Gene editing: A potential tool to enhance field crop production

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Abstract *— Genome editing of crops has been observed to be rapidly advancing technology to introduce targeted mutations in plant genomes. The advances in clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR associated (Cas) protein systems have enabled targeted genome editing for crop improvement as compared to the previous methods including zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) that were time consuming and expensive. This technology works by repairing the double stranded breaks (DSB) by non-homologous end joining (NHEJ) and homology directed repair (HDR) and targets the gene of interest more precisely. In this review, we highlight the basic mechanism of CRISPR Cas9 system including the adaptation of CRISPR Cas9 system and its variants in plant editing. A RNA guided endonuclease, Cas9 has been used for generating stable knock out and knock in mutants in several plant species. We further review the delivery systems and the applications of CRISPR in trait improvement of crops. We outline the future perspectives of CRISPR Cas9 genome editing for regulating the gene expression and increasing the editing efficiency in medicine and agriculture. Application of CRISPR Cas9 for non-GMO crop editing with desirable trait can lead to increased yield of crops under environmental stress conditions.*

Keywords — *CRISPR Cas, GMOs, TALEN, sgRNA*

I. INTRODUCTION

Modern methods of biotechnology are using biological, chemical and physical processes to study the molecular mechanisms and biological role of genes to manipulate them for the improvement of crop varieties. Plant molecular biologists have been using traditional breeding or single gene transfer techniques for yield improvement, stress resistance and for the introduction of other desirable traits in crops. The introduction of transgene into the host genome through breeding and traditional transformation techniques for producing genetically modified crops (GM crops) is non- specific and unstable and becomes a public fear especially when it is about eatable species of crops [1]. Various health and environmental safety concerns are associated with the GM crops due to random gene insertions which makes it difficult to cross the barriers for the rapid adaptation [2].

Genome editing techniques produce site-specific DNA cleavage. For genetic improvement in the organisms, some site specific nucleases (SSNs) produce double strand nick at the targeted region of the genome. These modified nucleases have a domain of non-specific nuclease that is merged with a sequence specific DNA binding domain. These nucleases can accurately cut the target DNA at a specific site and then breaks can be repaired by the process of non-homologous end joining (NHEJ) or homology-directed repair (HDR) that result in insertion/deletion and substitution mutations in the target regions, respectively [3, 4] (Fig. 1). NHEJ is imprecise and often generates gene knock-out mutations, whereas HR is precise and leads to gene knock-in or replacement when a donor DNA molecule is present. The site specific nucleases can be divided into three main classes including, Zinc finger nucleases (ZFNs) [5]**,** (TALENs) [6] and the CRISPR/Cas9 nuclease system [3]. As compared to the recombinant or transgenic processes that produced insertions randomly, the genome editing produces a defined mutant. The crops whose genomes were edited carried the DNA of the desired trait at a specific location Malzahn, Lowder [7].

Zinc finger nucleases (ZFNs) consist of two sequence-specific zinc-finger proteins flanking a target sequence and the C-terminal of each protein is followed by a FokI nuclease. ZFNs were used broadly for the genome editing for much longer time in the plant systems [1]. In the existing situation, ZFNs are not much chosen because of their low target specificity, laborious procedure, many off-targets chopping down and less number of existing target sites [8]. Transcriptional activator- like effector nucleases (TALENs) are edited by modifying transcription activator-like effector (TALE) domain repeats for the recognition of the desirable targets. TALENs consist of two sequence-specific TALEN proteins flanking a target sequence and the C-terminal of each protein is followed by a FokI nuclease. TALENs are difficult to intend and gather. They have been used for editing of plants genome including *Arabidopsis thaliana* [9] , tobacco [10] and Brachypodium [11] (Fig. 1).

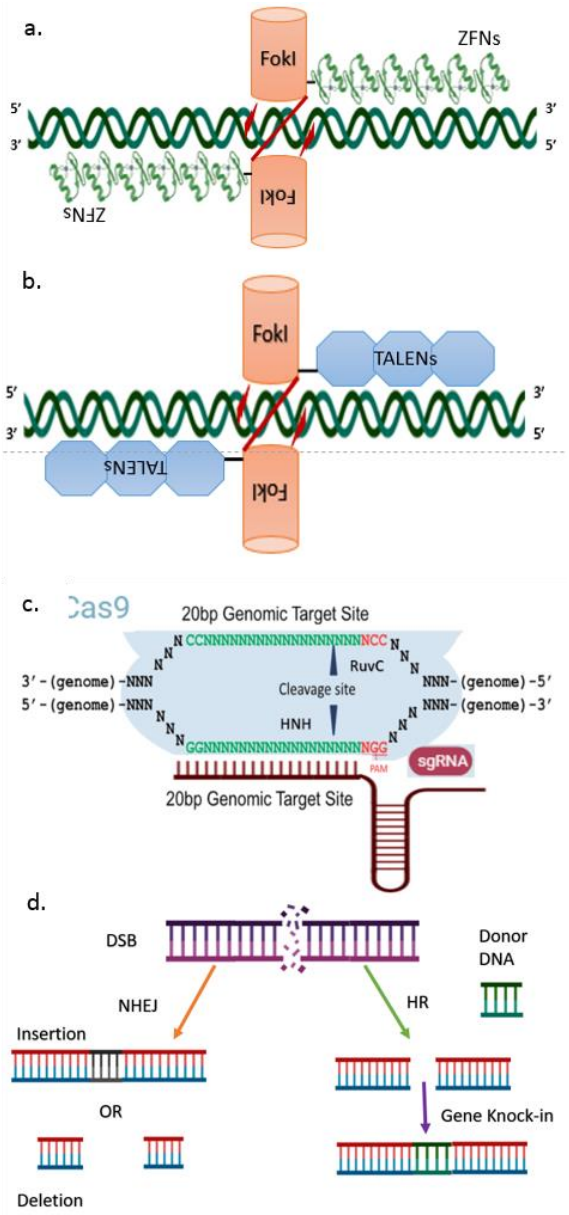

Fig. 1 An overview of genome editing in plants.

(A) The FokI dimer is guided by zinc-finger proteins to target the specific DNA site to chop. (B) The FokI dimer is guided by the TALEN proteins to target the specific DNA site to cut. (C) sgRNA guided nicking of CRISPR/ Cas9 system (D) The double strand break (DSB) can be repaired by non-homologous end joining (NHEJ) or by homologous recombination (HR).

CRISPR/ Cas is the third generation of genome editing technology which have design and methods that can be executed easily and are also much cost and time effective. CRISPR/Cas9 system is comprised of the Cas9 nuclease and sgRNA. The Cas9 nuclease is guided to the target cutting site by complementary pairing of sgRNA and a 20 bp target sequence, and the genomic DNA cut starts from the third base upstream of the PAM (NGG). The Cas9 nuclease contains two domains, HNH and RuvC-like, which cleave the DNA strands complementary (cut by the HNH domain) and non-complementary (cut by the RuvC-like domain) to the guide RNA.

II. MILESTONES IN THE DISCOVERY OF CRISPR/CAS SYSTEM

Thirty years ago, the first clustered regularly interspaced short palindromic repeat (CRISPR) was identified in *E.coli* during the study on alkaline phosphatase gene [12] and it opened an era of discoveries highlighted in Fig. 2 During that time it was difficult to predict the function of these repeated sequences until similar repeated pattern were discovered in archaea [13]. *Haloferax mediterranei* named them CRISPR sequences with the help of Ruud Jansen in 2002. In 2005, he discovered the sequence similarity between the CRISPR regions and sequence of bacteriophages as well as archaeal viruses. This discovery provided the clue of CRISPR's function as an adaptive immune system [14]. The CRISPR/ Cas9 system was developed from the bacteria's immune response system. They cleave the DNA of any outsider virus and hence protect themselves [15]. In May 2005, Alexander Bolotin while working on a bacterium *Streptococcus thermophilus* (whose genome has been sequenced) revealed an unfamiliar CRISPR locus [16]. The CRISPR array was similar to the systems previously reported but it contained some unique cas genes, with one gene expressing a large protein having a nuclease activity. They further identified that the sequence was similar to the viral genes, the spacers have a common conserved sequence at one end named as protospacer adjacent motif (PAM) functions in target recognition/identification.

CRISPR is basically a fragment of DNA consisting of tiny DNA repeats interspaced by some unpredictable (variable) sequences known as spacers. Cas genes were found to be linked with CRISPR loci, as these genes are present after the CRISPR locus [17]. In 2005, it was discovered that the spacers present within the CRISPRs were derived from any foreign viruses and plasmids [14, 18]. During the study of the mechanism of spacer uptake, some motifs were detected that are linked with spacer precursors (protospacers) from the DNA of an outsider bacteriophage [19, 20]. These motifs (PAMs) are small stretches of dinucleotides or trinucleotides present immediately or one position after the proto-spacers. They play a vital role in the identification of specific proto-spacers and in providing the direction and position of spacers integrated into the repeat sequences [21].

In 2006, a hypothetical scheme for CRISPR array as bacterial immune system was proposed by Eugene Koonin on the basis of the fact that the inserts in the spacer array were homologous to the phage DNA [22]. Philippe Horvath with coworkers while working on the response of *S. thermophilus* to phage attack proved that the CRISPR system is involved in

adaptive immunity. They incorporated new phage DNA into the CRISPR system, mounting a response against the next attack by phage. They revealed that Cas9 was the only protein involved in interference and inactivation of invading pathogens by the CRISPR system.

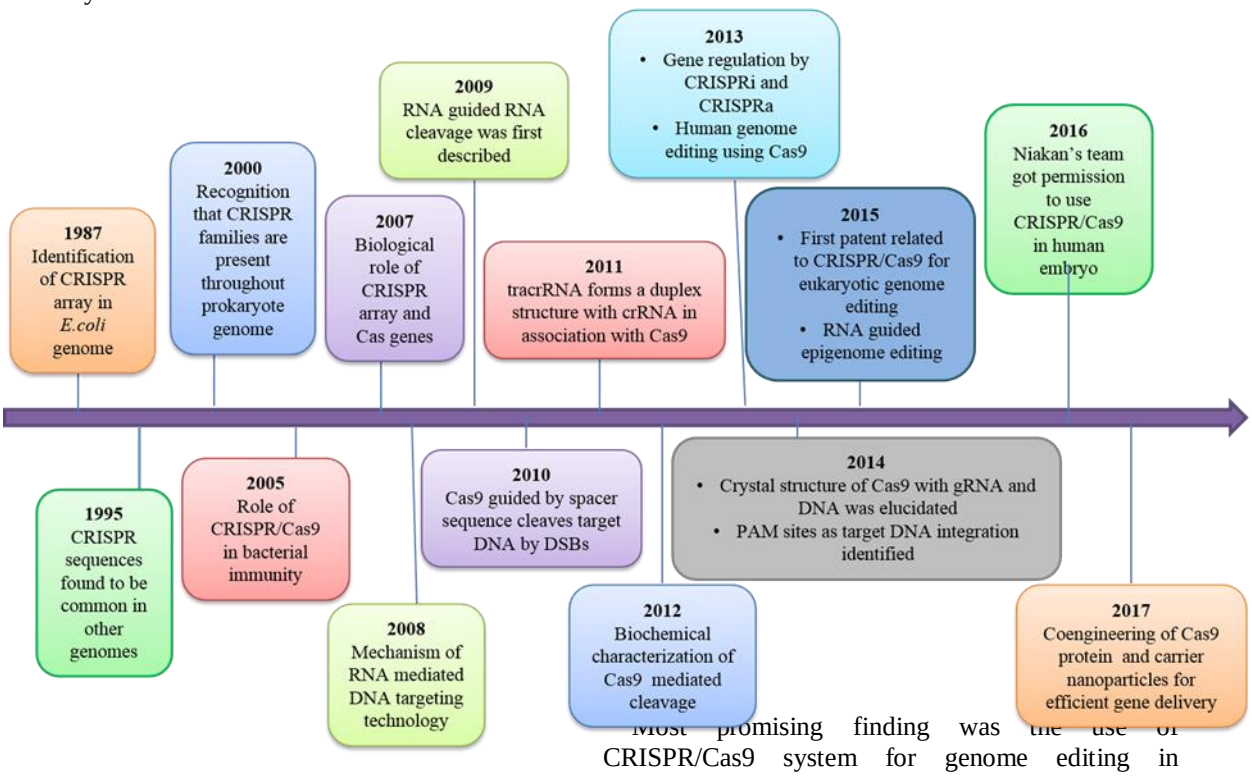

Fig. 2 Milestones in the discovery of CRISPR/Cas9 Technology

John van der Oost and colleagues in 2008 revealed that the spacer sequences of *E.coli* were transcribed to produce small RNAs named as CRISPR RNAs (crRNAs) that function in guiding the Cas proteins to the target [23]. *Sylvain moineau* with coworkers in 2010, revealed that CRISPR/Cas9 makes double stranded nicks in the DNA, particularly 3 bases upstream the PAM sequences and that Cas9 was the protein essential for the double stranded breaks in CRISPR/Cas9 system [24]. The final discovery on CRISPR system was made by Emmanuelle Charpentier in 2011 while sequencing the small RNA of *S. pyogenes* which contains a Cas9 mediated CRISPR system. They studied small RNA other than the crRNA and named it as trans-activating crRNA (tracrRNA). This tracrRNA makes a duplex with the crRNA that is involved in guiding Cas9 to the target DNA [25]. It was further revealed that the CRISPR system can function in other species when Siksnys and co-workers cloned the CRISPR/Cas expression cassette of *S. thermophilus* into *E. coli* which previously doesn't contain the CRISPR system [26]. Taking benefit from the heterologous systems in which CRISPR can work, Siksnys studied the mode of action of the Cas9 [27], they revealed that PAM was required for cleavage, the non-complementary strand and the complementary strands were cleaved by RuvC and HNH domain of Cas9 respectively.

Most promising finding was the use of CRISPR/Cas9 system for genome editing in eukaryotic cells [28] proving that Cas9 could be reprogramed to target the DNA of choice by altering the crRNA sequence. In 2013 the Targeted genome cleavage in human and mouse cells by two Cas9 orthologs from *S. pyogenes* and *S. thermophilus* was studied by Zhang group [28]. After this, a new era of genome editing using CRISPR/Cas9 system started and till now many species have been engineered using CRISPR/Cas9 systems.

III. MECHANISM OF CRISPR/CAS MEDIATED DEFENCE SYSTEM

There are three stages of CRISPR/Cas mediated defense system in nature: (a) adaptation, (b) expression and (c) interference (Fig. 3). In the first stage when a bacterium or virus infects the host, small DNA fragments are integrated into the CRISPR arrangement as the new spacers. In the second stage, a CRISPR RNA precursor (pre-crRNA) that is transcribed from a CRISPR locus is chopped down within the repeats with the help of Cas protein complex and mature crRNA molecules are formed. Every mature crRNA consists of a spacer flanked by tiny DNA repeats and known as a small guide RNA that direct Cas proteins to produce an antiviral response [23]. In the final stage, the crRNAs that is of 20 nucleotides binds with the target nucleic acids thus directing Cas proteins to demolish the complementary plasmid or virus target sequences that

match the spacers [29]. Cas nuclease cleaves 3–4 bases after the PAM sequence which plays a vital role in binding and nicking of the target DNA [30]. All paragraphs must be indented. All paragraphs must be justified, i.e. both left-justified and right-justified.

DNA [34]. Cas9 protein will only cut if the gRNA spacer sequence shows homology with the target DNA.

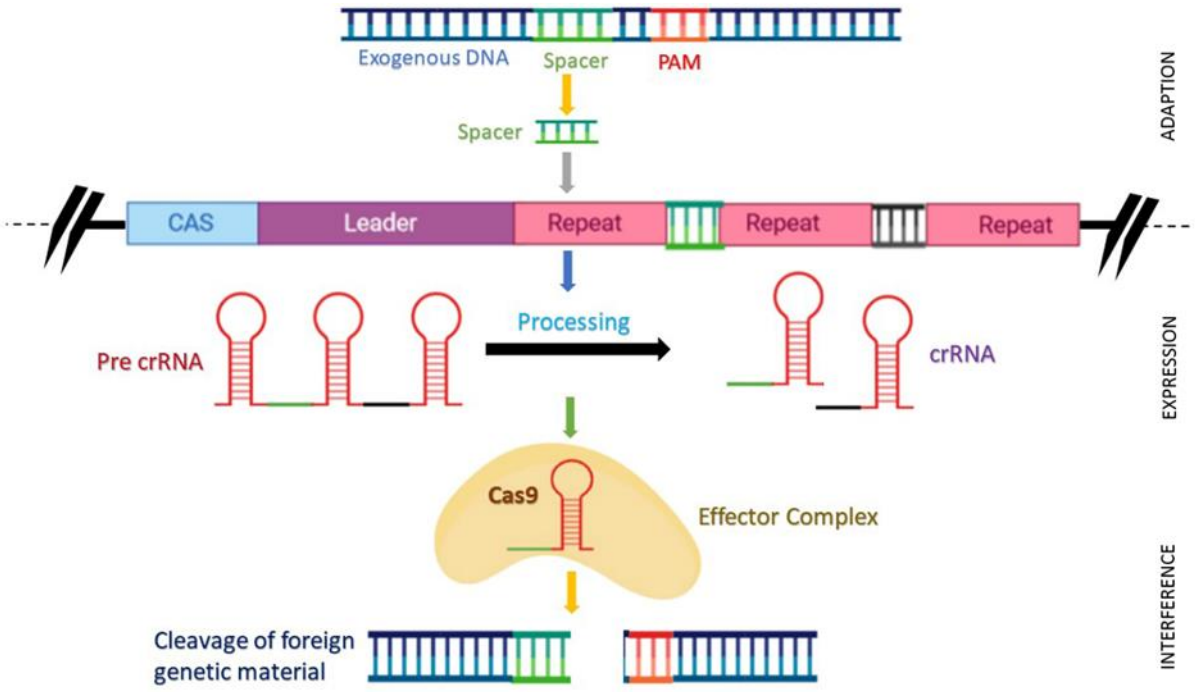

Fig. 3 CRISPR/Cas mediated innate immunity in prokaryotes.

In the engineered genome editing CRISPR systems, two components are present: a guide RNA (gRNA/sgRNA) and a CRISPR-associated endonuclease (Cas protein) [31]. The gRNA is a short synthetic RNA consisting of a sequence required for Cas-binding and a nearly 20 nucleotide spacer that defines the target DNA that has to be modified [3]. Thus, the genomic target of the Cas protein can be modified by simply editing the target sequence in the gRNA. The target DNA sequence should be unique or specific as compared to the remaining genome and the target protein should be present immediately flanking to a Protospacer adjacent motif (PAM) [32]. The PAM sequence acts as a necessary signal for Cas nuclease, but the exact sequence depends on which Cas protein is being used. After expression of CRISPR locus, a ribonucleoprotein complex is formed by the interactions between the scaffold of the gRNA and positively-charged grooves expressed on the surface of Cas9 protein [33].

The conformational change occurs in the Cas9 after binding of gRNA that converts the molecule from an inactive stage to the active stage. The spacer region of the gRNA remains free to bind with target

The complex undergoes a second conformational change after binding of the target to the nuclease domains, (RuvC and HNH) to cut the opposite strands of the target DNA [35]. The outcome of Cas9-mediated DNA cleavage is a double-strand break (DSB) within the target DNA (~3-4 nucleotides upstream of the PAM sequence) [36].

IV. TYPES OF CRISPR-CAS SYSTEMS

The CRISPR/Cas systems are classified into three major types on the basis of specific proteins which host possess to produce an immune response [29]. Type I and III systems use a Cas protein complex consisting of multiple proteins, on the other hand in the type II system the Cas9 is a large and a single specific multifunctional protein which is involved in the production of both crRNA and chopping down the target DNA. In comparison with the type I and III systems, the type II system is simple in construction and can be easily manipulated to function as a genome editing tool [29]. The locus of Type I has genes that translate into a large Cas3 protein having separate helicase and DNase activities. A newly discovered repeat associated mysterious Protein (RAMP) superfamily also contains a large number of

proteins like Cas5, Cas6 and Cas7 which are involved in the processing of long spacer transcript into the mature crRNA [37] . Whereas, the exception to this exists in Type1C- system where RNase activity was present. In many cases, Cas1 is fused with RecB nuclease domain of Cas4, where it played a role in spacer acquisition [37].

The type II system is a 'HNH' system (Streptococcus like) and also known as the Nmeni subtype for *Neisseria meningitidis* serogroup A or CasS4. This system contained a large Cas9 protein that not only generate crRNA but can also cleave the target DNA [38]. At the amino terminal, RuvC like two nuclease domains are present and a HNH domain is present in the center of the protein whose function is still unknown. From the previous analysis, HNH domain contained an endonuclease activity that always cleaved a target DNA [39]. The complete mechanism of Type II is still unknown but it is thought to be involved in the formation of duplex between tracrRNA and repeat part of pre-crRNA. The first cleavage takes place in this repeated region for processing of pre-crRNA. This step is catalyzed in the presence of Cas9 by the double stranded RNA-specific RNase III [25].

The type III CRISPR-Cas system is involved in the polymerase and RAMP activity that was responsible for cleaving the spacer-repeat transcripts comparable to Cascade complex. This system is categorized into subtypes III-A (called as CasS6) and III-B (called as polymerase RAMP domain) [40]. The type III-A is involved in the cleavage of targeted plasmid and type III-B is involved in RNA targeting. These two subtypes are involved in targeting nucleic acids but the exact mechanisms are still unknown. The ribonuclease in this system is the RAMP proteins apart from Cas2 proteins [41]. In addition to Cas6, Type III involves two more RAMPs for the processing of transcript. In many organisms, the type III is the only locus, containing polymerase RAMP activity forming a fully functional Type III system when combined with Cas1 and Cas2, that were likely to be involved in the spacer incorporation [42].

V. ENGINEERED CRISPR/CAS9 IN PLANT GENOME EDITING

The potential of CRISPR/Cas9 in editing the genome was initially revealed in 2012. This system has been used in approximately 20 crops till now for managing abiotic and biotic stresses as well as in improving the plant yield [43]. Many researchers have worked on the advantages of CRISPR/Cas9 for knocking in or out the particular genes involved in the biotic and abiotic stress tolerance. The process of genome editing in plants through CRISPR/Cas9 system is represented in Fig. 4.

The CRISPR/Cas9 developed for plant genome editing involves four phases. The first stage involves the formation of a gene specific gRNA (by fusing tracrRNA and crRNA). Many in silico methods are available for designing gRNA online [44] but they are not modified for plants specifically. More data collection and study is required to increase the accuracy to select gRNA in plants with greater efficiency Yin, Gao [44]. The Cas9 expression cassettes and constructs for gRNA are designed separately or integrated into a same vector. Ubiquitin6 (U6) and Ubiquitin3 (U3) small nuclear RNA promoters are used to express gRNA, these promoters help in transcription by RNA polymerase III. The guide RNA sequence must match the target sequence, except for the first nucleotide that could be 'G' in case of U6 promoter and 'A' for U3 promoter[45]. Nuclear localization signal (NLS) is required for the expression of Cas9 in the nucleus. Modified Cas9 system with optimized plant codon bias has been developed to improve its expression [46, 47]. Both, the gRNA and the Cas9 expression cassettes are ligated into the vector. This CRISPR/Cas9 system is then transferred to the plant cells in the form of a vector by either agrobacterium mediated transformation or gene gun method [44]. Both methods have been successfully used for delivering the CRISPR cassette into the immature embryos and calli of many crops as well as other plant tissues [46-49]. After the successful integration of CRISPR cassette into the plant genome, PCR and sequencing is done to confirm the transformed plants with the desired mutation [44]. Tobacco rattle virus (TRV) mediated gene delivery method has also been implied to transfer gRNA/Cas9 system into plant tissues without any non-specific effect[50].

VI. GENE KNOCKOUT/ KNOCKDOWN BY CRISPR CAS9

CRISPR Cas9/gRNA system can be used to obtain the desired insertion/deletion (indel) mutations in plant coding region by disrupting the reading frame of the gene [51]. This method has been employed to obtain gene knockout in plants like arabidopsis, sorghum, rice and tobacco [52-54]. Gene knockout using CRISPR uses the single guide RNA and Cas9 endonucleases to create double stranded breaks in target genes. The plant's DNA repair system i.e. NHEJ activates to repair these breaks but due to its error prone nature, creates deletions or insertions into the genome. This results in the silencing of the target genes [55].

Development in this system has generated a CRISPR/Cas9 toolbox for the knockout of non-coding target elements which has been a challenge in the plant genetics [51]. For example, this method could be used to knockout the mature microRNA sequence by indels, if PAM sequence is present. Thereby, disturbing the functionality of the mature microRNA. So, by introducing indels in the functional elements i.e. mature microRNA, cis element of promoter can be used for knockout of the non-coding fragments using Cas9/gRNA system.

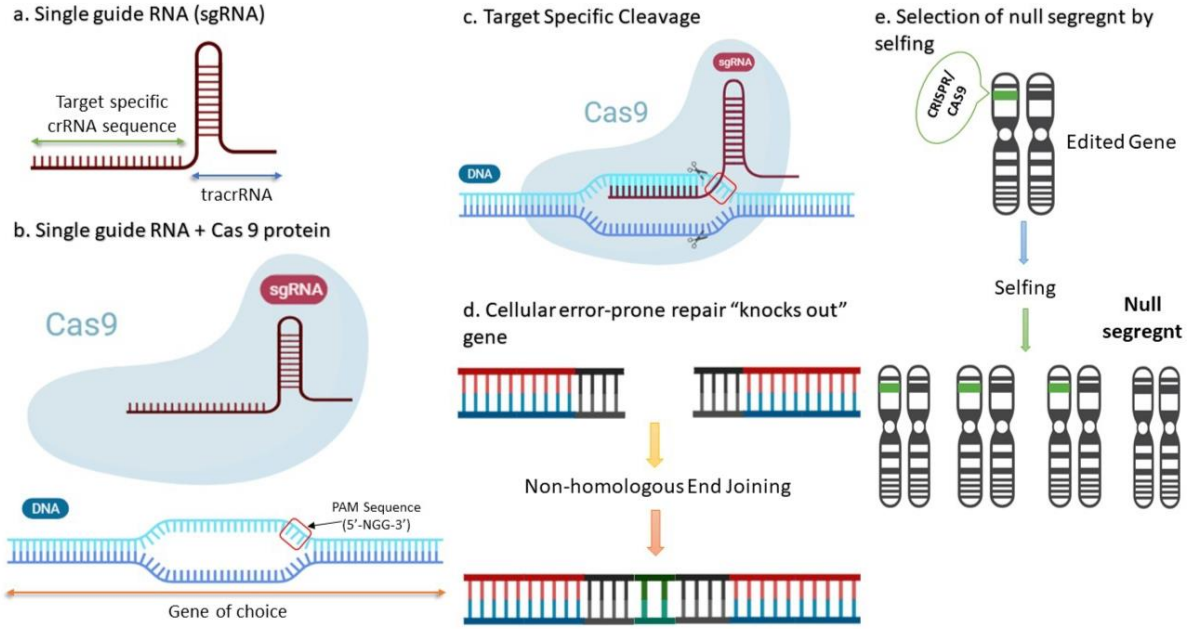

Fig. 4 Process showing CRISPR/Cas9 system as a powerful tool for crop improvement.

(A) Single guide RNA (sgRNA), (B) Engineered CRISPR/Cas9 nuclease with altered protospacer adjacent motif (PAM), (C) Target specific cleavage in plant genome, (D) Editing gene by non-homologous end joining (NHEJ) with CRISPR/Cas9 and (E) Selection of null segregates in the next generation

VII. GENE KNOCK-IN BY CRISPR/CAS9

Site directed mutagenesis and gene insertion at specific sites (knock-in) is of great importance in plant genome editing. Homology directed repair using a donor template DNA after the double stranded breaks results in the insertion of the desired gene into the specific region. In *A. thaliana* and rice protoplast [11], the site directed mutagenesis and knock-ins have been achieved by CRISPR/Cas9 system [52]. The generation of stable knock-in plants is still a challenge in most plants because it requires the co-delivery of the donor template into the cells which makes this process more complex. The NHEJ takes place at a much higher rate than HDR using the double stranded break repair [56]. Thus it requires more labour and selections to identify the real knock-in lines. Plant DNA virus can be used as a donor template to overcome these issues [57]. Gemini virus replicons due to their high copy number enhances the knock-in efficiency in plants [51]. However, CRISPR/Cas9 delivers simple procedure to generate double stranded breaks for HDR but still further insights to enhance the site directed mutagenesis for knock-in efficiency of plant genome is required.

VIII. CRISPR/CAS9 FOR POINT MUTATIONS

The fusion of Cas9 with cytidine deaminase was able to edit single base in the genome of mammals, yeasts and cereals crops by using single guide RNA (sgRNA) [58]. A substitution of single nucleotide can be targeted without foreign DNA donor and double strand break. Two fusion proteins were used like nCas9-PBE (Plant base editor) and dCas9- PBE [59]. Their composition consists of cytidine deaminase enzyme APOBEC1 (which is the variant, catalytically dCas9) and uracil glycosylase inhibitor. Both of these are used in base editing. There were the codon optimization for this fusion construct on the cereals plants and then cloned with the maize promoter Ubiquitin-1 to produce pnCas9-PBE and pdCas9- PBE [60].

In conclusion, C to T site specific base editing was occurred successfully in rice, wheat and maize by nCas9- PBE [61]. These plants consisted of deamination region having 7 bases of the protospacer and produced no indel mutations. The reports on base editing in rice suggested that this process is more efficient as compared to homologous recombination that create point mutation in crops [62].

IX. APPLICATIONS IN CROP IMPROVEMENT

The use of CRISPR/Cas9 system for agriculture is of great interest as it covers a wide range of applications in crop improvement from biotic and abiotic stress tolerance to improved yield, bio fortification and better plant quality.

Table 1. Application of CRISPR/Cas9 in agricultural crops.

Plant species	Targeted gene	Application Perspective	Reference
Citrus	Susceptibility gene CsLOB1 promoter	Citrus canker disease resistance	[63]
	PthA4 effector binding elements (EBEs) in the Type I CsLOB1 promoter (EBEPthA4-CsLOBP)	Canker disease tolerance	[63, 64]
Cucumber	eIF4E (eukaryotic translation initiation factors 4E)	Virus stress tolerance	[43]
Rice	OsMPK2, OsDEP1	Yield under stress	[11]
	OsDERF1, OsPMS3, OsMSH1, OsMYB5	Drought tolerance	[65]
	OsPRX2	Potassium deficiency tolerance	[66]
	OsERF922	Blast (caused by *Magnaporthe oryzae*) Tolerance	[67]
	SBEI (Starch branching enzyme) gene	Generation of high-amylose rice	[43]
	OsSAPK2	Drought, osmotic and salinity tolerance	[68]
	Grain size3 (GS3) and Grain number 1a (Gn1a)	Grain yield performance	[43]
	GW2, GW5 and TGW6 (Grain weight genes)	Increased grain weight	[43]
	OsEPSPS	Glyphosate resistance	[69]
	ALS (Acetolactate synthase) gene	Herbicide tolerance	[70]
	C287 gene	Imazamox (herbicide) resistance	[43]
	OsSWEET13	Bacterial blight tolerance	[71]
Wheat	TaVIT2	Iron Bio fortification	[72]
	TaDREB-2 and 3	Drought signaling	[73]
	TaMLO-A1 (wheat mildew resistance locus1)	Powdery mildew resistance	[74]
Maize	ARGOS8	Increased grain yield under drought stress	[75]
	ZmIPK1A ZmIPK and ZmMRP4	Phytic acid synthesis	[76]
	ALS2	Chlorsulfuron resistance	[48]
Tomato	BeYDV	Bean yellow dwarf virus (BeYDV) resistance	[77]
	SlMlo gene	Powdery mildew resistance	[78]
	SlIAA9 gene	Generation of parthenocarpic tomato plants	[43]
	ORFs and the IR sequence sDNA of virus	Tomato yellow leaf curl virus (TYLCV) and Merremia mosaic virus (MeMV)	[79]
	Rin (ripening inhibitor)	Fruit ripening	
	SlMAPK3	Drought tolerance	[80]
Potato	GBSS (Granule bound starch synthase)	Starch quality improved	[43]
	SSADH, CAT9, GABA-TP1, 2, 3	Enhanced GABA metabolite levels, abiotic stress tolerance	[81]
	ALS1 (Acetolactate synthase 1)	Herbicide resistance	[82]
Soybean	GmPDS11 and GmPDS18	Carotenoid biosynthesis	[83]
Flax seed	Enolpyruvylshikimate-3-phosphate synthase (EPSPS)	Glyphosate tolerance	[43]
Barley	Endogenous barley ENGase gene	N-glycans modification in cereal grains	
Cassava	MePDS	Carotenoid biosynthesis	[84]
Cotton	Cloroplastos alterados 1 (GhCLA1)	Increased mutation efficiency	[85]
	CLCuD IR and Rep Regions	Cotton leaf curl disease resistance	[86]

Biotic stress tolerance was targeted in various crops by gene knockout with Cas9/gRNA. Virus stress tolerance was studied in *Cucumis sativus* by targeting the eukaryotic translation initiation factors 4E (eIF4E) through CRISPR/Cas9. Gene knockout of OsERF922, SlMlo and TaMLO-A1 allele with Cas9/gRNA resulted in fungus stress tolerance in *Oryza sativa*, *Solanum lycopersicum* and *Triticum aestivum* respectively. Citrus canker resistance of *Citrus paradise* and *C. sinensis Osbeck* was analyzed by targeting PthA4 effector binding elements (EBEs) in the Type I promoter of CsLOB1 (*C. sinensis* lateral organ boundaries) gene [63, 64] while in *O. sativa* was made resistant to bacterial blight by targeting the susceptibility genes, Sucrose transporter gene (OsSWEET13)[71]. Abiotic factors like drought, salinity, high/low temperatures and herbicides affect the crops yield and performance. Various delivery methods have been employed for CRISPR/Cas9 mediated genome editing in *Linum usitatissimum*, *O. sativa*, and *S. tuberosum* to obtain herbicide tolerance[43].

Maize (*Zea mays*) is a significant cereal crop and 70% of the maize seed is phytic acid. CRISPR/Cas9 genome editing was utilized to produce novel allelic variants of ARGOS8 which improved maize grain yield under drought stress. This study showed the use of CRISPR/Cas9 as a tool for producing new variants and their utilization in crop improvement [75]. Moreover, editing done by CRISPR/Cas9 system in the protoplast of wheat for the two abiotic stress related genes called as wheat ethylene responsive factor 3 (TaERF3) and wheat dehydration responsive element binding protein 2 (TaDREB2) can produce stress tolerant novel variation in plant progeny expressing CRISPR-Cas9 [49]. *S. lycopersicum* is an ideal crop for checking gene editing by CRISPR/Cas9 system because the procedures for its efficient transformation are available and background information on the improvement of quality is also present. The overexpression of SIMPK3 regulates tolerance to drought in tomato. SIMPK3 was targeted by CRISPR/Cas9 system for improving drought tolerance. Likewise, ACETOLACTATE SYNTHASE1 (StALS1) mutation in potatoes resulted in herbicide resistant lines [82].

Major staple food crop in more than half of the world's population is rice (*O. sativa*) followed by other major crops: maize (*Z. mays*), potato (*S. tuberosum*), wheat (*T. aestivum*), tomato (*S. lycopersicum*), and barley (*Hordeum vulgare*). Bio fortification of staple crops is the need of hour to meet the world food requirements. Enhancement of seed oil (fatty acid) composition, reduced levels of polyunsaturated fatty acids and increased accumulation of oleic acid in the oil by targeting the fatty acid desaturase 2 (FAD2) genes of *Camelina sativa*, N-glycans modification in cereal grains (*H. vulgare*), generation of high-amylose rice by targeting SBEI and SBEIIb genes, biosynthesis of medical biomolecules like benzylisoquinoline alkaloids (BIAs) in *Papaver somniferum*, and biofortification of *S. tuberosum* by improving its starch quality through CRISPR-Cas9-mediated genome editing has been reported till date. The quality of potato starch is significant for numerous food applications as well as a great area of research. The granule-bound starch synthase (GBSS) gene was mutated in hexaploid potato to develop a waxy genotype. The genome edited potato showed the presence of amylopectin and complete absence of amylose, which confirmed that all the GBSS alleles were mutated [87].

The application of CRISPR-Cas9 system in crop yield improvement is of great interest. Pod shatter and control of dormancy in *Brassica oleracea* and *H. vulgare*, lignocellulose biosynthesis in *Dendrobium officinale*, increase in grain size, number and weight of *O. sativa*, generation of parthenocarpic tomato plants, improved rubber biosynthesis in hairy roots of *Taraxacum kok-saghyz* and reduction of the linkage drag during breeding procedure in *Zea mays* are the example of crops with improved yield [43].

X. FUTURE PROSPECTS

Bacteria have been known to alter the genome since ages. It took almost 30 years to reveal its function in bacterial immune system against the invading phages or plasmids for yogurt production [12, 19]. After the mechanism of CRISPR system was revealed, many additional applications of this system were derived. The ability of CRISPR system for precise RNA guided genome editing was described [3] and the CRISPR era started. This idea has been employed by scientists working in different fields and thousands of genomes have been edited from viruses to plants. Other than the classical CRISPR/Cas9 system from *S. pyogenes*, the Cas variants from many other species including *S. aureus* and *S. thermophilus* have been used for plant genome editing [88].

A CRISPR variant named Cas13a has been recently discovered that cuts the specific RNA instead of DNA and hence can be employed for RNA editing in bacteria as well as plants [89]. Further studies are required for its commercial applications in medicine and agriculture. Some studies of using Cas13a in bacteria showed RNA degradation but these effects are not reported in studies performed in plants [90, 91].

Beside the role of CRISPR system in knockins and knockouts, it can be used for regulating the gene expression by fusing the activators or repressors to DNA binding domains of the constructs used for editing genome (transcriptional activator-like effector (TALE), dCas) thereby regulating the endogenous gene expression [92]. Additionally, CRISPR/Cas9 system can be used to activate or repress plant gene transcription by fusing dCas9

(inactive) with sgRNA that targets the specific plant gene promoter [93, 94]. Recently, a bidirectional promoter was used to express the Cas9 and gRNA in opposite directions which increased the genome editing efficiency in rice [95]. So, these modifications could be employed to increase the genome editing efficiencies in many more crops.

XI. CONCLUSION

The CRISPR/Cas9 system is a major breakthrough in molecular biology. With the recent advances it is making its way in the field of agriculture industry. Through this technology it has become possible to produce disease and pest resistant plants of better quality and yield. CRISPR/Cas9 editing can also be employed to replace defective genes and in production of such plants that would be beneficial for human consumption. But there are still some concerns regarding the use of genome editing in crops. So, further studies are required to confirm the safety of using CRISPR/Cas9 edited crops. It will also raise some ethical concerns in the public using the genome edited crops. Proper education regarding the basis of this technology in editing crops, their benefits and health effects should be given to the public. This technology can progress only if it is properly understood and socially accepted. Laws and regulations regarding use of CRISPR/Cas9 will be needed for implementation of this technology worldwide (http://dx.doi.org/10.5772/intechopen.75024). This technology should be used to its fullest to get maximum benefit in the field of agriculture to cope against the world hunger.

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