**CRISPR/Cas: a new horizon in crop improvement**

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**ABSTRACT**

By the over expanding growth of human population, feeding this enormous number becomes the major issue in recent times. In such situations, augmentation in agricultural production is an urgent need of the hour in order to supply nutrition along with food to the humankind. Moreover, conventional breeding methods are not enough to meet this demand due to its high time-consumption and labour-intensive nature. Therefore, to vanquish these circumstances, CRISPR-Cas comes into the field of molecular approach to provide an alternative to the conventional procedures. CRISPR has played a crucial part in the increase in genome editing studies since recent past. Base editing, knock out or knock in of desirable genes, fine tuning of several genes, promotion of antiviral defence, and alteration of various demanding biochemical pathways through this specific gene editing system has proven its ability to spread new wings for accelerating crop advancement in last few years. In a nutshell, this chapter mainly aims at the execution of CRISPR/Cas system towards the revolutionary approaches of future plant breeding, along with a short overview and future endeavour.

**Keywords**—CRISPR/Cas; crop improvement; double-stranded break; future breeding; genome editing; hybrid breeding

1. **INTRODUCTION**

Classical or conventional approach was proven extremely successful times and again in plant breeding. Most of the high yielding varieties (HYVs) and hybrids available today are the resultant of the same. Throughout the years, it played a major role in crop improvement [1,2]. But to substantiate this rapidly growing human population these days in the diverse situation of changing climate coupled with scarcity of available water and arable land resources, some sort of innovations in crop breeding technology were required to accelerate productivity and sustainability in agriculture. The inability of conventional breeding to meet today’s demands for increasing crop production due to its highly time-consuming nature and laborious procedures highlighted hybridization, mutagenesis, and transgenic breeding as the leading strategies [3,4]. Transgenic breeding generates desired traits by transferring exogenous genes into the background of elite varieties [5]. Although it encompasses the capability to break the bottleneck of reproductive isolation, commercialization of genetically modified (GM) crops is limited by stringent Government guidelines considering future concerns [5,6]. Therefore, developing a novel variety through precise genome editing is a revolutionary approach for rapid improvement of agricultural crops.

Zinc finger nucleases (ZFN) were first adapted in 2005 while experimenting with tobacco plant [7], and five years later, transcription activator-like effector nucleases (TALENs) surfaced in the field of plant biotechnology as a genome-editing tool [8]. In 2013, three independent groups of researchers discovered the CRISPR/Cas9 system for using in rice (*Oryza* *sativa*), wheat (*Triticum aestivum*), rockcress (*Arabidopsis thaliana),* andbenthi (*Nicotiana benthamiana*), respectively [9,3]. Later, this has been introduced as an ingenious technique for rapid crop improvement due to its ability to create the specific introduction of targeted sequence variation at specific sites. Apart from ease of hybrid breeding, this approach can also knock in desired traits or knockout unwanted traits into elite varieties. Due to its multiplexing ability, multiple targets can be modified expeditiously, and immediate pyramiding of multiple traits into an elite background becomes feasible within a single generation. Unprecedentedly this system paves the way to create genetic diversity for breeding. High-sugar content tomatoes are very expensive because of its lengthy tedious procedure of production, though this reduces the size. Kawaguchi et al. [10] implemented gene editing to modify cell wall invertase inhibitor gene to accumulate higher quantity of sugar in the fruit. Digestion of the inhibitor through gene editing technology leads to 30 percent increase of sugar content than usual, without even effecting fruit size [10]. The first directly consumable genome-edited tomato was launched in Japan by Sanatech Seed [11] Sanatech Seed's “Sicilian Rouge” high gamma-aminobutyric acid (GABA) tomato was developed using CRISPR-Cas9 gene editing technology. The tomato contains five times more GABA than a regular tomato, an amino acid believed to aid relaxation and help lowering blood pressure [11]. The ministry of Japan declared that it will not be regulated as a genetically modified product [11]. Thus, for sustainable agriculture, the power of CRISPR and its variants remains undeniable.

1. **CRISPR/CAS: GENOME EDITING TOOL FOR PLANT SYSTEMS**
2. **An overview of CRISPR/Cas system**

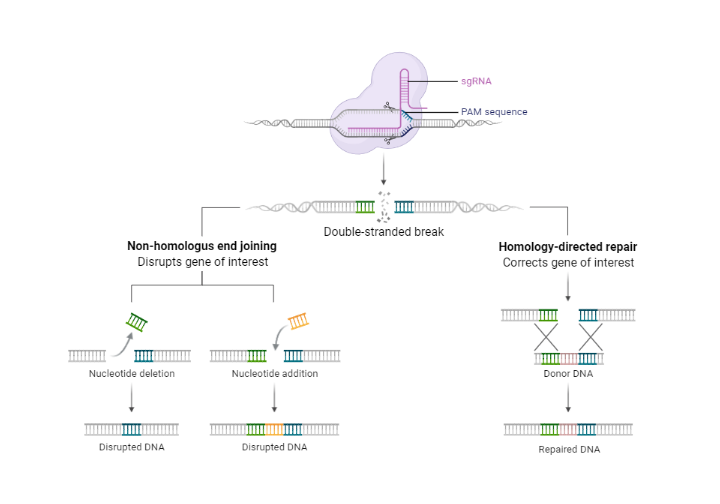
CRISPR/Cas system is a natural prokaryotic immune system present inside the bacteria to protect themselves from phage attack. CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats and Cas stands for CRISPR-associated protein, which is a kind of endonuclease. This system is known to provide a certain form of acquired immunity to the prokaryotes [3].

As bacteria face constant threat of infection and destruction from bacteriophage, they evolved a protective immune system called CRISPR in response to those. The CRISPR array, a short stretch of DNA in bacteria, is composed of an alternating repeated sequence and target specific spacer. These spacers contain the fragment of DNA of invading viruses collected from past infection. When a virus attacks bacteria, a new spacer is added into the growing CRISPR array. This process begins when a protein complex (known as Cas1-Cas2) identifies the invading viral DNA and cuts out a segment of DNA. This specific segment of DNA is called protospacer. These protospacer is added to the collection of CRISPR array and it reserves a memory of that particular phage infection, so that the bacteria can easily and immediately defend the next attack. This defense process starts with transcribing long CRISPR-RNA (pre-crRNA) from the spacer and repeats of the CRISPR array. Later, another RNA, named as transacting or spacer RNA, comes up and links with the crRNA for base pairing. The protein Cas9 binds on the dual RNA and trims them. Upon matching of the sequences of both crRNA and the viral DNA, Cas9 creates a double stranded cut on the viral DNA at precise position in order to destroy it. Further to mention, the juxta-positional presence of PAM (Protospacer Adjacent Motif) to the protospacer in viral DNA helps Cas9 to distinguish despite their similar sequences [3].

Six distinct types of bacterial CRISPR systems (type I, II, III, IV, V, and VI) has been identified in different type of bacterium, so far. Among these CRISPR/Cas9 belongs to type II. Another popular system type V where protein cpf1 can be found [3].

Recently, a multipurpose efficient mini-CRISPR genome editing tool was developed by Stanford University, which acts like a ‘molecular swiss knife’. It is compact and less than half the size of the conventional Cas proteins *viz*. Cas9 and Cas12a. CasMINI, a powerful dwarf among the CRISPR giants, can efficiently activate, delete, and edit genetic code, just like Cas12a. Now-a-days, this tool becomes very popular for cell engineering and gene therapy *ex vivo* and *in vivo* because of its robust nature [12].

1. **CRISPR-induced genome editing by DNA double-strand breaks (DSBs)**

Creation of double stranded breaks (DSBs) at target loci is the key feature of CRISPR/Cas. It generally undergoes either of the two main DNA repairing pathways, *i.e*. non-homologous end joining (NHEJ), and homology-directed repair (HDR) in order to introduce genomic modifications (Figure 1; Table 1) [13].

**Figure 1: Non-homologous end joining (NHEJ)- and Homology dependent repair (HDR)-mediated double stranded break repair in DNA (Source:** [**https://biorender.com**](https://biorender.com)**)**

The former one, without the requirement of homologous repair templates, can be implemented as a productive strategy for insertions of donor DNA sequences in a homology-independent manner, and thus, can be utilized for crop improvement through gene stacking [3]. Although, NHEJ is well recommended for large-scale knockout studies and it can be initiated in any phase of the cell cycle, the lack of precision creates a barrier towards sophisticated genome engineering. On the other hand, the latter one possesses the capability to insert or replace the sequence of interest into the targeted place of DNA. Further to mention, HDR can be initiated in the second and last phase of interphase *viz*. S and G2-phase of the cell cycle, respectively. Repairing of the DSB require a template with homology to the break site, that can either be the sister chromatid or an exogenous template [14].

CRISPR is not limited to the formation of DSBs, sequence-specific gene regulation, epigenome editing, and genome imaging [3]. dCas9 can also provide a unique stage for proteins recruitment. dCas9, fused with transcriptional repressor (e.g., the KRAB domain and SRDX domain) or transcriptional activators domains (*e.g.,* VP64, p65AD, and VPR), can regulate gene expression [15]. After dCas9 recruits epigenetic effectors such as the histone demethylase LSD1, histone acetyl transferase (HAT) p300, and ten-eleven translocation (TET) proteins, it modifies the epigenetic marks at DNA or histone level. This leads to the alteration of chromatin modification, which eventually results in change in various biological events *i.e.* gene expression, cell differentiation, etc. [16].

Beyond DSB-mediated genome editing, base-editing systems, independent of HDR or donor DNA, can introduce specific base changes. As instance, cytosine base-editor (CBE) system converts targeted C to U in genomic DNA [17]. Subsequently, Gaudelli et al. [18] developed adenine base editors (ABEs) for converting A to G in genomic DNA. Higher efficiency, generation of a very few numbers of non-specific products, lesser chromosomal aberrations etc. make base-editing systems more advantageous over the DSB mediated one [3].

1. **APPLICATIONS OF CRISPR/CAS IN PRECISION PLANT BREEDING**
2. **Knockout-controlled trait improvement**

Elimination of negative elements is considered as the most promising approach for genetic improvement. Therefore, knocking out of undesirable genes through CRISPR/Cas9 is the simplest and most common application (Figure 2a). Till date, several traits such as yield, quality, and biotic- and abiotic-stress resistance etc. have been improved by using CRISPR/Cas9. Rather, various breeding techniques like hybrid-breeding techniques and many more have also been enhanced, as reported [3].

1. **Yield improvement**

Yield is a complex dependent polygenic trait. Several traits such as grain number (*OsGn1a*), grain weight (*TaGW2*, *OsGW5*, *OsGLW2*, or *TaGASR7*), grain size (*OsGS3*), panicle size (*OsDEP1*, *TaDEP1*), and tiller number (*OsAAP3*) have negative impact on yield. In such circumstances, knocking out those traits using CRISPR/Cas9 was proven to be an effective tool for improving yield through creation of loss-of-function mutation of respective genes [19,20,21,22,23,24]. Simultaneous knockout of three grain-weight related genes in rice (*GW2*, *GW5*, and *TGW6*) led to trait pyramiding, which significantly enhanced grain weight as per the published report [12].

1. **Quality improvements**

Quality traits depends on the specific breeding requirements. Now-a-days, genome editing have impacted various quality parameters in several crops *i.e.* starch content, aroma, nutritional value, storage etc. Improvement in amylose content in rice for better cooking and eating quality was generated by knockout of *Waxy* gene by CRISPR/Cas9 [25]. By CRISPR/Cas9 knockout procedure, waxy corn line with higher yielding ability was developed by DuPont Pioneer for commercial purpose [26]. CRISPR/Cas9 was used to develop high-amylose containing and resistant starch rice through mutation of *SBEIIb, a* starch branching enzyme gene [27]. The study also suggested that consumption of high-amylose containing foods should benefit patients with dietary problems related to chronic noninfectious diseases [27].

2-acetyl-1-pyrroline (2-AP) is the principal compound behind the aroma, which adds higher market value to rice. A defect in betaine aldehyde dehydrogenase 2 (*BADH2*) gene causes the biosynthesis of 2-AP. An aromatic rice line with a similar 2-AP content (0.35–0.75 mg/kg) like a natural mutant aromatic rice variety was developed by using TALEN-targeted disruption of *OsBADH2* [28]. With evolution of CRISPR/Cas9 techniques, aromatic trait has been incorporated to more than 30 elite rice cultivars in major rice cultivating areas of China [3].

Almost 7% of individuals in western countries suffer from celiac disease, which is triggered by gluten protein in cereals. The major gluten-encoding gene family in wheat is the α-gliadin gene family, which consists of around 100 genes and/ or pseudogenes. By CRISPR/Cas9 editing, researchers developed low gluten wheat by simultaneous knocking out of most conserved domains of that particular gene family [29]. Other than wheat, CRISPR/Cas9 editing also created seeds of *Brassica napus* [30]and *Camelina sativa* [31,32] with increased oleic acid oil, longer shelf life in tomatoes [33,34], high-value tomatoes with elevated lycopene [35] or GABA content [36,37], and potato containing reduced levels of steroidal toxic glycoalkaloids [38].

1. **Biotic- and abiotic-stress resistance**

Several plants with increased biotic-stress resistance have been obtained via CRISPR/Cas9 knockout. For example, TALEN and CRISPR/Cas9 knocked out all six *TaMLO* alleles in order to develop increased resistance against powdery mildew in wheat [39]. Similarly, Nekrasov et al. [40] showed that CRISPR/Cas9 mediated knockout of *MLO* to develop powdery mildew resistant tomato. Knockout of *OsERF922* anddeletion of the *OsSWEET13* promoted blast- and bacterial leaf blight (BLB) resistant rice, respectively [41,42]. CRISPR/Cas9 also efficiently offered tungro disease–resistant *eif4g* rice [43], broad potyvirus–resistant *eif4e* cucumber [44], and cotton leaf curl disease–resistant *clcud* cotton [45].

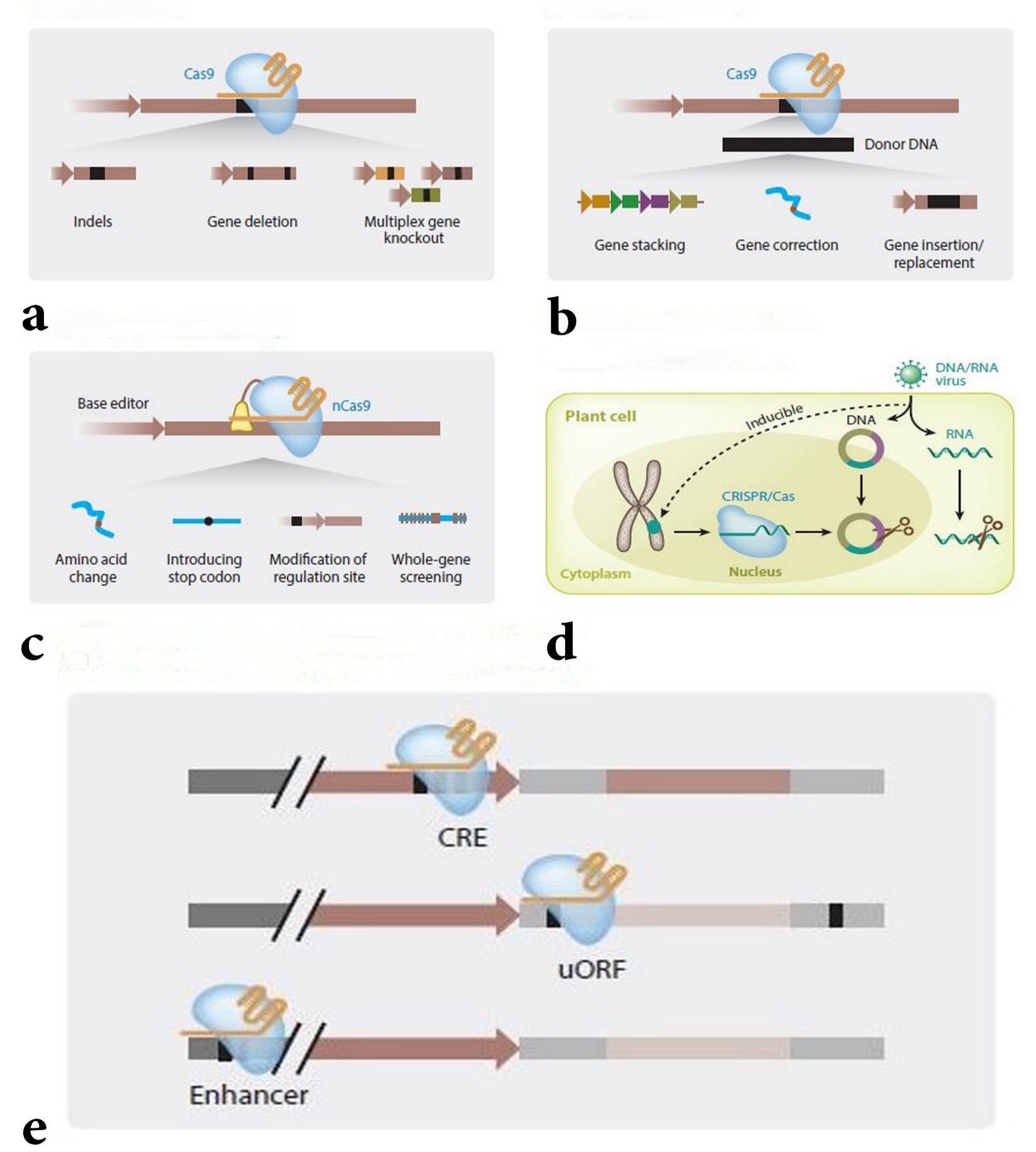
On the other hand, amongst all the abiotic factors, contamination of arable lands has prompted the need to prevent hazardous heavy metal accumulation in crops [3]. Breeders have generated rice variants with low amounts of cadmium (Cd), arsenic (As), and radioactive caesium (Cs), by knocking down OsARM1, OsNramp5, and OsHAK1, respectively [46,47,48]. In addition, pyl1/4/6 triple knockout rice generated by CRISPR/Cas9 editing exhibited higher grain yield, higher high-temperature tolerance, and lower preharvest sprouting than the wild type [49].

1. **Accelerating hybrid breeding**

The pre-requisite to high quality hybrid is male sterile lines. Development of thermo-sensitive male-sterile *tms5* lines in rice [50] and maize [51], photosensitive genetic male-sterile (GMS) *csa* rice [52], and *ms45* wheat [53], etc. are some instances of the progress in male sterile line development through CRISPR/Cas-mediated gene knockout system. Moreover, to overcome hybrid sterility in *japonica*-*indica* hybrids, *SaF/SaM*,and *OgTPR1* at the sterility locus *Sa,* and *S1* were disrupted, respectively [54,55]. Knockout of *Sc-I* allele in *Sc* gene in *indica* allele was reported to rescue male fertility in the aforesaid hybrids [56].

1. **Improvement through knock-in and replacement**

Many traits are conferred by addition, substitution, or change of expression of several nucleotides or genes. Knock-in or replacement of specific alleles facilitate breeding without linkage drag or generating non-existing allelic variants (Figure 2b) [57]. Moreover, it could also alter multiple elite traits through gene pyramiding in a singular variety [3].

Shi et al. [58] enhanced ARGOS8 expression by inserting or knocking in the GOS2 promoter replacing the natural ARGOS8 promoter through HDR. Under drought stress, the altered ARGOS8 variants had higher ARGOS8 transcript levels and higher yield. In another study, replacement of T317A into the ALC gene resulted in longer shelf life in the created tomato line [59]. On the other hand, the use of Gemini virus replicons reportedly increased repair template numbers to enhance gene-targeting efficiency in various crops [3]. It efficiently increased the frequency of insertion of Cauliflower mosaic virus 35S promoter upstream to *ANT1* gene, which was constitutively expressed in tomato in order to increase the anthocyanin content [60]. Moreover, substitution of principal enzymes in the conserved domains of endogenous acetolactate synthase (*ALS*) and 5-enolpyruvylshikimate-3-phosphate synthase (*EPSPS*) genes, the key target for gene editing, conferred resistance against several herbicides. As example, the substitution of double amino acid (T102I/P106S and T102I/P106A) in flax [61] and cassava [62] gave rise to HDR-mediated glyphosate resistance.

**Figure 2: Applications of CRISPR/Cas-based gene editing methodologies.**

**a** Gene knockout, **b** Gene knock-in/ replacement, **c** Base editing, **d** Antiviral resistance in plant breeding, **e** Fine tuning gene regulation. (Adapted from [3])

1. **Applications of base editors in plants:**

Base editing in coding regions is one of the major achievements to confer herbicide resistance (Figure 2c). Sulfonylurea- or imidazolinone-resistant rice [63], wheat [64], *Arabidopsis* [65], and watermelon [66] have been created by targeting *ALS* with a plant cytidine base editor, and haloxyfop-R-methyl resistant rice has been generated by targeting acetyl-coA carboxylase *ACCase* gene with a plant adenine base editor [67,68]. On the other hand, in RNA splicing processes, most eukaryotic mRNA follows the canonical GU/AG rule, where introns contain a splice donor site (GU) at the 5' end and a splice acceptor site (AG) at the 3' end. At consensus sequences, base editing can generate point mutation leading to the loss of particular splice forms or mis-splicing. Xue et al. [69], in their study, converted G to A in the splice donor site, which not only causes hypersensitivity to abscisic acid but also the constitutive retention of an intron of *AtHAB*. Kang et al. [70] disrupted the splicing acceptor site by following the opposite conversion, which ultimately guided to altered splicing of *AtPDS* mRNA.

1. **Antiviral plant breeding strategies**

CRISPR/Cas system provides a defense mechanism to cleave plasmids and viruses, that invade the primitive creatures *i.e.* archaea and bacteria (Figure 2d). Besides, it is well-known to confer virus resistance in plants too. For example, stable over expression of Cas9 and sgRNAs specifically target the genome of Gemini virus in order to inhibit its replication [71,72,73]. For developing Gemini virus-resistant plants, the intragenic sequences required for replication initiation was the ideal target for this system [73]. Further to indicate, the main backdrop of this antiviral system was the constitutive expression of Cas9/sgRNA, which led to off-target mutations, although a viral promoter with the ability to drive Cas9 expression could reportedly check this [74].

1. **Fine-tuning gene regulation in plants**

Modulation of gene expression for assessing gene functions at transcriptional, post-transcriptional or translational level can greatly facilitate plant breeding apart from creation of mutations (Figure 2e). An array of cis-regulatory elements which can be modified through genome editing generally control these processes. To date, genome editing for altering gene expression in plants has predominantly focused replacing promoters and deleting *cis-*regulatory elements [75,76]. Rodriguez-Leal et al. [77] edited the promoter regions of quantitative trait–associated genes viz. *SlCLV3, SlS*, and *SlSP*. These eventually led to continuous variation as well the selection of mutated alleles with enhanced yield.

**Table 1: CRISPR/Cas-mediated gene editing in various plants/ crops**

|  |  |  |  |
| --- | --- | --- | --- |
| Plants/ crops utilized | Targeted genes | Method of gene editing | References |
| *Arabidopsis thaliana* | *BRI1, JAZ1, GAI* | NHEJ | 78 |
| *GFP* | NHEJ | 79 |
| *CHLI1, CHLI2, TT4* | NHEJ | 80 |
| *ADH1, TT4, RTEL1* | NHEJ | 81 |
| *ADH1* | NHEJ, HDR | 82 |
| *TRY, CPC, ETC2* | NHEJ | 83 |
| *FT, SPL4* | NHEJ | 84 |
| *AtCRU3* | NHEJ | 85 |
| *At1g16210, At1g56650, At5g55580* | NHEJ | 86 |
| *Nicotiana benthamianum* | *NbPDS* | NHEJ | 87 |
| *NbFLS2, NbBAK1* | NHEJ | 88 |
| *Nbpds* | NHEJ | 89 |
| *NbPDS, NbIspH* | NHEJ | 90 |
| *XT* | NHEJ | 91 |
| *Oryza sativa* | *ROC5, SPP, YSA* | NHEJ | 78 |
| *OsSWEET11, OsSWEET14* | NHEJ | 79 |
| *OsMYB1* | NHEJ | 80 |
| *CAO1, LAZY1* | NHEJ | 92 |
| *OsMPK5* | NHEJ | 93 |
| *OsPDS, OsDEP1* | NHEJ, HDR | 94 |
| *OsBEL* | NHEJ | 95 |
| *OsPDS, OsPMS3, OsEPSPS,* | NHEJ | 96 |
| *SWEET1a, SWEET1b, SWEET11* | NHEJ | 97 |
| *ALS* | HDR | 98 |
| *CDKA1, CDKA2, CDKB1,* | NHEJ | 99 |
| *YSA, CDKB2* | NHEJ | 100 |
| *Solanum lycopersicum* | *SlAGO7* | NHEJ | 101 |
| *SHR, SCR* | NHEJ | 102 |
| *RIN* | NHEJ | 33 |
| *SlPDS, SlPIF4* | NHEJ | 103 |
| *S. tuberosum* | *StALS1* | NHEJ | 104 |
| *StIAA2* | NHEJ | 105 |
| *Triticum aestivum* | *TaMLO* | NHEJ | 106 |
| *Tainox, Tapds* | NHEJ | 89 |
| *TaLOX2* | NHEJ | 94 |
| *TaMLOA1, TaMLOB1, TaMLOD1* | NHEJ | 39 |
| *Zea mays* | *ZmIPK* | NHEJ | 107 |
| *LIG, MS26, MS45* | NHEJ | 108 |
| *Zmzb7* | NHEJ | 109 |
| *PSY1* | NHEJ | 110 |

**Abbreviations:** NHEJ: non-homologous end joining; HDR: homology-directed repair.

1. **FUTURE PROSPECTS**
2. **Utilizing the potential of plant synthetic biology**

Plant synthetic biology, an emerging field in plant biotechnology, will play an important role in traditional crop improvement through the development of different novel bio-production processes [111]. The first transgenic plant was generated more than 30 years ago, designating the age of designing plants with novel functions. The CRISPR/Cas system possesses great potential for improving plant architecture as well as synthetic biology. Several artificial DNA sequences *i.e*. promoters, transcriptional regulatory elements, and genome assemblies can be inserted into known plant genomes to alter cytological behavior in order to generate their novel functions. Moreover, the CRISPR/Cas system can be used for transferring the genetic elements involved in nod factor signaling pathways from legumes to cereals in order to fix atmospheric nitrogen. In addition, synthetic biology also aims at building regulatory circuits to manipulate plant behavior or to generate novel traits for improving crop productivity [112].

dCas9-mediated gene regulation via multiplex gene modification, and epigenome editing offers unprecedented opportunities for designing synthetic transcription factors. Those factors could be deployed to construct efficiently programmable complex gene circuits. Finely tuned gene expression in the C4 cycle was essential for increasing the carbon fixation efficiency through optimization of protein levels in C4 rice project [113].

1. **Speeding up the domestication of wild plants**

Key domestication events are mainly related to mutations, especially in the domestication genes. These mutational events brought about some marked effects on key phenotypes of several cereals. Among them, *vrs1* was responsible for spike number in barley [114]; *tga1* conferred naked kernels in maize [115]; and *Sh4*, *Rc*, *PROG1*, and *LABA1* produced non-shattering rachis, white pericarp, erect growth, and barbless awns, respectively in rice [116]. Firstly, the most attractive target for rapid domestication was the winter annual plant field pennycress (*Thlaspi arvense* L*.*, Brassicaceae). Genome-edited modifications of those genes controlling seed dormancy (*DOG1*), oil quality (*FAE1* and *FAE2*), glucosinolateaccumulation (*HAG1* and *GTR*2), and oil content (*DGAT* genes) greatly facilitated thedevelopment of elite domesticated varieties of the above species [117]. Secondly, modern tomato cultivars derived from intensive inbreeding were suffering from both biotic and abiotic stresses. Naturally stress tolerant wild tomato plants could serve as ideal materials for *de novo* domestication *via* precise genetic engineering technique. Two independent studies recently implemented this strategy to speed up the domestication process of wild tomato with regard to growth habit, flower and fruit production, and nutritional traits without losing the stress tolerance ability of the original one [118,119]. Lemmon et al. [120] edited a wild relative of tomato, named as ground cherry (*Physalis pruinosa*), to produce high yielding crop with larger fruit. In the upcoming future, new domesticated crops with increased tolerance to a wide range of challenging environmental condition should promote agricultural diversity and help solving many issues regarding sustainable agriculture.

1. **Improved specificity of CRISPR/Cas systems**

Ongoing discussion concerns the degree of off-target changes that occur in plant genomes with CRISPR/Cas-mediated genome editors and whether this must be fully rectified prior to application for trait development. Generally, outcrossing to different varieties is typical during commercial product development and seed multiplication. It enables exclusion of potential off-target effects with timelines that are still greatly reduced as compared to the standard cross-breeding approaches for crop development. In some studies, whole genome sequencing was performed to detect cleavage of off-target sites by Cas9 or Cpf1 nucleases in stably transgenic *Arabidopsis* [121], cotton [122], and rice [123], which revealied that both Cas9 and Cpf1 activities were highly specific. The study also suggested that low-level off-targeting could be avoided by designing highly specific sgRNAs. Several strategies have been designed to improve the specificity of Cas9-associated base editors *viz*. sgRNA guide sequences extension, APOBEC1 linked with Cas9-HF1, and base editor delivery via RNP (ribonucleoprotein) [124,125].

1. **Increased efficiency of precise gene editing mediated by HDR**

The need of simultaneous induction of DSBs as well as delivery of a repair template to a specific location within the genome was proven to be the major challenge of HDR-mediated gene editing. Several effective ways like manipulation of DNA repair pathways increased the HDR frequency in plant cells. Heterologous expression of several key proteins could enhance HDR efficiency, *i.e.* homologous pairing and DNA strand exchange proteins RAD52, RAD54, and RPA, resection protein RecQL4, Exo1 and Spo11, etc. [126]. Interestingly, expression of the RAD18-ΔSAP mutant generated a significant elevation in CRISPR-mediated HDR as compared to full-length RAD18. HDR stimulation in this variant occurred due to the inhibition of 53BP1 localization to DSBs.

Furthermore, HDR-associated genome editing could be enhanced through some plant delivery methods which prioritized donor delivery. In case of *Agrobacterium* sp., it exerted type IV secretion system to transfer virulence effector proteins into plant cells [127]. VirD2 protein could be linked with single-strand T-DNA through covalent bonding, and thus, it allowed the transfer process of T-DNA through the protein transfer mechanism. This mechanism could deliver CRISPR DNA or RNP alongside the donor templates to trigger HDR-mediated genome editing. This system could also be integrated with the non-integrating Gemini virus replicon system for increasing the copy number of donor templates inside cells [3].

1. **Controlling invasive species by gene drives through CRISPR/Cas**

Gene drives through CRISPR/Cas gene-editing system offer a strong practice for efficient spreading of heritable elements throughout the populations *via* sexual reproduction. These gene drives could eliminate or suppress the community of invasive species including pests and weeds, in order to introduce novel traits into the present population [3].

1. **CONCLUSION**

The unprecedented capability of generating genome-wide sequence-defined diversity in plants through genome editing has opened a new horizon in crop improvement programme. The simplistic, versatile, and robust nature 1point mutations, fine-tuning of gene regulatory elements, and many other modifications at any specific location of crop genetic structure. Additionally, it helps with antiviral breeding. However, quick discovery of the genetic bases of traits of interest, improved efficiency of gene targeting (gene insertion and replacement), effective delivery of CRISPR/Cas reagents to plant cells and subsequent plant regeneration with or without the need for tissue culture, and the availability of base editors with enhanced range and frequency of targets are all required for efficient transfer technologies from the bench to the field. Despite enormous social and ethical issues, this technology owns immense potential for futuristic approach in agriculture.

Succinctly, this system is gradually going to replace major portion of the conventional breeding approaches in future due to its numerous advantages over the latter one, especially less time consumption in this rapidly expanding human population. Therefore, this is the need of the hour as time is going to be most determining factor in future breeding programmes.

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