipooxygenase,which provide resistance to(Fusarium graminearum) (TaGW2)

MAIZE GENOME:- As maize is a major cereal crop of world for consumption it need to be developed to reach population needs.as maize consists of 70% of phytic acid as an anti-nutritional in monogastric animals and environmental pollutant.

(ZmIPK,ZmMRP4,ZmIPK) are responsible for synthesis of phytic acid (Liang et al., 2014) have reported involved in it and use of CRISPR in knocking out of the genes responsible for phytic acid synthesis and editing of phytoene synthase was explained by(Zhu et al., 2016) and PSY 1 is responsible for carotenoid bio-synthesis and psy-1 responsible for production of albino plants (Zmzb7) by this CRISPR which was demonstrated in albino maize plants for knocking out those targeted sites by (Feng et al., 2016).

CRISPR IN SOYBEAN GENOME

Soybean is one of the important oil seed crop and the seeds also contain many other physiological active and beneficial to humans

(Caiet al., 2015)

- Rj 4 is the gene responsible for the dominant nodulation restriction by many strains of Bradyrhizobium elkanii and this gene was eliminated by gene knock out experiment by CRISPR technology by (Tang et al., 2016)
- CRISPR use in disrupting the avirulent gene of disease causing organism (Avr4/6) in *phytophtora sojae*
- CRISPR knock out of GmFT2 gene which was stably heritable with homozygous GmFT2a mutants exhibit late flowering

COTTON GENOME EDITING:-

(Li et al., 2017c) demonstrated induced trunication episodes in cotton crop improvement controlling and PCR amplification with sequence analysis of GhMYB25, A and D in transgenic cotton

Now the gene editing is done to control wilt in cotton to show resistance by modifying the Gh14-3-3d gene through editing.

TOMATO:-

Tomato is economically important and it is an excellent crop for analysing genome editing as there is access for transforming technology and good background for quality (pan et al., 2016)

When it comes to ripening of tomato and regulating it this is the one of the toughest concern (Ito et al., 2015) but when the role of incRNA1459 was confirmed by CMGE; mutants showed repression of ethylene and carotenoid biosynthesis this technology has knocked out this problem.

CANGENOME EDITING BEATS THE GMO?

Definitely yes! Because there is a lot more difference between genetically modified and genome edited cultivar for example in India there Is an ethical dilemma regarding to the GMO that the introduced gene is of other organism may be toxic due to this reason the Bt brinjal was rejected for consumption but where as this new technology is totally different for example the gene edited cultivars are similar to mutagenesis it's an simple product of deleted several nucleotide sequences from its own genome there is no artificial introduction of other genetic material and even repair system is employed in repairing double stranded DNA break and having regulatory frame work unlike mutagenesis (Wolf et al., 2016) the outcome of this breeding process could thus resemble a transgenic crop more than a simple product of mutagenesis it's the main problem in differentiating them .

SOCIAL IMPACT:-

Continuous use of this technology need a very careful observation (Singh et al., 2016) when we consider the question of ethics it has less intense .but this technology can drastically transform present phase in agriculture by this technique breeders can produce n-resistance variety with both biotic and abiotic stress resistance and low cost to produce by fact that CRISPR CAS 9 are derived from same bacteria that already naturally reside in human gut making difficult to claim that anything foreign included in editing process.

Variance among genome edited plants thus next level of difficulty in defining exactly a "GMO" are all genome edited this technology will reach zero hungercrops are GMO"S by what criteria do we group and split new cultivars this conceptual more as suggests the end of "GMOS" as a workable frame for regulating plant breeding, human construction vary over time and space indeed nature makes its own transgenic plants in different how the society would understand it.

CONCLUSION

These new breeding technology can bypass the convectional breeding method which can target directly the trait of interest by CRISPR CAS 9 based on this genome editing is a break through technique by this an abnormal development in crop takes place from last 4-5 years it has been applied intensively in plants of both biotic and abiotic stress as well as to improve agronomic trait this technology will reach zero hunger goal and maintain population

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