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

Puranjoy Sar\*

Email id:sar.puranjoy1997@gmail.com

**ORCiD**:0000-0002-2571-7169

(<https://orcid.org/0000-0002-2571-7169>)

Biswajit Pramanik\*†

Email id: biswajit1996pramanik@gmail.com

**ORCiD**: 0000-0002-6016-8935 (<https://orcid.org/0000-0002-6016-8935>)

Sandip Debnath

Email id: sandip.debnath@visva-bharati.ac.in

**ORCiD**: 0000-0002-0234-6633

(<https://orcid.org/0000-0002-0234-6633>)

†**Corresponding author:** Email id: biswajit1996pramanik@gmail.com

\***Both authors should be considered as first author**

**Address:** Department of Genetics and Plant Breeding, Palli-Siksha Bhavana (Institute of Agriculture), Visva-Bharati, Sriniketan, West Bengal, India, Pin- 731236

**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 the utmost need for supplying nutrition along with food to the mankind. 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 role in the progress in genome editing studies since last few decades. Base editing, knock out or knock in of desirable genes, fine tuning of several genes, promotion of antiviral defense, 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 sorts of innovative breeding techniques were required for accelerating genetic gain, productivity as well as 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 foreign genes into the background of well-known mega-varieties [5]. Although genetically modified (GM) crops encompass the capability to overcome the bottleneck of reproductive isolation, their commercialization are still restricted by stringent Government guidelines considering future concerns [5, 6]. Therefore, developing a novel variety through precise genome editing is a revolutionary approach for rapid advancement of agricultural crops.

Zinc finger nucleases (ZFN) were first discovered in 2005 while experimenting with tobacco plant [7], and five years later, transcription activator-like effector nucleases (TALENs) surfaced as a genome-editing tool in the field of plant biotechnology [8]. In 2013, three independent groups of researchers discovered the CRISPR/Cas9 system for using in wheat (*Triticum aestivum*), rice (*Oryza sativa*), rockcress (*Arabidopsis thaliana),* and benthi (*Nicotiana benthamiana*), respectively [9, 3]. Later, this has been introduced as an ingenious technique for rapid crop improvement due to its ability to create variation by introducing targeted sequence at specific sites. Apart from ease of hybrid breeding, this approach can also knock in desired traits or knockout unwanted traits into elite varieties. Its multiplexing nature helps to modify multiple targets expeditiously, and therefore, multiple traits can be pyramided into an elite background within a single generation. Unprecedentedly, this system paves the way of creating genetic diversity too. 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]. Sanatech Seed in Japan first launched the genome-edited directly consumable tomato [11] CRISPR/Cas mediated gene editing was used to produce “Sicilian Rouge”, which was a γ-aminobutyric acid (GABA) rich (five times higher than the regular one) tomato. GABA, an inhibitory neurotransmitter, reported helps in controlling blood pressure and diabetes [11]. The ministry of Japan withdrew the rules and regulations of GM products for this GABA rich tomato [11]. Thus, for sustainable agriculture, the power of CRISPR and its variants remains undeniable.

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

CRISPR/Cas system is an innate prokaryotic immune system inside the bacterial cells to provide protection against phages. CRISPR refers to Clustered Regularly Interspaced Short Palindromic Repeats and Cas represents 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 struggles from infection of bacteriophage for their destruction, they evolved a protective layer of immune system called CRISPR, in response to those. The CRISPR array, a short DNA stretch inside bacteria, is composed of a repeated sequence altered by target specific spacer. These spacers contain DNA fragments of previously invading viruses. The CRISPR array keeps growing with the newly added spacer segment after every virus attack. Identification of the viral DNA by a protein complex, namely Cas1-Cas2, initiates removal of a specific segment of DNA, *i.e.* protospacer. These protospacer, after addition with the CRISPR array, reserves a memory of that particular phage infection, so that the bacteria can easily and immediately defend the next attack. The transcription of long CRISPR-RNA (pre-crRNA) starts the defense process. Later, transacting or spacer RNA, pairs bases with the crRNA. The protein Cas9 binds on the dual RNA in order to trim 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 orientation of PAM (Protospacer Adjacent Motif) nearby protospacer in viral DNA helps Cas9 to distinguish despite their sequence similarities [3].

Six distinct types of bacterial CRISPR systems (type I-VI) have been discovered in different types of bacteria, so far, among which 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’. Its compactness makes it fifty-percent of the conventional Cas proteins *viz*. Cas9 and Cas12a, in size. 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 *in vivo* and *ex vivo* gene therapy as well as cell engineering 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 basic feature of CRISPR/Cas. It generally undergoes either of the two principal 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 following a homology-independent manner, and therefore, can be utilized for crop improvement through gene pyramiding [3]. Although, NHEJ is well recommended for knockout studies at a large scale and it can be initiated in any cell cycle stages, the lack of precision creates a barrier towards sophisticated genome engineering. On the other hand, the latter one possesses the capability to add 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, respectively. An exogenous template or sister chromatid can serve as a template for repairing DSB due to its homology of sequences [14].

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

Beyond DSB-mediated genome editing, these systems, independent of HDR or donor DNA, can introduce specific base changes. As instance, cytosine base-editor (CBE) system makes a targeted transition (C🡪U) in genomic DNA [17]. Subsequently, Gaudelli et al. [18] discovered 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 PLANT BREEDING**
2. **Knockout-controlled trait development**

Elimination of negative elements has been reported as one of most promising approaches for genetic advancement. Therefore, CRISPR/Cas9 can be commonly applied to knock out undesirable genes from the genetic background (Figure 2a). Till date, it successfully improved several quantitative traits like quality, resistance to abiotic and biotic stresses, and overall yield. Rather, various breeding techniques like hybrid-breeding techniques and many more have also been enhanced, as reported [3].

1. **Yield improvement**

Yield is a dependent polygenic complex trait. Several traits such as grain number (*OsGn1a*), grain size (*OsGS3*), grain weight (*OsGW5*, *TaGW2*, *TaGASR7, or OsGLW2*), panicle size (*TaDEP1, OsDEP1*), and tiller number (*OsAAP3*) have negative impact on grain yield. In such circumstances, knocking out those traits using CRISPR/Cas9 was proven to be a proficient 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*) generated trait pyramiding for significant enhancement of grain weight as per the published report [12].

1. **Quality improvements**

Quality characters depend on the specific breeding pre-requisites. Now-a-days, genome editing have impacted various quality parameters in several crops *i.e.* amylose content, aroma, nutritional value, duration and condition of storage etc. Improvement in amylose content in rice for better cooking and eating quality was induced by *Waxy* gene knockout by CRISPR/Cas9 [25]. By this knockout procedure, line of waxy corn coupled with higher yielding ability was developed by DuPont Pioneer for commercial purpose [26]. CRISPR/Cas9 also produced high-amylose containing rice through mutation of *SBEIIb, a* starch branching enzyme gene [27]. The study also suggested that consumption of enhanced amylose containing foods should strengthen the dietary habit of the patients of various chronic noninfectious diseases [27].

2-acetyl-1-pyrroline (2-AP) is the principal volatile compound behind the aroma in rice, which leads to increase in consumer demand as well as market value. A mutation in betaine aldehyde dehydrogenase 2 (*BADH2*) gene causes its biosynthesis. An aromatic rice line with a nearly comparable 2-AP content (0.35–0.75 mg/kg) like a natural mutant aromatic rice variety was developed by executing TALEN-induced disruption of *OsBADH2* [28]. With evolution of CRISPR/Cas9 techniques, aromatic trait has been incorporated into almost 30 elite rice genotypes in China [3].

Almost 7% of the population in western countries suffers 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 pseudo-genes. Researchers already developed low gluten wheat through CRISPR/Cas9 editing technology by simultaneous knocking out of most conserved domains of that particular α-gliadin 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 developed biotic-stress resistance have been achieved *via* CRISPR/Cas9 knockout. For example, TALEN along with CRISPR/Cas9, knocked out all six *TaMLO* alleles for developing increased resistance against powdery mildew in wheat [39]. Similarly, Nekrasov et al. [40] reported that knockout of *MLO via* CRISPR/Cas9 leadsto develop powdery mildew resistance in tomato. Knockout of *OsERF922* and deletion of the *OsSWEET13* resulted in blast- and bacterial leaf blight (BLB) resistant rice, respectively [41, 42]. CRISPR/Cas9 also efficiently offered rice tungro virus resistant *eif4g* rice [43], potyvirus–resistant *eif4e* cucumber [44], and resistant *clcud* cotton for cotton leaf curl virus [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 already developed rice variants with low amounts of heavy metal likes 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 leads to higher yield, higher temperature tolerance, and lower pre-harvest sprouting than the wild type [49].

1. **Accelerating hybrid breeding**

For developing high quality hybrid, the pre-requisite is to development of male sterile lines. Development of such 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 knocking out genes by CRISPR/Cas 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/ or replacement**

Several yield-attributing traits are conferred by addition, substitution, or change of expression of several nucleotides or genes. Knock-in or replacement of specific alleles facilitates breeding excluding linkage drag or 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 the expression of ARGOS8 by knocking in the GOS2 promoter instead of the regular ARGOS8 promoter through HDR. Under drought stress, the altered ARGOS8 variants possessed high level of transcription as well as 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 elevated the insertion frequency of Cauliflower mosaic virus (CaMV) 35S constitutive promoter upstream to *ANT1* gene, which was constitutively expressed in tomato in order to increase the anthocyanin content [60]. Moreover, substitution in the conserved domains of various principal enzymes likes endogenous 5-enolpyruvylshikimate-3-phosphate synthase (*EPSPS*) and acetolactate synthase (*ALS*) genes, the key target for gene editing, conferred resistance against several herbicides like glyphosate or those based on sulfonylurea. 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 at coding sequences is one of the major achievements for developing herbicide resistance (Figure 2c). Imidazolinone or sulfonylurea-resistant rice [63], wheat [64], *Arabidopsis* [65], and watermelon [66] have been created by targeting *ALS* with a plant cytidine base editor, and plant adenine base editor targeted acetyl-coA carboxylase *ACCase* gene to generate haloxyfop-R-methyl resistant rice [67,68]. On the other hand, in RNA splicing processes, most eukaryotic mRNA follows the systematic GU/AG rule, where introns possess a splice donor site (GU), and a splice acceptor site (AG) at 5’ and 3' end, respectively. At consensus sequences, base editing can induce point mutation, which leads to the loss or alteration of particular splice forms. Xue et al. [69], in their study, performed transition (G🡪A) in the splice donor site, which not only causes hypersensitivity to abscisic acid (ABA) but also constitutively retain an intron of *AtHAB*. Kang et al. [70] distorted the splicing acceptor site by following the opposite conversion, which ultimately leads to change in splicing pattern of *AtPDS* mRNA.

1. **Antiviral plant breeding strategies**

CRISPR/Cas system offers a defense mechanism to cleave viruses and plasmids, that attack the primitive creatures *i.e.* archaea and bacteria (Figure 2d). Besides, it is well-known to provide virus resistance in various plant species too. As instance, continuous over expression of Cas9 and sgRNAs specifically target the Gemini virus genome 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 constrains 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 genome-edited cis-regulatory elements generally controls these processes. Till date, genome editing for altering gene expression in plants has predominantly focused on either promoter replacement or deletion of *cis*-regulatory elements [75, 76]. Rodriguez-Leal et al. [77] edited the promoter regions of quantitative trait–associated genes *viz*. *SlS*, *SlCLV3,* and *SlSP*. These eventually led to continuous variation as well as 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] |
| *OsPMS3, OsPDS, OsEPSPS,* | NHEJ | [96] |
| *SWEET1b, SWEET1a, SWEET11* | NHEJ | [97] |
| *ALS* | HDR | [98] |
| *CDKA2, CDKA1, 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. (Table adapted from [111])

1. **FUTURE PROSPECTS**
2. **Potential utilization of plant synthetic biology**

Now in plant biotechnology, an emerging field is synthetic biology, which will play a major role in crop improvement through development of different novel bio-production processes [112]. In 1982, the discovery of the first transgenic plants indicated the beginning of designer plants with novel functionalities. The CRISPR/Cas possess the tremendous potential not only to improve plant architecture but also the synthetic biology. Several DNA sequences (*viz*. promoters, enhancers, repressors, transcriptional regulatory elements, etc.) can be incorporated into the genome of interest to alter the behaviour for generating their novel functions. For example, this CRISPR/Cas system can be deployed to transfer the DNA sequences involving nod factor signaling pathways from legumes to cereals for atmospheric nitrogen fixation. Additionally, synthetic biology also aims to build regulatory circuits for manipulating vegetative behavior or to generate novel traits for improving various economically important traits in plants [113].

Multiplex gene modification and epigenome editing *via* dCas9-controlled gene regulation provides the plausibility to design various synthetic transcription factors. Those factors could be used to build 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 [114].

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

Domestication events are mainly related to various modifications, especially mutation in those genes. These mutational events brought about some marked changes on key phenotypes of several cereals. Their changes become favorable for humankind leads to selection of those traits. Among them, six-rowed spike 1(*vrs1*) was responsible for increasing spike number in barley [115]; *tga1* conferred naked kernels in maize [116]; and *Rc*, *Sh4*, *PROG1*, and *LABA1* leads to formation of white pericarp, non-shattering rachis, erect growth, and barbless awns, respectively, in rice [117]. At very first, domestication was experimented on the winter annual plant field pennycress (*Thlaspi arvense* L*.*, Brassicaceae). Modifications of genes by genome editing are controlling seed dormancy (*DOG1*), glucosinolate accumulation (*HAG1*, and *GTR2*), oil quality (*FAE1*, and *FAE2*), and oil content (*DGAT*). They significantly aid in the creation of exceptional domesticated forms of the aforesaid species [118]. Secondly, the modern tomato which bred through intensive inbreeding programme, was suffering from various biotic and abiotic stresses. Naturally available stress tolerant genes in wild tomato could be the perfect resource for *de novo* domestication *via* precise genome editing technique. This technique was recently used in two separate investigations to accelerate the domestication process of wild tomato regarding flower and fruit production, growth habit, and nutritional traits with intact stress tolerance [119, 120]. Lemmon et al. [121] edited *Physalis pruinosa* (ground cherry), a wild relative of tomato, to produce high yielding tomato with larger fruit size. In near future, newly domesticated crops with increased tolerance to an array of challenging environmental condition will promote crop diversity and help solving many issues regarding sustainable agriculture.

1. **Enhancing the precision of CRISPR/Cas systems**

There is an ongoing debate on the extent of off-target changes through CRISPR/Cas-mediated gene editing in plant genomes and whether these need to be entirely fixed before being applied for trait development or not. Generally, a typical seed multiplication process undergoes outcrossing of numerous varieties. In comparison to the conventional cross-breeding procedures, it permits the removal of potential off-target impacts with timescales that are indeed significantly shortened. Whole genome sequencing was examined in various studies to detect the off-target site cleavage by Cas9 or Cpf1 nucleases in transgenic *Arabidopsis* [122], cotton [123], and rice [124]. These studies revealed high specificity of both Cas9 and Cpf1. These studies also suggested that by designing highly specific sgRNAs, low-level off-targeting could be eluded. Numerous strategies have been implemented to increase the specificity of Cas9-associated base editors *viz*. sgRNA guide sequences extension, Cas9-HF1-connected APOBEC1, along with base editor delivery *via* RNP (ribonucleoprotein) [125, 126].

1. **Precise gene editing *via* HDR**

The need of simultaneous development of DSBs as well as location-specific repair template delivery within the genome was proven to be the major constrain of HDR-mediated gene editing. Several potential 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.* proteins involved in homologous pairing and nuclear strand exchange like RAD52, RAD54, and RPA, resection protein RecQL4 (helicase), Spo11 and Exo1, etc. [127]. It is interesting to note that the expression of the mutant RAD18-ΔSAP conferred a significant enhancement of CRISPR-mediated HDR as compared to the regular one. In this variant, HDR stimulation 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 used type IV secretion system for transferring virulence effector proteins [128]. The covalent bond formation between VirD2 and single-stranded T-DNA promotes its transfer process*via* protein transfer mechanism. This mechanism has the ability to transfer CRISPR DNA or RNP accompanied by the donor templates by triggering HDR-liaise genome editing. The integration between CRISPR/Cas with the non-integrating Gemini virus replicon system is able to increase the copy number of donor templates [3].

1. **Restricting invasive species by gene drives**

Gene drives, through CRISPR/Cas genome-editing system, offer not only a strong practice for efficient proliferation of heritable elements throughout the populations *via* sexual reproduction but also the speeding up in this process. These gene drives could eliminate or suppress the community of prey organisms including pests and weeds, altering the virulence of pathogen, and in order to introduce novel characters into the present population [3].

1. **CONCLUSION**

The unprecedented capability of generating genome-wide sequence-defined diversity in plants through genome editing has unlocked a new horizon in crop improvement programme. The simplistic, versatile, and robust nature of CRISPR/Cas makes it a powerful tool. It can improve crop genetic structure by precise point mutations, finetuning of gene regulatory elements, and many other changes at any specific location. Additionally, it helps to construct the mutant library and had tremendous potential in the antiviral breeding. However, quick detection of the genetic bases of traits of interest, improved efficiency of gene targeting (gene insertion and/ or replacement), successful transfer of CRISPR/Cas reagents to plant cells and succeeding plant regeneration irrespective of tissue culture, and the accessibility of base editors with enhanced range and frequency of targets are required for efficient transfer technologies from the *in vitro* to *ex vitro*. Despite enormous social and ethical issues, this technology owns immense potential for futuristic approach in agriculture.

 Now there is a need to reexamine the laws governing genome-edited crops in various countries. Recently, on 30thMarch, 2022, the Indian Ministry of Environment, Forest and Climate Change has announced that genome-edited crops without exogenous DNA are free from being classified as transgenic crops. There are three categories of gene-editing: SDN1, SDN2 and SDN3. The first two, involves “knocking off” or “over expressing” certain traits in a genome without any insertion of foreign gene. The third one, will be treated as GMO due to it involve insertion of foreign genes. The Memorandum states, “SDN1 and SDN2 genome-edited products free from exogenous introduced DNA be exempted from biosafety assessment in pursuance of rule 20 of the Manufacture, Use, Import, Export and Storage of Hazardous Microorganisms/Genetically engineered Organisms or Cells Rules 1989.”

CRISPR-Cas has tremendous potential to transform agriculture by developing climate smart varieties and increasing nutritional value and yield of the crops. This can improve the genetic gain to meet the food demand of an increasing world population and fed the humankind. Specificity and less time consumption make CRISPR-Cas advantageous over other approaches. Therefore, this is the need of the hour as time is going to be most determining factor in future breeding programmes.

**REFERENCES**

1. P. Satya, and S. Debnath, “[Hybrid rice: a two way solution for food security and economic improvement](https://scholar.google.com/citations?view_op=view_citation&hl=en&user=_dYlLdUAAAAJ&pagesize=80&citation_for_view=_dYlLdUAAAAJ:qjMakFHDy7sC),” Int. J. Agric. Environ. Biotechnol, vol. 2, pp. 489-491, April, 2009.
2. S. Satpathy, S. Debnath, and A. Mishra, “Study on character association *Lens culinaris* Medik.,” Electron. J. Plant Breed, vol. 12, pp. 58-65, April, 2021.
3. K. Chen, Y. Wang, R. Zhang, H. Zhang, and C. Gao, “CRISPR/Cas genome editing and precision plant breeding in agriculture,” Annu. Rev. Plant Biol, vol. 70, pp. 667–697, March 2019.
4. S. Debnath, A. Sarkar, K. Perveen, N.A. Bukhari, K.K. Kesari, A. Verma, et al., “Principal component and path analysis for trait selection based on the assessment of diverse lentil populations developed by Gamma-irradiated physical mutation,” Biomed Res. Int, vol. 2022, pp. 1-14, July, 2022.
5. S. Debnath, and R. Sadhukhan, “Genetically modified crops: an overview,” SATSA Mukhopatra (Annual Tech Issues), vol. 18, pp. 117-125, 2014.
6. J.R. Prado, G. Segers, T. Voelker, D. Carson, R. Dobert, et al., “Genetically engineered crops: from idea to product,” Annu. Rev. Plant Biol, vol. 65, pp. 769–790, February 2014.
7. D.A. Wright, J.A. Townsend, R.J. Jr Winfrey, P.A. Irwin, J. Rajagopal, et al., “High-frequency homologous recombination in plants mediated by zinc-finger nucleases,” Plant J. vol. 44, pp. 693–705, August 2005.
8. M. Christian, T. Čermak, E.L. Doyle, C. Schmidt, F. Zhang, et al., “Targeting DNA double-strand breaks with TAL effector nucleases,” Genetics, vol. 186, pp. 757–761, July 2010.
9. J.F. Li, J.E. Norville, J. Aach, M. McCormack, D. Zhang, et al., “Multiplex and homologous recombination-mediated genome editing in *Arabidopsis* and *Nicotiana benthamiana* using guide RNA and Cas9,” Nat. Biotechnol, vol. 31, pp. 688–691, August 2013.
10. K. Kawaguchi, R.Takei-Hoshi, I. Yoshikawa, et al., “Functional disruption of cell wall invertase inhibitor by genome editing increases sugar content of tomato fruit without decrease fruit weight,” Sci Rep, vol. 11, pp. 21534, November 2021.
11. H. Ezura, “Letter to the editor: the world’s first CRISPR tomato launched to a Japanese market: the social-economic impact of its implementation on crop genome editing,” Plant Cell Physiol, vol. 63, pp. 731-733, June 2022.
12. R. Xu, Y. Yang, R. Qin, H. Li, C. Qiu, et al., Rapid improvement of grain weight via highly efficient CRISPR/Cas9-mediated multiplex genome editing in rice. J. Genet. Genom, vol. 43, pp. 529–532, August 2016.
13. L.S. Symington, and J. Gautier, “Double-strand break end resection and repair pathway choice,” Annu. Rev. Genet, vol. 45, pp. 247–271, September 2011.
14. J. Salsman, and G. Dellaire, “Precision genome editing in the CRISPR era,” Biochem. Cell Biol, vol. 95, pp. 187–201, September 2016.
15. A.A. Dominguez, W.A. Lim, and L.S. Q, “Beyond editing: repurposing CRISPR-Cas9 for precision genome regulation and interrogation,” Nat. Rev. Mol. Cell Biol, vol. 17, pp. 5–15, January 2015.
16. G. Kungulovski, and A. Jeltsch, “Epigenome editing: state of the art, concepts, and perspectives,” Trends Genet, vol. 32, pp. 101–113, February 2016.
17. A.C. Komor, Y.B. Kim, M.S. Packer, J.A. Zuris, and D.R. Liu, “Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage,” Nature, vol. 533, pp. 420–424, May 2016.
18. N.M. Gaudelli, A.C. Komor, H.A. Rees, M.S. Packer, A.H. Badran, et al., “Programmable base editing of A \_T to G \_C in genomic DNA without DNA cleavage,” Nature, vol. 551, pp. 464–471, November 2017.
19. M. Li, X. Li, Z. Zhou, P. Wu, M. Fang, et al., “Reassessment of the four yield-related genes gn1a, dep1, gs3, and ipa1 in rice using a CRISPR/Cas9 system,” Front. Plant Sci, vol. 7 pp. 377, March 2016.
20. S. Li, F. Gao, K. Xie, X. Zeng, Y. Cao, et al., “The OsmiR396c-OsGRF4-OsGIF1 regulatory module determines grain size and yield in rice,” Plant Biotechnol. J, vol. 14, pp. 2134–2146, November 2016.
21. J. Liu, J. Chen, X. Zheng, F. Wu, Q. Lin, et al., “GW5 acts in the brassinosteroid signaling pathway to regulate grain width and weight in rice,” Nat. Plants, vol. 3, pp. 17043, April 2017.
22. K. Lu, B. Wu, J. Wang, W. Zhu, H. Nie, et al., “Blocking amino acid transporter OsAAP3 improves grain yield by promoting outgrowth buds and increasing tiller number in rice,” Plant Biotechnol. J, vol. 16, pp. 1710–1722, October 2018.
23. Y. Zhang, Z. Liang, Y. Zong, Y. Wang, J. Liu, et al., “Efficient and transgene-free genome editing in wheat through transient expression of CRISPR/Cas9 DNA or RNA,” Nat. Commun, vol. 7, pp. 12617, August 2016.
24. Y. Zhang, D. Li, D. Zhang, X. Zhao, X. Cao, et al., “Analysis of the functions of TaGW2 homoeologs in wheat grain weight and protein content traits,” Plant J, vol. 94, pp. 857–866, June 2018.
25. J. Zhang, H. Zhang, J.R. Botella, J.K. Zhu, “Generation of new glutinous rice by CRISPR/Cas9- targeted mutagenesis of the Waxy gene in elite rice varieties,” J. Integr. Plant Biol, vol. 60, pp. 369–375, May 2018.
26. E. Waltz, “CRISPR-edited crops free to enter market, skip regulation,” Nat. Biotechnol, vol. 34, pp. 582, June 2016.
27. Y. Sun, G. Jiao, Z. Liu, X. Zhang, J. Li, et al., “Generation of high-amylose rice through CRISPR/Cas9- mediated targeted mutagenesis of starch branching enzymes,” Front. Plant Sci, vol. 8, pp. 1298, March 2017.
28. Q. Shan, Y. Zhang, K. Chen, K. Zhang, and C. Gao, “Creation of fragrant rice by targeted knockout of the OsBADH2 gene using TALEN technology,” Plant Biotechnol. J, vol. 13, pp. 791–800, August 2015.
29. S. Sanchez-Leon, J. Gil-Humanes, C.V. Ozuna, M.J. Gimenez, C. Sousa, et al., “Low-gluten, non-transgenic wheat engineered with CRISPR/Cas9,” Plant Biotechnol. J, vol. 16, pp. 902–910, April 2018.
30. A. Okuzaki, T. Ogawa, C. Koizuka, K. Kaneko, M. Inaba, et al., “CRISPR/Cas9-mediated genome editing of the fatty acid desaturase 2 gene in *Brassica napus*,” Plant Physiol. Biochem, vol. 131, pp. 63–69, October 2018.
31. W.Z. Jiang, I.M. Henry, P.G. Lynagh, L. Comai, E.B. Cahoon, and D.P. Weeks, “Significant enhancement of fatty acid composition in seeds of the allohexaploid, *Camelina sativa*, using CRISPR/Cas9 gene editing,” Plant Biotechnol. J, vol. 15, pp. 648–657, May 2017.
32. C. Morineau, Y. Bellec, F. Tellier, L. Gissot, Z. Kelemen, et al., “Selective gene dosage by CRISPRCas9 genome editing in hexaploid *Camelina sativa*,” Plant Biotechnol. J, vol 15, 729–739, June 2017.
33. Y. Ito, A. Nishizawa-Yokoi, M. Endo, M. Mikami, and S. Toki, “CRISPR/Cas9-mediated mutagenesis of the RIN locus that regulates tomato fruit ripening,” Biochem. Biophys. Res. Commun, vol. 467, pp. 76–82. November 2015.
34. R. Li, D. Fu, B. Zhu, Y. Luo, and H. Zhu, “CRISPR/Cas9-mediated mutagenesis of lncRNA1459 alters tomato fruit ripening,” Plant J, vol. 94, pp. 513–524, May 2018.
35. X. Li, Y. Wang, S. Chen, H. Tian, D. Fu, et al., “Lycopene is enriched in tomato fruit by CRISPR/Cas9- mediated multiplex genome editing,” Front. Plant Sci, vol. 9, pp. 559, April 2018.
36. S. Nonaka, C. Arai, M. Takayama, C. Matsukura, and H. Ezura, “Efficient increase of γ-aminobutyric acid (GABA) content in tomato fruits by targeted mutagenesis,” Sci Rep, vol. 7, pp. 7057, August 2017.
37. R. Li, R. Li, X. Li, D. Fu, B. Zhu, et al., “Multiplexed CRISPR/Cas9-mediated metabolic engineering of γ-aminobutyric acid levels in *Solanum lycopersicum*,” Plant Biotechnol. J, vol 16, pp. 415–427, February 2018.
38. M. Nakayasu, R. Akiyama, H.J. Lee, K. Osakabe, Y. Osakabe, et al., “Generation of α-solanine-free hairy roots of potato by CRISPR/Cas9 mediated genome editing of the St16DOX gene,” Plant Physiol. Biochem, vol. 131, pp. 70–77, October 2018.
39. Y. Wang, X. Cheng, Q. Shan, Y. Zhang, J. Liu, et al., “Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew,” Nat. Biotechnol, vol. 32, pp. 947–951, September 2014.
40. V. Nekrasov, C. Wang, J. Win, C Lanz, D. Weigel, and S. Kamoun, “Rapid generation of a transgene-free powdery mildew resistant tomato by genome deletion,” Sci. Rep, vol. 7, pp. 482, March 2017.
41. J. Zhou, Z. Peng, J. Long, D. Sosso, B. Liu, et al., “Gene targeting by the TAL effector PthXo2 reveals cryptic resistance gene for bacterial blight of rice,” Plant J, vol. 82, pp. 632–643, May 2015.
42. F. Wang, C. Wang, P. Liu, C. Lei, W. Hao, et al., “Enhanced rice blast resistance by CRISPR/Cas9- targeted mutagenesis of the ERF transcription factor gene OsERF922,” PLOS ONE, vol. 11, pp. e0154027, April 2016.
43. A. Macovei, N.R. Sevilla, C. Cantos, G.B. Jonson, I. Slamet-Loedin, et al., “Novel alleles of rice eIF4G generated by CRISPR/Cas9-targeted mutagenesis confer resistance to Rice *tungro spherical virus*,” Plant Biotechnol. J, vol. 16, 1918–1927, November 2018.
44. J. Chandrasekaran, M. Brumin, D. Wolf, D. Leibman, C. Klap, et al., “Development of broad virus resistance in non-transgenic cucumber using CRISPR/Cas9 technology,” Mol. Plant Pathol, vol. 17, pp. 1140– 1153, July 2016.
45. H. Butt, A. Eid, Z. Ali, M.A.M. Atia, M.M. Mokhtar, et al., “Efficient CRISPR/Cas9-mediated genome editing using a chimeric single-guide RNA molecule,” Front. Plant Sci, vol. 8, pp. 1441, August 2017.
46. M. Nieves-Cordones, S. Mohamed, K. Tanoi, N.I. Kobayashi, K. Takagi, et al., “Production of low-Cs+ rice plants by inactivation of the K+ transporter OsHAK1 with the CRISPR-Cas system,” Plant J, vol. 92, pp. 43–56, October 2017.
47. L. Tang, B. Mao, Y. Li, Q. Lv, L. Zhang, et al., “Knockout of OsNramp5 using the CRISPR/Cas9 system produces low Cd-accumulating indica rice without compromising yield,” Sci. Rep, vol. 7, pp. 14438, October 2017.
48. F.Z. Wang, M.X. Chen, L.J. Yu, L.J. Xie, L.B. Yuan, et al., “OsARM1, an R2R3 MYB transcription factor, is involved in regulation of the response to arsenic stress in rice” Front. Plant Sci, vol. 8, pp. 1868, October 2017.
49. C. Miao, L. Xiao, K. Hua, C. Zou, Y. Zhao, et al., “Mutations in a subfamily of abscisic acid receptor genes promote rice growth and productivity,” PNAS, vol. 115, pp. 6058–6063, June 2018.
50. H. Zhou, M. He, J. Li, L Chen, Z. Huang, et al., “Development of commercial thermo-sensitive genic male sterile rice accelerates hybrid rice breeding using the CRISPR/Cas9-mediated TMS5 editing system,” Sci. Rep, vol. 6, pp. 37395, November 2016.
51. J. Li, H. Zhang, X. Si, Y. Tian, K. Chen, et al., “Generation of thermosensitive male-sterile maize by targeted knockout of the ZmTMS5 gene,” J. Genet. Genom, vol. 44, pp. 465–468, September 2017.
52. Q. Li, D. Zhang, M. Chen, W. Liang, J. Wei, et al., “Development of japonica photo-sensitive genic male sterile rice lines by editing carbon starved anther using CRISPR/Cas9,” J. Genet. Genom, vol. 43, pp. 415–419, June 2016.
53. M. Singh, M. Kumar, M.C. Albertsen, J.K. Young, and A.M. Cigan, “Concurrent modifications in the three homeologs of Ms45 gene with CRISPR-Cas9 lead to rapid generation of male sterile bread wheat (*Triticum aestivum* L.),” Plant Mol. Biol, vol. 97, pp. 371–383, July 2018.
54. Y. Xie, B. Niu, Y. Long, G. Li, J. Tang, et al., “Suppression or knockout of SaF/SaM overcomes the Sa-mediated hybrid male sterility in rice,” J. Integr. Plant Biol, vol. 59, pp. 669–679, September 2017.
55. Y. Xie, P Xu, J. Huang, S. Ma, X. Xie, et al., “Interspecific hybrid sterility in rice is mediated by OgTPR1 at the S1 locus encoding a peptidase-like protein,” Mol. Plant, vol. 10, pp. 1137–1140, August 2017.
56. R. Shen, L. Wang, X. Liu, J. Wu, W. Jin, et al., “Genomic structural variation-mediated allelic suppression causes hybrid male sterility in rice,” Nat. Commun, vol. 8, pp. 1310, November 2017.
57. M. Luo, B. Gilbert, and M. Ayliffe, “Applications of CRISPR/Cas9 technology for targeted mutagenesis, gene replacement and stacking of genes in higher plants,” Plant Cell Rep, vol. 35, pp. 1439–1450, July 2016.
58. J. Shi, H. Gao, H. Wang, H.R. Lafitte, R.L. Archibald, et al., “ARGOS8 variants generated by CRISPR/Cas9 improve maize grain yield under field drought stress conditions,” Plant Biotechnol. J, vol. 15, pp. 207–216, February 2017.
59. Q.H. Yu, B. Wang, N. Li, Y. Tang, S. Yang, et al., “CRISPR/Cas9-induced targeted mutagenesis and gene replacement to generate long shelf-life tomato lines,” Sci. Rep, vol. 7, pp. 11874, September 2017.
60. T. Cˇermak, N.J. Baltes, R. Cˇegan, Y. Zhang, D.F. Voytas, “High-frequency, precise modification of the tomato genome,” Genome Biol, vol. 16, pp. 232, December 2015.
61. N.J. Sauer, J. Narvaez-Vasquez, J. Mozoruk, R.B. Miller, Z.J. Warburg, et al., “Oligonucleotide-mediated genome editing provides precision and function to engineered nucleases and antibiotics in plants,” Plant Physiol, vol. 170, pp. 1917–1928, April 2016.
62. A.W. Hummel, R.D. Chauhan, T. Cermak, A.M. Mutka, A. Vijayaraghavan, et al., “Allele exchange at the EPSPS locus confers glyphosate tolerance in cassava,” Plant Biotechnol. J, vol. 16, pp. 1275–1282, July 2017.
63. Z. Shimatani, S. Kashojiya, M. Takayama, R. Terada, T. Arazoe, et al., “Targeted base editing in rice and tomato using a CRISPR-Cas9 cytidine deaminase fusion,” Nat. Biotechnol, vol. 35, pp. 441–443, May 2017.
64. Y. Zong, Q. Song, C. Li, S. Jin, D. Zhang, et al., “Efficient C-to-T base editing in plants using a fusion of nCas9 and human APOBEC3A,” Nat. Biotechnol, vol. 36, pp. 950–953, November 2018.
65. Y.Y. Chen, Z.P. Wang, H.W. Ni, Y. Xu, Q.J. Chen, L.J. Jiang, “CRISPR/Cas9-mediated base-editing system efficiently generates gain-of-function mutations in Arabidopsis,” Sci. China Life Sci, vol. 60, pp. 520–523, May 2017.
66. S. Tian, L. Jiang, X. Cui, J. Zhang, S. Guo, et al., “Engineering herbicide-resistant watermelon variety through CRISPR/Cas9-mediated base-editing,” Plant Cell Rep, vol. 37, pp. 1353–1356, September 2018.
67. C. Li, Y. Zong, Y. Wang, S. Jin, D. Zhang, et al., “Expanded base editing in rice and wheat using a Cas9-adenosine deaminase fusion,” Genome Biol, vol. 19, pp. 59, December 2018.
68. C. Xue, H. Zhang, Q. Lin, R. Fan, and C. Gao, “Manipulating mRNA splicing by base editing in plants,” Sci. China Life Sci, vol. 61, pp. 1293–1300, November 2018.
69. B.C. Kang, J.Y. Yun, S.T. Kim, Y. Shin, J. Ryu, et al., “Precision genome engineering through adenine base editing in plants,” Nat. Plants, vol. 4, pp. 427–431, July 2018.
70. Z. Ali, A. Abulfaraj, A. Idris, S. Ali, M. Tashkandi, and M. Mahfouz, “CRISPR/Cas9-mediated viral interference in plants,” Genome Biol, vol. 16, pp. 238, December 2015.
71. N.J. Baltes, A.W. Hummel, E. Konecna, R. Cegan, A.N. Bruns, et al., “Conferring resistance to geminiviruses with the CRISPR–Cas prokaryotic immune system,” Nat. Plants, vol. 1, pp. 15145, September 2015.
72. X. Ji, H. Zhang, Y. Zhang, Y. Wang, and C. Gao, “Establishing a CRISPR-Cas-like immune system conferring DNA virus resistance in plants,” Nat. Plants, vol. 1, pp. 15144, September 2015.
73. Z. Ali, S. Ali, M. Tashkandi, S.S. Zaidi, and M.M. Mahfouz, “CRISPR/Cas9-mediated immunity to geminiviruses: differential interference and evasion” Sci. Rep, vol. 6, pp. 26912, May 2016.
74. X. Ji, X. Si, Y. Zhang, H. Zhang, F. Zhang, et al., “Conferring DNA virus resistance with high specificity in plants using a virus-inducible genome editing system,” Genome Biol, vol. 19, pp. 197, December 2018.
75. A. Piatek, Z. Ali, H. Baazim, L. Li, A. Abulfaraj, et al., “RNA-guided transcriptional regulation in planta via synthetic dCas9-based transcription factors,” Plant Biotechnol. J, vol. 13, pp. 578–589, May 2015.
76. A. Peng, S. Chen, T. Lei, L. Xu, Y. He, et al., “Engineering canker-resistant plants through CRISPR/Cas9-targeted editing of the susceptibility gene CsLOB1 promoter in citrus,” Plant Biotechnol. J, vol. 15, pp. 1509–1519, December 2017.
77. D. Rodríguez-Leal, Z.H. Lemmon, J. Man, M.E. Bartlett, and Z.B. Lippman, “Engineering quantitative trait variation for crop improvement by genome editing,” Cell, vol. 171, pp. 470–480, October 2017.
78. Z.Y. Feng, B.T. Zhang, W.N. Ding, X.D. Liu, D.L. Yang, P.L. Wei, et al., “Efficient genome editing in plants using a CRISPR/Cas system,” Cell Res, vol. 23, pp. 1229–1232, October 2013.
79. W.Z. Jiang, H.B. Zhou, H.H. Bi, M. Fromm, B. Yang, and D.P. Weeks, “Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in *Arabidopsis*, tobacco, sorghum and rice,” Nucleic Acids Res, vol. 41, pp. e188, November 2013.
80. Y.F. Mao, H. Zhang, N.F. Xu, B.T. Zhang, F. Gou, and J.K. Zhu, “Application of the CRISPR–Cas system for efficient genome engineering in plants,” Mol. Plant, vol. 6, pp. 2008-2011, November 2013.
81. F. Fauser, S. Schiml, and H. Puchta, “Both CRISPR/Cas-based nucleases and nickases can be used efficiently for genome engineering in *Arabidopsis thaliana*,” Plant J. vol. 79, 348-359, July 2014.
82. S. Schiml, F. Fauser, and H. Puchta, “The CRISPR/Cas system can be used as nuclease for in planta gene targeting and as paired nickases for directed mutagenesis in *Arabidopsis* resulting in heritable progeny,” Plant J. vol. 80, pp. 1139-1150, December 2014.
83. H.L. Xing, L. Dong, Z.P. Wang, H.Y. Zhang, C.Y. Han, B. Liu, et al., “A CRISPR/Cas9 tool kit for multiplex genome editing in plants,” BMC Plant Biol, vol. 14, pp. 327, December 2014.
84. Y. Hyun, J. Kim, S.W. Cho, Y. Choi, J.S. Kim, and G. Coupland, “Site- directed mutagenesis in Arabidopsis thaliana using dividing tissue- targeted RGEN of the CRISPR/Cas system to generate heritable null alleles,” Planta, vol. 241, pp. 271-284, January 2015.
85. R.A. Johnson, V. Gurevich, S. Filler, A. Samach, and A.A. Levy, “Comparative assessments of CRISPR-Cas nucleases' cleavage efficiency in planta,” Plant Mol. Biol, vol. 87, pp. 143-156, January 2015.
86. X.L. Ma, Q.Y. Zhang, Q.L. Zhu, W. Liu, Y. Chen, R. Qiu, et al., “A robust CRISPR/Cas9 system for convenient, high-efficiency multiplex genome editing in monocot and dicot plants,” Mol. Plant, vol.8, pp. 1274-1284, August 2015.
87. V. Nekrasov, B. Staskawicz, l.D. Weige, J.D. Jones, and S. Kamoun, “Targeted mutagenesis in the model plant *Nicotiana benthamiana* using Cas9-guided endonuclease,” Nat. Biotechnol, vol. 31, pp. 691-693, August 2013.
88. L.G. Lowder, D.W. Zhang, N.J. Baltes, J.W. Paul, X. Tang, X.L. Zheng, et al., “A CRISPR/Cas9 tool box for multiplexed plant genome editing and transcriptional regulation,” Plant Physiol, vol. 169, pp. 971-985, October 2015.
89. S.K. Upadhyay, J. Kumar, A. Alok, R. Tuli, “RNA-guided genome editing for target gene mutations in wheat,” G3-Genes Genomes Genet, vol. 3, pp. 2233-2238, December 2013.
90. K.Q. Yin, T. Han, G. Liu, T. Chen, Y. Wang, Y.Z. Alice, et al., “A geminivirus-based guide RNA delivery system for CRISPR/Cas9 mediated plant genome editing,” Sci. Rep, vol. 5, pp. 14926, October 2015.
91. M. Vazquez-Vilar, J.M. Bernabe-Orts, A. Fernandez-del-Carmen, P. Ziarsolo, B. Jose, A. Granell, et al., “A modular tool box for gRNA–Cas9 genome engineering in plants based on the Golden Braid standard,” Plant Methods, vol. 12, pp. 10, December 2016.
92. J. Miao, D.S. Guo, J.Z. Zhang, Q.P. Huang, G.J. Qin, X. Zhang, et al., “Targeted mutagenesis in rice using CRISPR-Cas system,” Cell Res, vol. 23, pp. 1233-1236, October 2013.
93. K.B. Xie, and Y.N. Yang, “RNA-guided genome editing in plants using a CRISPR–Cas system,” Mol. Plant, vol. 6, pp. 1975-1983, November 2013.
94. Q.W. Shan, Y.P. Wang, J. Li, and C.X. Gao, “Genome editing in rice and wheat using the CRISPR/Cas system,” Nat. Protoc, vol. 9, pp. 2340-2395, October 2014.
95. R.F. Xu, H. Li, R.Y. Qin, L. Wang, L. Li, P.C. Wei, et al., “Gene targeting using the *Agrobacterium tumefaciens*-mediated CRISPR–Cas system in rice,” Rice, vol. 7, pp. 5, December 2014.
96. H. Zhang, J.S. Zhang, P.L. Wei, B.T. Zhang, F. Gou, Z.Y. Feng, et al., “The CRISPR/Cas9 system produces specific and homozygous targeted gene editing in rice in one generation,” Plant Biotechnol. J, vol. 12, pp. 797-807, August 2014.
97. H.B. Zhou, B. Liu, D.P. Weeks, M.H. Spalding, and B. Yang, “Large chromosomal deletions and heritable small genetic changes induced by CRISPR/Cas9 in rice,” Nucleic Acids Res, vol. 42, pp. 10903-10914, September 2014.
98. M. Endo, M. Mikami, and S. Toki, “Bi-allelic gene targeting in rice,” Plant Physiol, vol. 170, pp. 666-677, February 2016.
99. M. Endo, M. Mikami, and S. Toki, “Multigene knockout utilizing off-target mutations of the CRISPR/Cas9 system in rice, Plant Cell Physiol, vol. 56, pp. 41-47, January 2015.
100. M. Mikami, S. Toki, and M. Endo, “Parameters affecting frequency of CRISPR/Cas9 mediated targeted mutagenesis in rice, Plant Cell Rep, vol. 34, pp. 1807-1815, October 2015.
101. C. Brooks, V. Nekrasov, Z.B. Lippman, and J.V. Eck, “Efficient gene editing in tomato in the first generation using the clustered regularly interspaced short palindromic repeats/CRISPR-associated9 system,” Plant Physiol, vol. 166, pp. 1292-1297, November 2014.
102. M. Ron, K. Kajala, G. Pauluzzi, D.X. Wang, M.A. Reynoso, K. Zumstein, et al., “Hairy root transformation using *Agrobacterium* *rhizogenes* as a tool for exploring cell type-specific gene expression and function using tomato asa model,” Plant Physiol, vol. 166, pp. 455-469, October 2014.
103. C.T. Pan, L. Ye, L. Qin, X. Liu, Y.J. He, J. Wang, et al., “CRISPR/Cas9- mediated efficient and heritable targeted mutagenesis in tomato plants in the first and later generations,” Sci. Rep, vol. 6, pp. 24765, April 2016.
104. N.M. Butler, P.A. Atkins, D.F. Voytas, and D.S. Douches, “Generation and inheritance of targeted mutations in potato (*Solanum tuberosum* L.) using the CRISPR/Cas system,” PLoS One, vol. 10, pp. e0144591, December 2015.
105. S.H. Wang, S.B. Zhang, W.X. Wang, X.Y. Xiong, F.R. Meng, and X. Cui, “Efficient targeted mutagenesis in potato by the CRISPR/Cas9 system,” Plant Cell Rep, vol. 34, pp. 1473-1476, September 2015.
106. Q.W. Shan, Y.P. Wang, J. Li, Y. Zhang, K.L. Chen, Z. Liang, et al., “Targeted genome modification of crop plants using a CRISPR-Cas system,” Nat. Biotechnol, vol. 31, pp. 686-688, August 2013.
107. Z. Liang, K. Zhang, K.L. Chen, and C.X. Gao, “Targeted mutagenesis in *Zea mays* using TALENs and the CRISPR/Cas system,” J. Genet. Genom, vol. 41, pp. 63-68, February 2014.
108. S. Svitashev, J.K. Young, C. Schwartz, H.R. Hao, and S.C. Falco, “Targeted mutagenesis, precise gene editing and site-specific gene insertion in maize using Cas9 and guide RNA,” Plant Physiol, vol. 169, pp. 931-945, October 2015.
109. C. Feng, J. Yuan, R. Wang, Y. Liu, J.A. Birchler, and F.P. Han, “Efficient targeted genome modification in maize using CRISPR/Cas9 system,” J. Genet. Genom, vol. 43, pp. 37-43, January 2016.
110. J.J. Zhu, N. Song, S.L. Sun, W.L. Yang, H.M. Zhao, W.B. Song, et al., “Efficiency and inheritance of targeted mutagenesis in maize using CRISPR-Cas9,” J. Genet. Genom, vol. 43, pp. 25-36, January 2016.
111. X. Liu, S. Wu, J. Xu, C. Sui, J. Wei, “Application of CRISPR/Cas9 in plant biology,” Acta Pharm. Sin. B, vol. 7, pp. 292-302, May 2017.
112. J.L. Nemhauser, and K.U. Torii, “Plant synthetic biology for molecular engineering of signaling and development,” Nat. Plants, vol. 2, pp. 16010, March 2016.
113. B. Jusiak, S. Cleto, P. Perez-Pinera, and T.K. Lu, “Engineering synthetic gene circuits in living cells with CRISPR technology,” Trends Biotechnol, vol. 34, pp. 535–547, July 2016.
114. S. von Caemmerer, W.P. Quick, and R.T. Furbank, “The development of C4 rice: current progress and future challenges,” Science, vol. 336, pp. 1671–1672, June 2012.
115. T. Komatsuda, M. Pourkheirandish, C. He, P. Azhaguvel, H. Kanamori, et al., “Six-rowed barley originated from a mutation in a homeodomain-leucine zipper I-class homeobox gene,” PNAS, vol. 104, pp. 1424–1429, January 2007.
116. H. Wang, A.J. Studer, Q. Zhao, R. Meeley, and J.F. Doebley, “Evidence that the origin of naked kernels during maize domestication was caused by a single amino acid substitution in tga1,” Genetics, vol. 200, pp. 965–974, July 2015.
117. P. Civáň, and T.A. Brown, “Origin of rice (Oryza sativa L.) domestication genes,” Genet. Resour. Crop Evol, vol. 64, pp. 1125–1132, August 2017.
118. J.C. Sedbrook, W.B. Phippen, M.D. Marks, “New approaches to facilitate rapid domestication of a wild plant to an oilseed crop: example pennycress (*Thlaspi arvense* L.),” Plant Sci, vol. 227, pp. 122–132, October 2014.
119. T. Li, X. Yang, Y. Yu, X. Si, X. Zhai, et al., “Domestication of wild tomato is accelerated by genome editing,” Nat. Biotechnol, vol. 36, pp. 1160–1163, December 2018.
120. A. Zsögön, T. Čermák, E.R. Naves, M.M. Notini, K.H. Edel, et al., “*De novo* domestication of wild tomato using genome editing,” Nat. Biotechnol, vol. 36, pp. 1211–1216, December 2018.
121. Z.H. Lemmon, N.T. Reem, J. Dalrymple, S. Soyk, K.E. Swartwood, et al., “Rapid improvement of domestication traits in an orphan crop by genome editing,” Nat. Plants, vol. 4, pp. 766–770, October 2018.
122. Z. Feng, Y. Mao, N. Xu, B. Zhang, P. Wei, et al., “Multigeneration analysis reveals the inheritance, specificity, and patterns of CRISPR/Cas-induced gene modifications in *Arabidopsis*,” PNAS, vol. 111, pp. 4632–4637, March 2014.
123. J. Li, H. Manghwar, L. Sun, P. Wang, G. Wang, et al., “Whole genome sequencing reveals rare off-target mutations and considerable inherent genetic or/and somaclonal variations in CRISPR/Cas9-edited cotton plants,” Plant biotechnol. J, vol. 17, pp. 858-868, May 2018.
124. X. Tang, G. Liu, J. Zhou, Q. Ren, Q. You, et al., “A large-scale whole-genome sequencing analysis reveals highly specific genome editing by both Cas9 and Cpf1 (Cas12a) nucleases in rice,” Genome Biol, vol. 19, pp. 84, December 2018.
125. D. Kim, K. Lim, S.T. Kim, S.H. Yoon, K. Kim, et al., “Genome-wide target specificities of CRISPR RNA-guided programmable deaminases,” Nat. Biotechnol, vol. 35, pp. 475–480, May 2017.
126. H.A. Rees, A.C. Komor, W.H. Yeh, J. Caetano-Lopes, M. Warman, et al., “Improving the DNA specificity and applicability of base editing through protein engineering and protein delivery,” Nat. Commun, vol. 8, pp. 15790, June 2017.
127. M. McVey, V.Y. Khodaverdian, D. Meyer, P.G. Cerqueira, and W.D. Heyer, “Eukaryotic DNA polymerases in homologous recombination,” Annu. Rev. Genet, vol. 50, pp. 393–421, November 2016.
128. D.E. Voth, L.J. Broederdorf, and J.G. Graham, “Bacterial type IV secretion systems: versatile virulence machines,” Future Microbiol, vol. 7, pp. 241–257, February 2012.

**Authors’ contribution:** PS conceived the idea and collected data; PS and BP wrote and edited and the manuscript; BP did some addition and correction to the manuscript; PS prepared the table; BP added the pictorial diagrams; SD revised the manuscript. All authors read, finalized and approved the manuscript.

**Funding:** None

**Conflict of interest:** None