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Genome editing a robust way of modifications, a brief introduction of its tools

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Abstract

Biotechnology is an emerging field. Different biotech tools are used for making improvements in organism's genetic makeup. This modifying or repairing process of genetic makeup is called genome editing. Different tools are used in this editing system for making desired changes in the genetic makeup. These tools included ZFNs, TALENs and CRISPER. As biotech is robust way of modifications so these advance tools are more helpful than conventional means or tools in field of crop sciences. ZFNs and TALENs have same working principle and cause sit specific breakage and repairing. CRISPER is also used for site directed mutagensis or improvements with more advancements. Locus is found in *E. coli*. These all tools are widely used and has many applications in crop sciences as well as including successful stories to meet the demand or requirements of population.

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Introduction

Agriculture is main concern for food and other basic needs of life. Demand for basic requirements is increasing with ever increasing world population. Elferink and Schierhorn (2019) reported that population may reach 9.7 billion and food demand is expected to increase from 59% to 98% by 2050. Much advancement have been made in field of agriculture via conventional and non-conventional ways but there is further room for improvements in this field for tackling the adversely changing climatic conditions and higher crop production (Soda *et al.,* 2018). As wheat and maize are staple food in many region of world that's why improvement in wheat, maize specifically and for disease resistance, heat, cold, and herbicide tolerance of crop plants have been made (Zaidi *et al.,* 2018).

Conventional ways for crop improvement require more time as compared to modern molecular techniques and tools (Abdelrahman *et al.,* 2018). Molecular tools provide precise ways to replace or modify the genes in desired region of the genome (Abdelrahman *et al.,* 2018). Today's Biotechnology is robust way for making genetic improvement in crop plants. It has been made easy to understand structure and function of gene (Giovannetti *et al.*, 2005). Proteomics, interactomics, transcriptomics and epigenomics are some tools or techniques used in functional genomics for study of genes (Gasperskaja and Kučinskas). Genome editing is a technique of biotechnology use for making modifications in genome of organisms. Modifications are made at specific regions of DNA. DNA segments are cut with restriction enzymes, desired sequence is added in that region of genome and rejoined using ligate enzyme.

A trait of economic and agronomic importance is targeted to get novel end product (Piatek *et al.*, 2018). Target specific genome editing system brings improvement in quality, production, biotic and a-biotic stress resistance (Kamburova *et al.,* 2017). Editing system aims at meeting the demands of growing populations, by introduction of efficient and cost-effective agriculture (Shah *et al.,* 2018).

In last era efficient genomic editing tools like zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (Reyon *et al.*), clustered regularly interspaced short palindromic repeats-CAS9 (Zhang *et al.*), Oligonucleotide-directed mutagenesis (ODM) and Cleaved genomic sites has been used (Chen and Gao 2013; Kamburova*et al.,* 2017; Shah *et al.*, 2018).Plant breeders and biotechnologists are working for transformation of crops for sustainable agricultural production (Piatek*et al.,* 2018), crops with improved genetic makeup which can tolerate harsh condition of biotic and a-biotic stresses (shah *et al.,* 2018). They also introduced CRISPER CAS type 2 in plants for study of their medicinal benefits (Liu *et al.,* 2017).

It is a genome editing tool and more efficient and less time and labor consuming as compared to ZENs and TALENs consisting of Cas 9 protein which is from *Strptococcus pyogens* bacteria. This technique is easy to use and there is no need of promoters or optimization of codon usage (Woo *et al.,* 2015).

This review covers genome editing, different tools for genome editing with main focus of CRISPER that is advance tool of editing system. Mechanism of Cas 9 protein, types of CRISPER Cas, application of CRISPER Cas 9 in plant science and future prospective or strategies in regard of genome editing tool CRISPER Cas9 has also been discussed with examples.

Genome editing

Genome editing is a process used for modifying and repairing the DNA within organism or a cell. Restriction endonucleases enzyme are used for specific cleavage in DNA molecule. Endonuclease enzymes do their job precisely in short genome like bacteria but in large genome they lose their specificity of work. To overcome such problems artificial enzymes such as oligonucleotides are prepared.

They bind and cleave DNA at specific site, then after inserting other desired segment DNA is rejoined by using of ligase enzymes (Knorre and Vlasov 1985).

Target specific cleavage and binding is accomplished by designing the chimeric nucleases. These are complex proteins with one or two structural units. One unit catalyze the cleavage of DNA, and the second is make selective binding to specific nucleotide sequences of target molecule as well as provide the nuclease action to this site (Palpant and Dudzinski 2013; Jankele and Svoboda 2014). Following is the simplest diagram for understanding of genome editing system.

Different tools used in genome editing system

Zinc finger nucleases (ZFNs)

Zinc finger nuclease is a powerful tool for site specific DNA breakage and rejoining (Gaj *et al.,* 2013). Main property of ZFNs is it creates double strand breaks in DNA segment (Cantos *et al.,* 2014). ZFNs are programmed nucleases, Plasmid *Agrobacterium tumefaciens* is used for encoding. Fok1 is use for target specific breakage in double stranded DNA (Woo *et al.,* 2015).

Table 1. Applications of Zinc Finger Nucleases in crop plant.

Plant	Studied traits	References
Oryza sativa	Twenty eight loci are identified for precise integration	(Cantos *et al.,* 2014)
Zea mays	Target integration into endogenous loci, which leads to the herbicide resistance	(Shukla *et al.,* 2009)
Nicotianatabacum	Introduction of genes (SuRA and SuRB) for herbicide resistance at loci SuR	(Townsend *et al.,* 2009)
Arabidopsis thaliana	ADH1 and TT4 Genes are modified (TT4 , less anthocyanin in seed coat and ADH1, allyl alcohol resistance)	(Zhang *et al.,* 2010)
Arabidopsis thaliana	Mutation is created in ABI4 for precise reverse genetic study	(Osakabe*et al.,* 2010)

Transcription activator like effector nucleases

Transcription activator like effector nucleases has been used for biomedical research and genetic engineering (Woo *et al.,* 2015). It can be easily engineered. TALENs has great scope in biological and genetic diseases research because it make alternation in genes efficiently and quickly (Joung and Sander 2013). TALENs has same working principle as Zinc Finger Nucleases. TALEN are made up with repeating motif of 33-34 amino acid and for recognition of specific nucleotides two variable positions are available (Deng *et al.*, 2014). Specific cutting of the genome is achieved by assembling arrays of these TALs and joining them to a FokI nuclease. Double strand breaks induced by forkI, it could inactivate the gene or can be used for desired DNA insertion. This whole activity is achieved by combining two TALENs (Cermak *et al.*, 2011). TALENs have high specificity than ZFN (Reyon *et al.,* 2012).

Table 2. Applications of Transcription activator like effector nucleases in crop plants**.**

Plant	Targeted traits	References
Saccharum officinarum	Target mutagenesis for Improvement in saccharification. Mediated mutants shown 19.7% decrease in lignin with 43.8% improvement in saccharification	(Kannan *et al.,* 2017)
Triticum aestivum	Three homoalleles were added for resistance against powdery mildew.	(Wang *et al.,* 2014)
Oryza sativa	Mutation were induced by using TALEN for development of plants having heritable mutagenesis	(Zhang *et al.,* 2016)
Oryza sativa	Modifications were made in plant genome for resistance against bacterial blight.	(Li *et al.,* 2012)
Arabidopsis thaliana	Target mutations were made in following genes TT4, DSK2B, ADH1, NATA2 and MAPKKK2	(Christian *at al.,* 2013)
Arabidopsis thaliana	chimeric transcriptional repressor gene were generated	(Mahfouz *et al.,* 2012)

Clustered regularly interspaced short palindromic repeat

Clustered regularly interspaced short palindromic repeat (Gaj *et al.*)is an advance tool in editing system for mutation or editing in genome at specific site (Ito *et al.,* 2015; Rath *et al.,* 2015). CRISPER locus is first observed in E. coli (Ishaino *et al.,* 1987). It is present in archea (84%) and bacteria (45%) (Grissa *et al.,* 2007). Crisper is an array of short repeated sequences which are separated by spacers of unique sequences. CRISPER is found on both chromosomal and plasmid DNA. Spacers are often derived from nucleic acid of viruses and plasmid. It gives an idea that CRISPER is a part of anti-virus system. Spacers are used to recognize new viruses (Bolotein *et al.,* 2005; Mojica *et al.,* 2005).

Table 3. Applications of CRISPER Cas 9 in plants.

CROP	TARGET	ACIEVEMNET	REFRENCE
Potato	Three sites was targeted GTI 1, GTI 2, GTI 3	Starch quality has been altered with knock out of granule-bound starch synthase (GBSS) genes	(Andersson *et al.,* 2016)
Brassicanapus	two copies of BnWRKY11 were used to create muatatio in BnWRKY11	BnWRKY70 may function as a regulating factor to negatively control the Sclerotinia resistance and CRISPR/Cas9 system could be used to generate germplasm	(Sun *et al.,* 2018)
Cotton	an endogenous gene GhCLA1and Discosoma red fluorescent protein2(DsRed2)	DsRed2 and GhCLA1 was targeted to check the efficiency of CRISPER. Results matched well with Sanger sequencing results. There was no off-target editing. The results proved that the CRISPR/Cas9 is highly efficient system And reliable for allotetraploid cotton genome editing.	(Wang *et al.,* 2018)
Chardoanny	Two single guide RNAs (sgRNAs) were designed to target distinct sites of the L-idonate dehydrogenase gene (IdnDH).	Study was with aim, to check precise genome editing of CRISPER Cas. Results were showed that it is efficient and precise way for editing in grapes.	(Ren *et al.,* 2016)
Cucumber	C and Ntermini were targeted for disruption of functions in eIF4E gene(eukaryotic translation initiation factor 4E). Cas9/sgRNA technology was used.	By using CRISPER Cas technology non transgenic virus resistance cucumber was developed. Results showed that this can be used in other cops with target the DNA to make them virus resistance.	(Chandrasekaran *et al.,* 2016)
Wheat	Individual expression vector constructed by targeting seven sites in three genes (Pinb, DAI and waxy)	Results showed that our Agrobacterium-mediated CRISPR/Cas9 system can be used for targeted mutations and facilitated wheat genetic improvement.	(Zhang *et al.,* 2018)
Wheat	The mutations were targeted in the inositol oxygenase (inox) and phytoene desaturase (pds) genes using cell suspension culture of wheat and in the pds gene in leaves of Nicotiana Benthamiana.	The expression of duplex cgRNA with Cas9 targeting two sites in the same gene resulted in deletion of DNA fragment between the targeted sequences and this provide powerful method for engineering in plants.	(Upadhyay *et al.,* 2013)
Maize	targeting five different genomic regions: upstream of the liguleless1 (LIG1) gene, male fertility genes (Ms26 and Ms45), and acetolactate synthase (ALS) genes (ALS1 and ALS2).	Study demonstrated the utilization of Cas9-guide RNA technology for editing of plant genetic makeup for meeting up with demands and future needs of research	(Svitashev *et al.,* 2015)
Rice	stomatal developmental gene EPFL9	This study demonstrated the application of CRISPR-Cas9/Cpf1 to precisely target genomic locations and develop transgene-free homozygous heritable gene edits and confirms that the loss of function analysis of the candidate genes emerging from different systems biology based approaches, could be performed, and therefore, this system adds value in the validation Of gene function studies.	(Yin *et al.,* 2017)

Crisper Casis a precise way of genome editing for target mutagenesis (Svitashev *et al.,* 2015; Wang *et al.,* 2018; Zhang *et al.,* 2018).

CRISPER associated (Yin *et al.*) Genes that code a protein that is essential for immune response are required for proper functioning of CRISPER system. These genes found adjacent to CRISPER (Barranngo *et al.,* 2007; Brouns *et al.,* 2008).Cas 9 protein present in prokaryotes (Mojica *et al.,* 2000). Cas 9 attach on flank region of CRISPER in genetic makeup (Mojica *et al.,* 2009). CRISPER consists of three

components crRNA (CRISPER RNA), Cas9 nuclease and Trans activating crRNA (trRNA) (Mojica *et al.,* 2009). The CRISPER Cas mediated defense mechanism can be divide into three stages, first stage is adaptation that leads to insertion of new spacers, second stage is expression, the system gets ready for acting by expressing the Cas gene and transcribing the CRISPER into long precursors crisper RNA (cr-RNA). The pre cr-RNA is processed into mature cr-RNA by CAS protein and other elements.

Because modification in genome has been made with this tool And It helps plants to survive in harsh climatic conditions (Abdelrahman *et al.,* 2018).

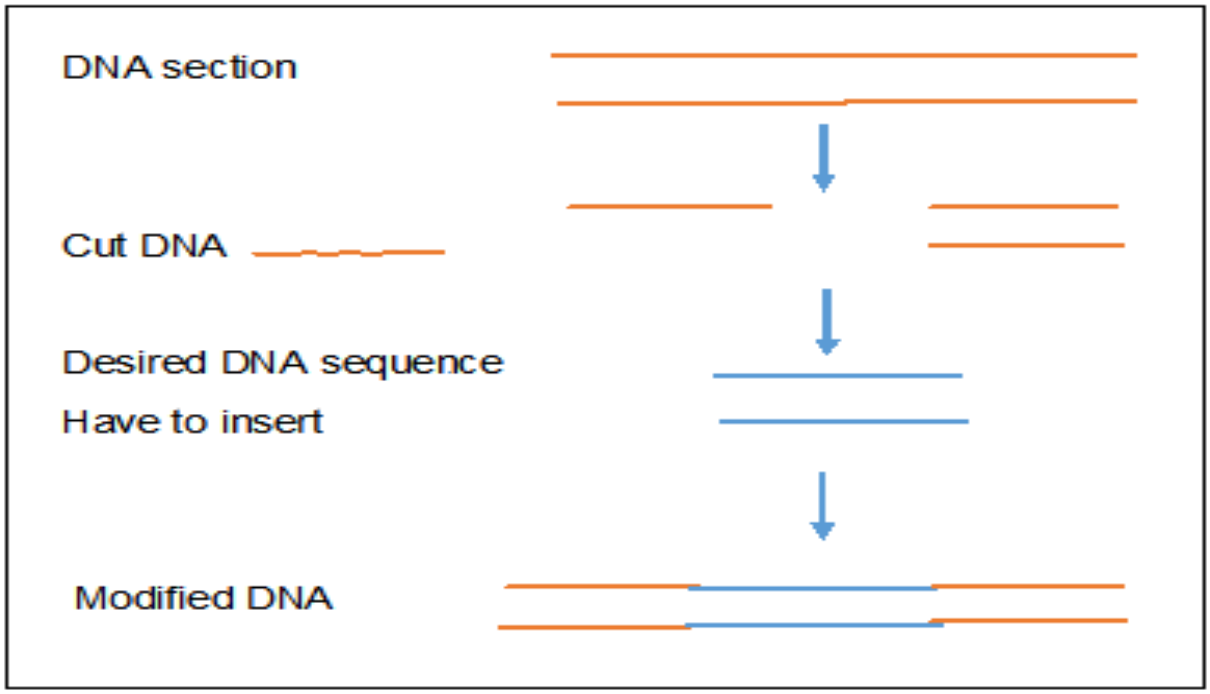

Fig. 1. Genome editing.

There are three types of CRISPR-Cas system: type I, type II and type III. Types I and III are found in both bacteria and archea, type II is unique and present in bacteria. The bacterial type II CRISPR/Cas system is the most studied and best characterized in which Cas9 protein is the critical component. In this system, CRISPR loci are transcribed as a precursor CRISPR RNA (pre-crRNA) containing the full set of CRISPR repeats and embedded invader-derived sequences. A trans-activating crRNA (tracrRNA) binds to the repeat sequences of the pre-crRNA to form a duplex RNA, which is then cleaved by a double-stranded RNA-specific ribonucleases RNase III, the Cas9 protein (Golkar 2016).

Types of CRISPER Cas 9

Type I CRISPR–Cas system

Type I contain the Cas3 gene, which encodes a large protein with distinct helicase and DNAase activities.

These genes encode proteins that form Cascadelike complexes with different compositions (Cristobal *et al.*, 2014). These complexes contain numerous proteins that have been included in the RAMP superfamily (Horvath *et al.*, 2010).

RAMP superfamily is linked to CRISPR loci which contains six genes, cmr, cmr2, cmr3, cmr4, cmr5 and cmr6 and present in a range of bacteria and archea.

In the Cascade complex, a RAMP protein with RNA endonuclease activity has been identified as the main enzyme that catalyze the processing of the long spacer-repeat-containing transcript into a mature crRNA (Cristobal *et al.*, 2014 and Horvath *et al.*, 2010).

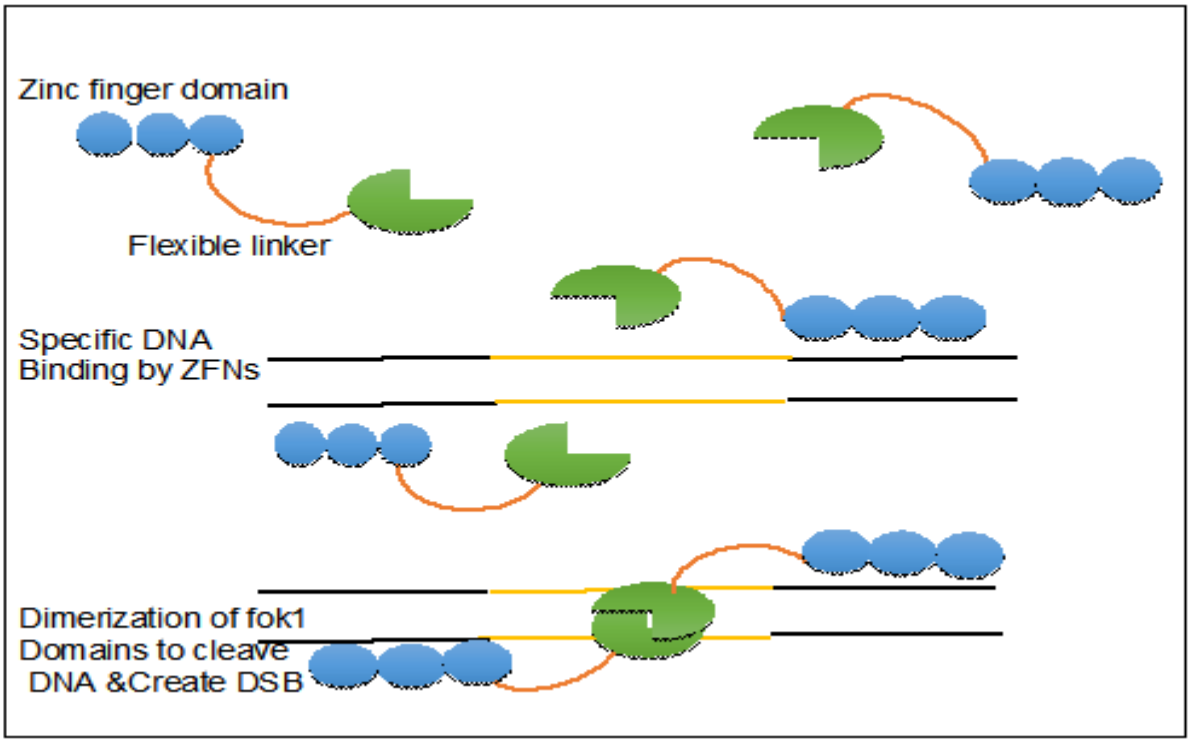

Fig. 2. ZFNs mechanism (Papaiannou *et al.*, 2012).

Type II CRISPR–Cas system

The type II CRISPR mechanism is unique compared to other because it includes the `HNH'-type system and Cas9 is required for gene silencing (Zhu *et al.*, 2015). During the destruction of target DNA, the HNH and Ruv C-like nuclease domains cut both DNA strands, thus generating double-stranded breaks (DSBs) at sites defined by a 20 nucleotide target sequence.

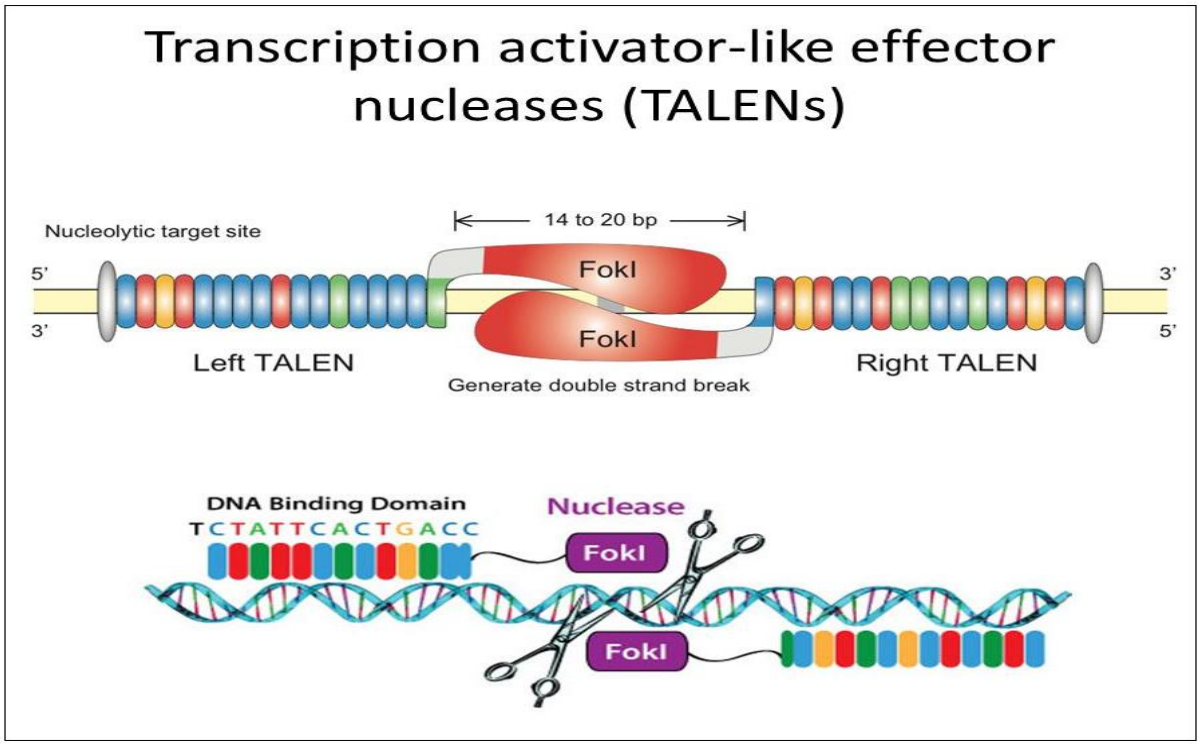

Fig. 3. Transcription activator like effector nucleases (Cunningham 2016).

The HNH domain cleaves the complementary strand, while the RuvC domain cleaves the non-complementary strand (Cong *et al.,* 2013; Cristobal *et al.*, 2014 and Zhu *et al.,* 2015). Type II seems to be sufficient for generating crRNA and improving the target DNA. Cas9's function in both of these steps and relies on the presence of two nuclease domains: a Ruv C-like nuclease domain located at the amino terminus and an HNH-like nuclease domain that resides in the mid-region of the protein (Zhu *et al.,* 2015 and Chylinski*et al.,* 2014).

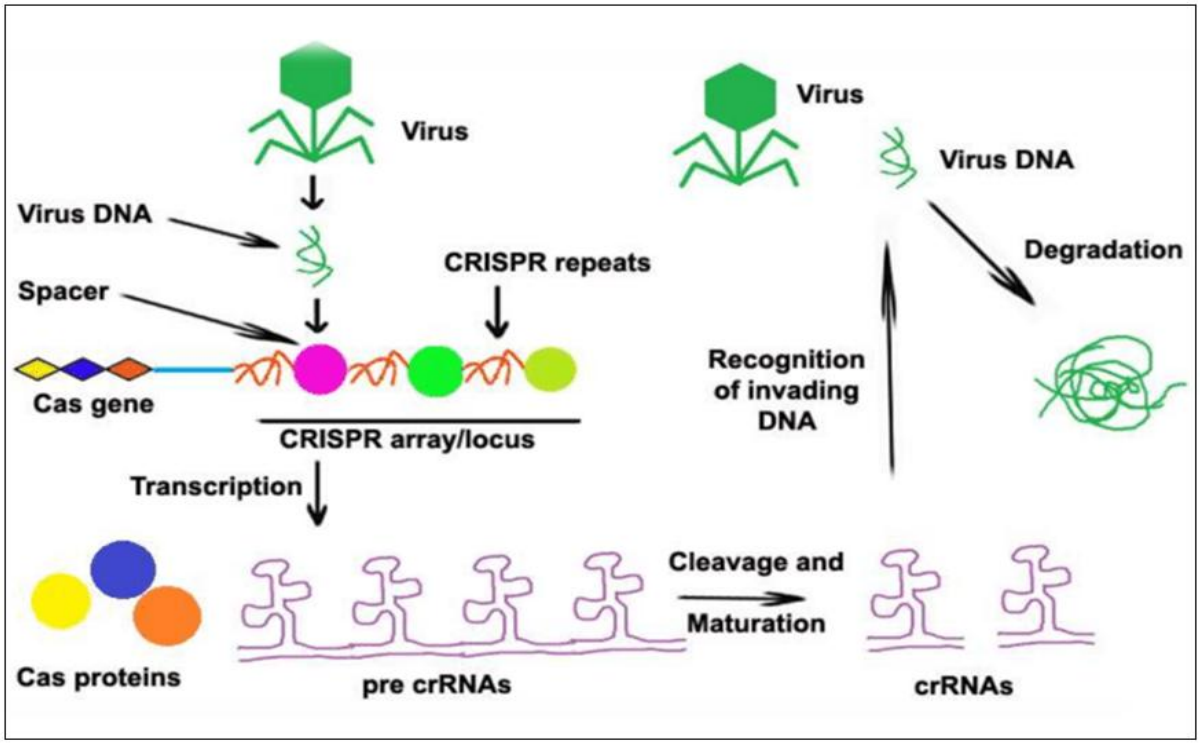

Fig. 4. Functioning of CRISPER Cas 9 (Ghimire 2017).

Type II systems cleave the pre-crRNA through a mechanism that involves duplex formation between a trans-crRNA and part of the repeat in the pre-crRNA; the first cleavage in the pre-crRNA processing pathway subsequently occurs in this repeat region. This cleavage is catalyzed by the housekeeping, double-stranded RNAspecific RNase III in the presence of Cas9 (Chylinski*et al.,* 2014).

Type III CRISPR-Cas system

Type III CRISPR-Cas systems contain polymerase and RAMP modules in which at least some of the RAMPs seem to be involved in the processing of the spacer-repeat transcripts, analogous to the Cascade complex. Type III systems can be further divided into subtypes III-A (Mtube or CASS6) and III-B (polymerase-RAMP module) (Makarova *et al.,* 2011 and Deltcheva *et al.,* 2011).

Mechanism of Cas9 protein

The most important step in genome editing is selection of target specific DNA sequence. Two biological macromolecules, guide RNA and Cas9 protein, interact to form a complex that can identify target sequences.

The Cas9 protein is responsible for locating and cleaving target DNA, both in natural and in artificial CRISPR Cas systems. The Cas9 protein has six domains, REC I, REC II, Bridge Helix, PAM Interacting, HNH and RuvC (Jinek *et al.,* 2014; Nishmasu *et al.,* 2014).The Rec I domain is the largest and is responsible for binding of guide RNA. The role of the REC II domain is not yet well understood. The arginine-rich bridge helix is crucial for initiating cleavage activity upon binding of target DNA (Nishmasu *et al.,* 2014).

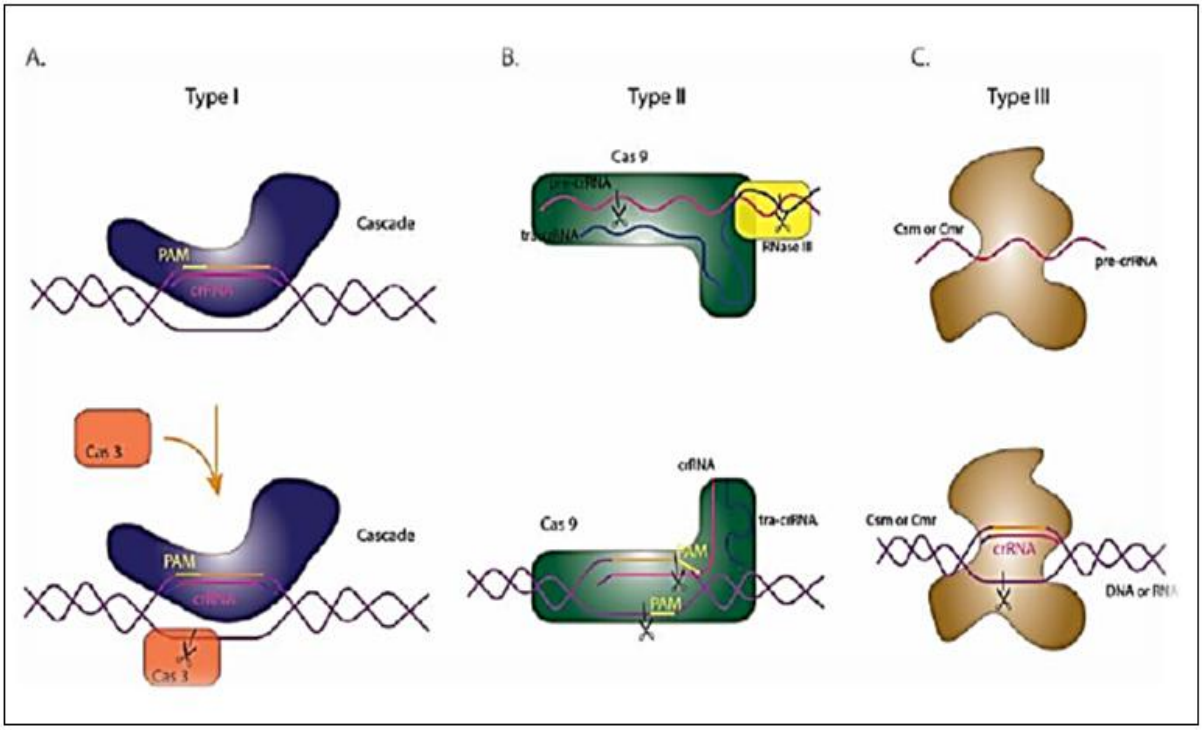

Fig. 5. Different types of CRISPER Cas genome editing system (Golkar 2016).

The PAM-Interacting domain confers PAM specificity and is therefore responsible for initiating binding to target DNA (Anders *et al.,* 2014; Jinek *et al.,* 2014; Nishmasu *et al.,* 2014; Sternberg *et al.,* 2014). The HNH and RuvC domains are nuclease domains that cut single-stranded DNA. They are highly homologous to HNH and RuvC domains found in other proteins (Jinek *et al.,* 2014; Nishmasu *et al.,* 2014). The Cas9 protein remains inactive in the absence of guide RNA (Jinek *et al.,* 2014). In engineered CRISPR systems, guide RNA is comprised of a single strand of RNA that forms a T-shape comprising of one tetra loop and two or three stem loops (Jinek *et al.,* 2014; Nishmasu *et al.,* 2014). The guide RNA is engineered to have a 5′ end that is complimentary to the target DNA sequence. This artificial guide RNA binds to the Cas9 protein and, upon binding, induces a conformational change in the protein.

Once the Cas9 protein is activated, then it searches for target DNA by binding with sequences that match its protospacer adjacent motif (Anders *et al.*) sequence (Sternberg *et al.,* 2014). A PAM is a two- or three-base sequence located within one nucleotide downstream of the region complementary to the guide RNA. PAMs have been identified in all CRISPR systems, and the specific nucleotides that define PAMs are specific to the particular category of CRISPR system (Mojica *et al.,* 2009). The PAM in *Streptococcus pyogenes* is 5′-NGG-3′ (Jinek *et al.,* 2012). When the Cas9 protein finds a potential target sequence with the appropriate PAM, the protein will melt the bases immediately upstream of the PAM and pair them with the complementary region on the guide RNA (Sternberg *et al.,* 2014). If the complementary region and the target region pair properly, the RuvC and HNH nuclease domains will cut the target DNA after the third nucleotide base upstream of the PAM (Anders *et al.,* 2014).

Conclusion

Genome editing is a technique of biotechnology used for alternation and modification of an organism's genetic makeup. Endonucleases and ligase enzymes are used for cutting and ligation of DNA respectively. Different tools of editing system like ZFN, TALEN and CRISPER are used for making improvements in crop plants. ZFN and TALEN were used in past. CRISPER is advanced tool involving cas 9 protein in

this system. CRISPER is found to be the most precise way of editing and target mutagenesis in multiple plants. Different studies have been conducted aiming at production of high yielding and stress resistant crop plants having high quality and medicinal uses. There is great room for improvement of technologies for meeting the enormously increasing food demands worldwide.

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