REVIEW

Emerging tools and paradigm shift of gene editing in cereals, fruits, and horticultural crops for enhancing nutritional value and food security

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Funding information
National Institute of Plant Genome Research and Department of Science and Technology-SERB, Grant/Award Number: SRG/2019/000505; Indian National Science Academy (INSA), Grant/Award Number: SP/YSP/150/2018/231

Abstract

Gene editing using sequence-specific nucleases, particularly CRISPR/Cas ribonucleoprotein, has drawn enormous attention in plant research in recent years. Nearly a decade ago, Cas9 protein was initially discovered for a role in adaptive immunity in bacteria. Owing to vast potential, a large number of reports came out in a short span of time, comprising the identification of Cas protein from different bacterial sources, new Cas9 variants with reduced off-targets, multiplexing, base editing, prime editing, and RNA manipulation in plants. Studies revealed that CRISPR/Cas-based gene editing can play a major role in ensuring food security *via* developing resilient commercial crops with improved yield and nutritional value. Use of the CRISPR/Cas9 system for creating mutation in genes and regulatory regions of promoter generated a number of alleles with variable phenotypes, which can serve as an excellent genetic resource in the breeding program. In this review, we provide a recent overview of state-of-art discoveries in the CRISPR/Cas system comprised of new Cas proteins, modifications of existing Cas9, refinements in CRISPR/Cas-induced gene editing, applications, and outcome emphasizing on major cereals and horticultural crops. We also highlight the current global policy framework for the regulation of gene-edited crops.

KEYWORDS
CRISPR/Cas9, crop, DNA repair, DSB, gene editing, NHEJ, ribonucleoprotein

1 | INTRODUCTION

The modification of a specific trait in the crop is not a new concept but a continuous process being practiced from centuries through crop breeding. The selective cultivation and trait introgression within preferred varieties led to enhanced productivity and better performance of crops. Apart from traditional plant breeding, the advent of recombinant DNA technology in the past century and its application geared the pace of crop improvement attempts but mostly *via* transgenesis. For the same purpose, gene-editing tools are widely being used for creating desired genetic and phenotypic variation in the plant nowadays. In general, precise and tailored changes in a specific sequence in the genome are referred to as genome/gene editing. The mutation within a specific region of the genome can be achieved during DNA breaks repair, which occurred *via* nonhomologous end joining (NHEJ) when site-directed nucleases introduce double-strand breaks

(DSBs) at directed position (Baltes & Voytas, 2015; Osakabe & Osakabe, 2015). Exploiting this unique feature, researchers have developed numerous strategies for genome editing by utilizing NHEJ breaks repair mechanism, which is normally vulnerable to mutations. These techniques use some sequence-specific nucleases, such as Zinc-Finger endonucleases (ZFNs). In these nucleases, endonuclease domain is fused with zinc-finger DNA-binding domains and recognizes a 3-bp module (Kim et al., 1996). Similarly, in transcription activator-like effector nucleases (TALENS), endonuclease domains are paired with transcription activator-like effector domains that recognize single base pairs (Christian et al., 2010). Parallel to these approaches, another sequence-specific nuclease-based technique has been developed in the recent past, popularized as Clustered Regularly Interspaced Short Palindromic. The CRISPR/Cas9 system harnessed the potential of RNA-guided nucleases, Cas9, which is responsible for maintaining bacterial adaptive immunity through capturing and recognizing invasive DNA with the help of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and neutralizing the invading pathogen.

Despite the fact that several reports of ZFNs mediated genome editing in various organisms, construction of the large modular proteins, including two ZFNs that bring *Fok*1, endonuclease, in the vicinity to target DNA, is a tedious and expansive process. Also, this method limits the widespread application of ZFNs (Puchta & Fauser, 2014). In the same way, TALENs are engineered enzymes originally derived from TAL effectors of *Xanthomonas* sp. The DNA-binding domain of TAL is fused with *Fok*1 nuclease. TALENs are considered as relatively a better option than ZFNs; however, the construction of novel TALE arrays for each gene is not cost-effective for gene editing. In addition, requirements of a pair of proteins to recognize both sense and antisense DNA strands for DSB using TALENS make it unsuitable for multiple gene editing (Langner et al., 2018; Voytas, 2013). In contrast, CRISPR/Cas9-based genome editing is a simple, easy in the application; hence, it emerged as an effective tool of genome editing in a wide range of organisms compared with ZFNs and TALENS.

The discovery of Cas9 nuclease from *Streptococcus pyogenes* laid the foundation for the development of the CRISPR/Cas9 system for genome editing (Barrangou et al., 2007; Bhaya et al., 2011). There are only two components essential for the CRISPR/Cas9 system; DNA endonuclease (monomeric Cas9) and a customizable single guide RNA (sgRNA), which recognizes the target DNA by Watson-Crick base pairing. The sgRNA is customizable according to the target sequence consisting of the proto-spacer adjacent motif (PAM) of CRISPR RNA (crRNA) and a trans-activating crRNA (tracrRNA) in bacteria. Later, in vitro research demonstrated that single guide RNA (sgRNA) is sufficient to guide Cas9 protein. Thus, it can replace the need for dual crRNA and tracrRNA (Jinek et al., 2012). Upon formation, Cas9-sgRNA complex searches target DNA double helix for the presence of canonical PAM flanking to potential gRNA complementary sequence, which leads to the establishment of gRNA-DNA heteroduplex followed by Cas9 nickase-mediated cleavage of DNA strands (Jinek et al., 2012).

The CRISPR/Cas9 tools have been frequently applied to make knockout mutant of genes, gene corrections, base editing, prime editing, gene replacement, and site-specific transgene incorporation of the desired gene in a flexible manner with high specificity in plants. In recent years, an unprecedented number of reports have shown successful genome editing stories in various crops to ensure food security and quality enhancement. The gene editing or precision breeding seems to be the only option to feed the rising human population under the pressure of the limiting land, climate change, pathogen, and abiotic stress conditions. In the present review, we provide an overview of recent comprehensive advancements in CRISPR/Cas9 system, exciting applications and in crop improvements. In addition, we also describe limitations and future perspectives of this technology, including regulation and acceptability-related policies of various countries concerning genome-edited crops.

2 | Cas9 PLAYS A CENTRAL ROLE IN GENE EDITING

The concept of CRISPR/Cas9-mediated genome editing came after one of the most fundamental discoveries of this century, leading to the characterization of the novel nuclease Cas9, a class II bacterial adaptive immune system from *S. pyogenes* (Barrangou et al., 2007; Bhaya et al., 2011). Some prokaryotic organisms, including the archaea, had evolved slew of weapons comprising restriction endonucleases and Cas9 system for contending threats of genetic invaders such as viruses. Around one-third of the bacterial population and about 90% of known archaea retain cluster of short DNA repeats separated with "spacer" and a series of nearby genes encoding Cas proteins (Bhaya et al., 2011). Bacteria use these spacers as a form of memory to identify the viruses and plasmids. However, the evolutionary process by which bacteria gain this information is not much known. Some studies reported that *Sulfolobus tokodaii*, archaea, devotes nearly 1% of its genome as spacers to be used in CRISPR/Cas immunity (Gophna & Brodt, 2012; Ledford, 2017).

In general, the Cas9-gRNA complex recognizes nuclease-specific PAM sequence (5′-NGG-3′) in the genome (Figure 1). Though the Cas9-gRNA complex recognizes an additional PAM sequence such as 5′-NAG-3′ and 5′-NGA-3′, these PAMs resulted in high off-target mutagenesis in the plant (Zhang et al., 2014). Interestingly, the requirement of the PAM sequence for Cas9 protein also depends

FIGURE 1 An outline of CRISPR/ Cas system and the gene editing. Ribonucleoprotein complex searches the target DNA region based on sequence complementarity and proto-spacer adjacent motif (PAM) sequence. Cas protein left the double-stranded breaks which are repaired via endogenous nonhomologous end joining (NHEJ) that may left mismatch/ mutation and bystander mutation whereas a desired mutation can be introduced through homology-dependent recombination (HDR) donor template or prime editors
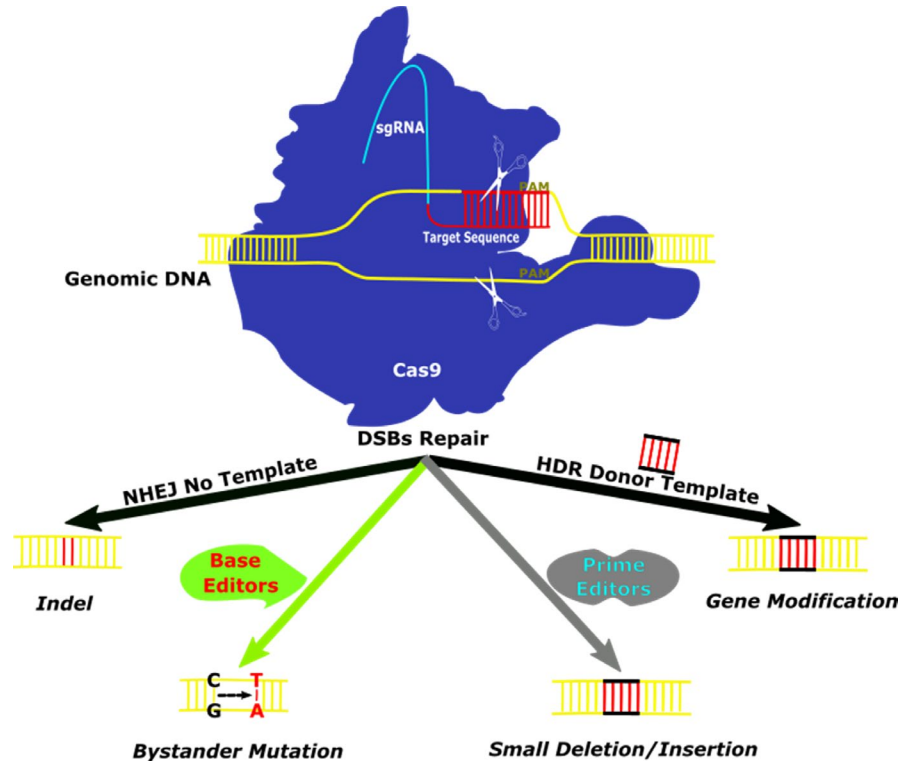
on the source of protein. For instance, Cas9 protein of *Staphylococcus aureus* (SaCas9) and *Staphylococcus thermophilus* (StCas9) uses 5′-NNGRRT-3′ and 5′-NNGGAA-3′ as PAM sequence (where R represents A or G), respectively (Nishimasu et al., 2014) whereas Cas9 from *Acidaminococcus* recognizes 5′-TTTV-3′ PAM (Zetsche et al., 2015). The variants of engineered Cas9 protein, namely xCas9 and SpCas9-NG, have been developed, which can recognize only "NG" PAM (Hu et al., 2018; Hua et al., 2019). A classical study reported that heterologous expression of a CRISPR3/Cas system from *Streptococcus thermophilus* could be transferred to *Escherichia coli*, which prevented further plasmid transformation and laid the foundation of the development of the CRISPR/Cas9 system (Sapranauskas et al., 2011).

3 | DISCOVERY OF NEW Cas PROTEINS

Due to the vast applicability of the Cas9-based genome-editing tool, researchers are consistently developing new Cas nucleases from different bacterial sources and additional variants of Cas protein. The five new programmable CRISPR class II nucleases (Cpf1, C2c1, C2c3, CasY, and CasX) are reported from different bacterial sources targeting mainly DNA (Shmakov et al., 2015, 2017). Further, numerous class II type VI nucleases (C2c2 and C2c6) have also been characterized, which target RNAs molecules (Abudayyeh et al., 2016, 2017). Burstein et al. (2017) reported the

presence of Cas9 protein in the archaeal domain of life using genome resolved metagenomics. In this study, two compact Cas proteins, namely CasX (required PAM; "TTCA") and CasY (Cas12d) were discovered (Burstein et al., 2017). The CasX, also known as Cas12e, displays less sequence similarity in RuvC domain of nuclease and left a staggered cut after DSBs in DNA (Liu, Orlova, et al., 2019). However, direct DNA cleavage and genome editing activity of CasY were not observed until a recent study reported that short-complementarity untranslated RNA (scoutRNA) and crRNA are required for optimal target DNA cleavage of CasY nuclease (Harrington et al., 2020). Owing to certain features such as compact size, dominant RNA content, and minimal trans-cleavage activity, CRISPR/CasX seems to provide an edge over other nucleases in future gene-editing tools.

The Cas12a (also known as Cpf1, CRISPR *Prevoltella* and *Francisella*1) is a newly studied class II nuclease, which cleaves similar to Cas9 protein but recognized "TTTN" PAM sequence. Therefore, it can serve as a valuable nuclease for editing in "T" rich region of the genome. Using Cas12a for genome editing offers additional benefits over Cas9, which comprises the least off-targets and high efficiency of indels and generates biallelic mutation in T0 generation in the plant (Xu et al., 2017; Zaidi et al., 2017). The characterization of Cas13a (also known as C2c2), a type VI-A endonuclease, was another exciting discovery in this area. The Cas13a has RNA-guided RNA ribonuclease and a distinct RNase activity (East-Seletsky et al., 2016). These properties of Cas13a protein help to develop future RNA-targeting tools for applications in RNA biology and therapeutics (Gootenberg et al., 2017; O'Connell, 2019).

4 | ADVANCEMENT OF CRISPR/Cas9 TOOL FOR GENOME EDITING

As described in Introduction, synthetic sgRNA comprised of crRNA and tracrRNA can mimic natural crRNA-tracrRNA hybrid, which directs Cas9 protein for DSB in the DNA molecule in bacteria (Hsu et al., 2013). In the beginning, different sizes of tracrRNA sequences were fused to assess variants of sgRNA (+48), sgRNA (+67), and sgRNA (+85) for enhancing Cas9 protein in vivo cleavage activity (Hsu et al., 2013; Jinek et al., 2012). Subsequent research demonstrated that only 20 bp of the leader sequence of sgRNA is capable to direct Cas9 protein to cleave the complementary target sequence and thus referred to as guide RNA (gRNA). Based on this knowledge, numerous vectors have been constructed for efficient cloning of the gRNA; a typical sgRNA cassette is 400–500 bp long containing RNA polymerase III promoter, gRNA, and Pol III terminator (Xie et al., 2015). The apropriate selection of promoter and terminator combination is also necessary in order to get maximum gene edit events (Box 1).

4.1 | Development of vectors for multiplexing

To achieve multiplex gene editing, separate sgRNA units are required to be combined in a transformation vector that can be driven by different Pol III promoters. Keeping this in mind, numerous vector systems have been developed. Several programmed sgRNAs can be easily cloned; for example, pYPQ131, pYPQ138, and pYPQ148 series vectors are developed for cloning of eight different gRNA (Lowder et al., 2015). Similarly, Ma et al. (2015) have developed a robust CRISPR/Cas9 vector system in which multiple sgRNA expression cassettes can be easily assembled through Golden Gate ligation or Gibson Assembly. This system has been successfully tested for creating biallelic mutation in rice and Arabidopsis and provided a versatile toolbox for studying functions of multiple genes in a plant (Ma et al., 2015). A study reported pEN chimera-based vectors can combine four sgRNAs through the Golden Gate cloning method which may increase to 18 sgRNA (Houbaert et al., 2018). The major limitation of gene multiplexing for combining several transcriptional units of sgRNA in the same vector increases size of the vector, ultimately affecting transformation efficiency (Xie et al., 2015). Overcoming this, strategies have been employed to cleave polycistronic mRNAs into individual sgRNA transcripts, including bacterial RNA cleaving enzymes such as CRISPR/Cas Subtype Ypest protein 4 (Csy4), host endogenous tRNA-processing enzymes and ribozymes. A study showed that the fusion of Csy4 (from

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Box 1   Promoters and terminators combination determine mutation frequency

In CRISPR/Cas9 vectors, *Cas9* and sgRNA expression are driven by RNA polymerase (Pol) II and Pol III promoters, respectively. Several reports revealed that the constitutive expression of *Cas9*, either under the control of ubiquitin or cauliflower mosaic virus (CaMV35S) can create successful mutation events in plants. It is also evident that transgenic lines obtained through the CRISPR/Cas9 system are mostly mosaic in T1 generation (Schiml et al., 2014). To overcome this issue, targeting egg cell/one-cell embryos can be a possibility during *in planta* transformation of the plant. Considering this fact, a study revealed that it would be possible to get homozygous or biallelic mutants in the T1 generation through the precise expression of Cas9 protein in egg cells/one-cell stage embryos by using the promoter of egg cell-specific (EC1.2) gene in Arabidopsis (Wang et al., 2015). A report revealed that the expression of *Cas9* under cell division-specific promoters *YAO* and *CDC45* resulted in high mutation rates (80.9%–100%) in the T1 generation. The percentage of inherited mutations to successive generations was also high accounted for 4.4%–10% (T1 generation) and 32.5%–46.1% (T2 generation) in Arabidopsis (Feng et al., 2018). Furthermore, an appropriate combination of promoter and terminator is also crucial for Cas9 protein abundance. In another study, the efficiency of terminators such as rbcS E9T (from *Pisum sativum*) and Nos have been analyzed to check the abundance of Cas9 protein and reported that triple mutants can only be obtained using rbcS E9T but not with NosT in T1 generation (Wang et al., 2015). Some studies demonstrated that promoters of *ubiquitin 1* gene and *U3* or *U6* for expression of *Cas9* and sgRNA resulted in high mutation efficiency (13% or 2%) in T0 generation in the case of maize (Feng et al., 2016; Zhu et al., 2016), while another study showed that the use of maize *ubiquitin 1* promoter for rice codon-optimized *Cas9* and rice U6 promoter for the expression of sgRNA further increased biallelic mutations at a frequency of 22%–58% in maize, indicating the significance of the promoter–terminator combination (Char et al., 2017). Overall it is amenable that a careful selection of promoter–terminator in a vector is required for more gene edits events and biallelic mutant in a single generation.

Pseudomonas aeruginosa) with dCas9 protein can successfully process polycistronic mRNAs into sgRNA in the mammalian cell (Tsai et al., 2014). Harnessing conserved endogenous tRNA-processing enzymes is another way for the processing of sgRNAs transcripts. Some reports illustrated that polycistronic-mRNA can also be processed into individual gRNA transcripts utilizing ribozyme (Gao & Zhao, 2014).

In addition to retaining DNA nickase activity, Cas12a protein contains RNA cleavage ability for processing of own crRNA, which can be effective for simultaneous gene editing of four genes using a single crRNA array in mammalian cells (Zetsche et al., 2017). The maturation of crRNA by Cas12a protein does not require tracrRNA rendering advantage for developing a simple and easy multiplexing vector system (Fonfara et al., 2016). Wang et al. (2017) constructed the CRISPR/Cas12a tool in pCAMBIA series vector and edited four members of receptor-like kinase family simultaneously with much precision and least off-targets (Wang et al., 2017). An interesting study reported that Cas12a enzyme activity was inhibited by bacteriophage-derived proteins known as anti-CRISPRs (acrs) *via* multiple ways, including inhibition dsDNA recognition and dimerization of Cas12a protein and triggering cleavage of gRNA bound with Cas12a (Knott et al., 2019). Since Cas12a protein is a popular tool for gene editing as well in molecular diagnostics nowadays, more research is required to understand the impact of acrs in the area of biotechnological use of CRISPR/Cas12a for gene editing in the future.

4.2 | Development of vectors for easy selection of heritable mutants

During transformation, a large proportion of mutations generated by CRISPR/Cas9 are somatic, which is generally not transferred to progenies. Further, it is impossible to know whether mutations are somatic or inherited from the previous generation upon stable integration of CRISPR/Cas9 cassette in the genome. Therefore, it is necessary to confirm heritable mutations among edited lines for this, screening of lines having the desired mutation from the pool of Cas9-free segregating population is a common method for ensuring germline mutation. Such screening is mostly performed based on restriction surveyor analysis or sequencing of edited gene and both are laborious and costly methods. Gao et al. (2016) devised a method in which the CRISPR/Cas9 cassette contains a fluorophore reporter (mCherry) under the control of seed-specific At2S3 promoter. The fluorescence-based visual screen of seeds in T2 generation greatly facilitated the screening of Cas9 protein-free lines and stably transmitted mutations

(Gao et al., 2016). Furthermore, a modified CRISPR/Cas9 system has been devised in pKSE401 vector in which sGFP was inserted as a fluorescence tag enabling rapid identification of positive primary transformants and Cas9-free mutants in later generations (Tang et al., 2018). The screening of GFP positive mutants among T1 population and negative Cas9-free mutant in later generations need a fluorescent microscope and seems inconvenient under certain conditions. Considering this, the DsRED fluorescence marker system has been developed for a rapid selection of Cas9-free homozygous edited plants in a single generation in rice, tomato, and Arabidopsis by seeing DsRED fluorescence in seeds (Aliaga-Franco et al., 2019).

5 | APPLICATIONS OF CRISPR/CAS9 IN GENE EDITING

In the beginning, the CRISPR/Cas9 system was primarily used for creating precise knockout mutations in a plant. However, later research proved the usefulness of this system in various ways such as allele creation, gene insertion, base editing, prime editing, codon editing, and many more (Figure 2). In this section, we provide comprehensive information about diverse ways of CRISPR/Cas gene editing.

5.1 | Mutant generation by targeting coding region

The most widespread use of the CRISPR/Cas system is to create the insertion-deletion (Indel) mutations in protein-coding regions of a gene by harnessing endogenous NHEJ potential of the cell. The nickase activity of Cas9 protein left the DSBs, which is repaired by an error-prone cellular machinery that often leads to indels in the desired sequence. The presence of indels in open reading frames resulted in frameshift mutation of proteins and the most likely introduction of premature stop codons, which ultimately cause the translation of truncated protein. Hence for creating such a loss of function mutant, it is always advisable to target the region close to the initiation codon or first exons. This strategy is very useful in studying the function of gene by creating loss of function allele in plants. In an elegant study, Houbaert et al. (2018) have generated several single and double mutants of *POLAR* and *POLARlike* genes in Arabidopsis for proving the scaffolding activity of these proteins for BIN2 and other GSK3 like kinases, and their role in stomatal development. Apart from creating indels, the CRISPR/Cas9 system is also applied for the deletion of large DNA fragments ranging from 1.7 to 13 Kb in Arabidopsis (Wu et al., 2018), soybean (Cai, Chen, Sun, et al., 2018), rice (Zhou et al., 2014), tomato, and *Nicotiana* (Ordon et al., 2017).
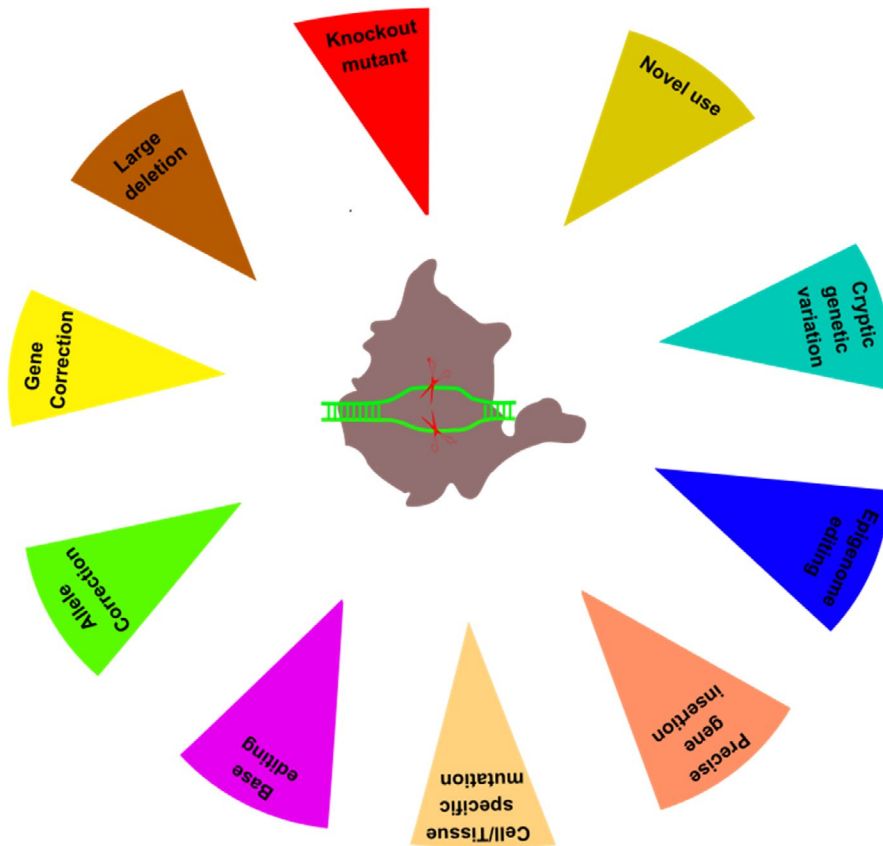
FIGURE 2 The depiction of gene editing events accomplished using CRISPR/Cas system in plant

5.2 | Promoter editing

Considering the CRISPR/Cas system ability to introduce indels in the desired sequence, attempts have been made to modify the regulatory motif in the promoter region of crucial genes for altering the transcript abundance. Indels creation within an important motif in the promoter region caused interference in the binding of RNA polymerase/accessory proteins, which eventually activated or repressed mRNA transcripts of the corresponding gene. This strategy was foremost applied for creating several alleles of the self-pruning gene in tomato (Rodriguez-Leal et al., 2017). In an elegant study, *Solanum pimpinellifolium*, a wild relative of tomato resistant to bacterial blight, drought, and salt stresses, has been domesticated using promoter editing of multiple genes regulating entirely different traits (Li et al., 2018). Similarly, several alleles of *Wx* gene, responsible for amylose synthesis in the endosperm, were created using CRISPR/Cas9-based editing of promoter region to improve the cooking quality of rice (Huang et al., 2020). In another study, the CRISPR/Cas9 system was employed to edit the *Xa13* promoter to create a number of alleles displaying a range of bacterial blight resistance in rice (Li, Li, Zhou, et al., 2020). Given the pluripotent nature of some genes regulating diverse developmental processes, promoter editing seems to be a viable option for creating multiple alleles that differ in variable expression

rather than direct targeting of the coding sequence, which left mostly knockout mutant.

5.3 | Desired mutation and gene insertion

Gene editing is not confined to only creating indels and large deletions in the genome at targeted loci but includes new ways of genome modification such as insertion of desired sequences, genes at specified loci, and allele replacement. These modifications can be accomplished *via* homology-dependent recombination (HDR) rather than nonhomologous end joining (Figure 1). In HDR, the donor template containing both the flanking sites of a target is usually co-delivered with sgRNA and CRISPR/Cas9. It has been found that mutation efficiency using HDR largely varied depending on the degree of delivery of donor repair template (DRT) molecule (Li et al., 2013). As proof of concept, RNA template DRT-HDR was initially tested in yeast and later in mammalian cells, reporting a successful gene modification event (Keskin et al., 2014; Storici et al., 2007). A study revealed that DRT can be fused with sgRNA to make chimeric RNA molecules containing sequences for target recognition as well as direct the HDR in rice protoplast (Butt et al., 2017). Harnessing both RNA nicking and DNA cleavage activity of Cas12a protein, researchers have devised a ribozyme-based system

to produce two crRNAs and RNA transcripts of DRTs from a single transcript and demonstrated that RNA transcript templated homologous recombination can be achieved *via* DRT-HDR in rice (Li et al., 2019). Recently, Lu et al. (2020) have developed a method of gene replacement and efficient gene insertion with high efficiency in rice. Authors used a chemically modified donor DNA and CRISPR/Cas9 system along with promoter and enhancer to insert a large DNA sequence (2049 bp) and replace a gene in the rice genome very efficiently (Lu et al., 2020). In a nice study, targeted insertion of a 5.2 kb carotenoid biosynthesis cassette within preferred genomic loci has been achieved using an optimized CRISPR/Cas9-based method resulting marker-free rice plants accumulate high carotenoid content in the grains (Dong et al., 2020). In a next-level improvement, Barone et al. (2020) used the heat-inducible expression of *Cas9* for generating DSBs and successfully mobilized marker-free donor template in the maize genome. Following this method, they have obtained nonchimeric and heritable gene edit events with high frequency in T0 generation in maize (Barone et al., 2020). The use of HDR-based gene modification further expands the ability of gene editing and leading to a great scope of novel applicability of gene editing.

5.4 | Prime editing

Prime editing (PE) is the outcome of the recent development in gene-editing arsenal, which is referred to as "search and replace" technology. Using this tool, it can be possible to introduce targeted insertion, deletion, and all the base transversions without double-strand breaks in DNA. In this system, gRNA scaffold is replaced with prime editing guide RNA (pegRNA) and modified Cas9 protein is combined with reverse transcriptase altogether. The unique feature of peg RNA is that it contains a primer binding site (PBS), the desired sequence that will be introduced in target gene and spacer sequence complementary to one strand of DNA (Anzalone et al., 2019). The comparative study of PE efficiency with either Cas9-based HDR or base editors exhibited similar results but with higher efficiency in human cell lines. One of the key advantages of prime editing is the flexibility of required PAM sequences as it can incorporate mutations (>30 bp) away from the site of nicking (Anzalone et al., 2019). Moreover, PE using Cas9 protein is found to left lesser off-targets in genome compared to simple Cas9 nuclease-based mutations. Owing such unique abilities, PE has proven worth to correct the point mutations responsible for the generation of various alleles associated with diseases in humans. The PE is thought to be a milestone in the refinement of precision tools that will be universally applied for gene editing in organisms. The PE holds great therapeutic potential in terms of precise gene and allele correction in

humans. However, report for the use of PE system in plant seems to be exciting and this system needs to be adapted for the application in the modification of plant genome to open the new future possibilities. A very recent study reported the applicability of prime editors in plants by showing that prime editors successfully induced point mutations, insertions, and deletion in cereals such as rice and wheat (Lin et al., 2020). Due to versatility of the PE, we can foresee a diverse application of PE as a wonderful tool in functional genomics and equally useful in crop breeding.

5.5 | Programmed single base editing

Apart from the mentioned applications, the CRISPR/Cas system is also used for single base editing when coupled with base editors. Base editing is a kind of gene engineering which can be achieved without DSBs and the NHEJ process (Figure 1). In general, base editors comprise of: (a) CRISPR-dCas9 which is catalytically inactive, (b) cytidine deaminase, which converts C to U and is single-stranded specific, (c) a uracil glycosylase inhibitor, and (d) a nickase, which cleaves the nonedited DNA strand in the wobble of DNA duplex made by dCas9 (Komor et al., 2016). Unlike to majority of C•G to A•T editors, researchers have also developed adenine base editors (ABE) that can convert A•T to G•C base pairs and such activity has been initially proved in bacteria and human cells (Gaudelli et al., 2017). Using the base editors, Zong et al. (2017) have precisely converted cytosine to thymine and succeeded to achieve the mutations through base editors in maize, wheat, and rice (Zong et al., 2017). Similarly, agronomic important genes namely *NRT1.1B* and *SLR1* encoding nitrate transporter and Della protein was engineered by base editors in rice (Lu & Zhu, 2017). For relaxing the requirement of PAM sequence, a new ABE system using SpCas9-NGv1 has been developed wherein engineered SpCas9 recognizes NG as PAM and widening the range of targets (Negishi et al., 2019). This tool was successfully implemented to induce A•G base substitutions at target sites leading to bystander mutations in the rice genome (Negishi et al., 2019). Nevertheless, a few reports indicate that cytosine base editors (CBE) caused mutations in several off-targets in mammals. A study revealed that CBE using rat APOBEC1 enzyme can cause extensive cytosine deamination in the genome as identified by transcriptome analysis in human cells showing that tens of thousands of C changed to U in the transcript with varying frequencies (Grunewald et al., 2019). Based on these reports, it is amenable that researchers need to be cautious about the frequent use of base editors either in diagnostics or in other applications until the development of engineered or advance variants with reduced off-targets of deaminase activity. To increase the diversity of base editing, cytidine deaminase with adenosine deaminase

were combined with nCas9 and uracil DNA glycosylase inhibitor to achieve C:G>T:A and A:T>G:C simultaneously (Li, Zhang et al., 2020). This system is referred as saturated targeted endogenous mutagenesis editors (STEMEs). It is feasible to generate diverse mutations, including base substitutions and in-frame indels using STEMEs, which provide additional benefit for analysis of protein function and accelerate the development of novel agronomic traits. In a nice report, herbicide-tolerant rice germplasm was created using a base editing-mediated gene evolution approach by targeting *OsALS1* gene (Kuang et al., 2020). The translational application of the base editing system is remarkable in the development of low amylose rice cultivar by tuning *Wx* gene expression *via* targeting the promoter region (Xu et al., 2020). It should be noted that localized mutations can be induced without any break in the genome with base editors, making it a popular tool; however, there is still a need for improvements in this tool in order to the large application for crop improvement.

5.6 | Cell type-specific and conditional mutation

The word "mutant" refers to the complete *in planta* loss of function of a specific gene. There is a lack of a system to create a somatic mutant when one wishes to abolish a gene function in certain cell files and tissues. A study reported that a large number of genes (around 10%) present in the Arabidopsis genome are indispensable, indicating functional study of these genes is not possible *via* conventional loss of function mutant analysis (Lloyd et al., 2015). Keeping this in mind, a report described a method known as CRISPR tissue-specific knock-out (CRISPR-TSKO) system in which cell/tissue-specific somatic mutation can be obtained by using a cell/tissue-specific promoter for driving *Cas9* transcription in Arabidopsis. Such kind of improvements in the CRISPR/Cas9 system provides unprecedented flexibility to the researchers and facilitating the diverse use of this system in plant research (Decaestecker et al., 2019). Continuing to the previous approach, investigation of gene function is greatly relying on phenotypic observation of loss of function mutants. For this purpose, chemical, radiation and T-DNA insertional, and now CRISPR, mutants are largely being used, even though it is not possible to use null mutants for the study of a gene, which is not viable and showing pleiotropic developmental defects. There is a list of such crucial genes in the Arabidopsis genome whose functions cannot be studied due to the nonviable nature of the null mutant. Dealing with this, researchers have recently developed a CRISPR/Cas9-based tool for generating inducible and conditional mutants in somatic cells, which seems to be very helpful for investigating the role of nonviable genes in the plant (Wang, Ye, et al., 2020).

5.7 | Novel use of CRISPR/Cas9: Beyond simple cut and repair

Besides using the CRISPR/Cas9 system for above-mentioned gene editing, reports indicate the novel and diverse use of this system, including fluorescence tagging of endogenously expressing the protein. By employing the CRISPR/Cas9, HDR-mediated DNA knock-in approach, Wang et al. (2017) demonstrated in-frame fusion protein of *actin 1* (Os03g50885) and *glutathione S-transferase* (Os05g02530) can be produced in rice (Wang et al., 2017). In the same context, Miki et al. (2018) reported that it is feasible to achieved precise knock-ins mutant, especially in-frame fusion proteins and amino acid substitutions. They have successfully employed the CRISPR/Cas9 HDR method for generating N-terminal as well as C-terminal reporter tagging of targeted genes such as ROS1-GFP, ROS1-Luc, DME-GFP, and GFP-DME fusions in Arabidopsis and transgenic lines, strongly showing the respective fluorescence upon illumination (Miki et al., 2018). The application of site-directed in-frame fusion proteins seems to apply for transcriptional regulation and protein localization for cell biology and studying mechanistic details of targeted genes. Nevertheless, the lack of PAM sequences at the appropriate sites is considered a major obstacle to the wide application of tagging of targeted genes. In a recent study, Sharma, Badola, Bhatia, Sharma and Trivedi (2020) have shown that editing of miR858a and miRNA-encoded peptide can lead to enhance flavonoid biosynthesis and plant growth and development in Arabidopsis (Sharma et al. 2020).

By utilizing the property of precise targeting by sgRNA guided Cas9 protein, researchers have developed a system for the transcriptional activation of genes without creating gene edits using inactivated Cas9 protein referred to as dCas9. For this, a vector was constructed, which contains dCas9 fused with transcriptional activator proteins such as VP64 and p65AD at C-terminal along with the gRNA module. Lowder et al. (2015) developed the pco-dCas9-VP64 system and significantly activated the AtPAP1 (2–7 fold) and miR319 (3- and 7.5-fold) compared with control Arabidopsis plants suggesting that this system is sufficient to increase the transcripts of coding as well noncoding genes (Lowder et al., 2015). An interesting feature of a variant of native Cas9 protein called RNA processing Cas9 (R-Cas9) is that it recognizes and cuts the target RNA molecules enabling researchers to achieve post-transcriptional silencing of desired mRNA using the CRISPR/RCas9 system. It may be envisioned that the RCas9-mediated gene silencing could be applied in a range of applications including from manipulation of RNA substrates to tracking of RNA molecules in live cells by fusing a fluorophore with dead RCas9 that is very much helpful to understand the transcriptional dynamics within the cells (Nelles et al., 2015; O'Connell et al., 2014).

6 | GENE EDITING AND IMPROVEMENT OF CEREALS AND HORTICULTURAL CROPS

The application of the CRISPR/Cas technology accelerated trait modification, precision breeding, and domestication of food crops. In this section, we describe the major accomplishments of this technique in food crops and illustrated in Figure 3 and Table 1.

6.1 | Cereals

6.1.1 | Rice

Rice is an important cereal consumed by the large human population of the world, especially in the East-Asian region. As per data from the United Nations Food and Agriculture Organization in the year 2016, rice is the third most widely cultivated crop after maize and sugarcane. In the recent past, numerous studies have performed using a CRISPR/Cas system to ensure food security by developing edited rice with increase yield, reduced susceptibility toward pathogens, high nutraceutical value, and improved quality. The bacterial blight, caused by *Xanthomonas oryzae* pv. *oryzae*, is one of the most serious concerns for rice growers in Asia and Africa. The past research has revealed that bacteria secrete some transcription

FIGURE 3 Summary of major accomplishments of CRISPR/Cas9 for ensuring food security, improving quality and nutraceutical value of crops and fruits

activator-like effectors biomolecules that specifically bind to the promoter of sucrose transporter genes (*SWEET11*, *SWEET13*, and *SWEET14*) in the host. The simultaneous mutation in the promoter region of *SWEET* genes by CRISPR/Cas9 hindered the binding with transcription activator-like effectors, which conferred the resistance to bacterial infection and thus restricting bacterial invasion (Eom et al., 2019; Oliva et al., 2019). Under the field trials, such edited rice varieties in IR64 and Ciherang-Sub1 background exhibited a robust and broad-spectrum bacterial resistance in rice (Oliva et al., 2019). Using the same approach, a bacterial blight-resistant variety of rice was generated via editing of promoter elements of *Xa13* gene, resulting in excess mRNA or protein accumulation of downstream gene (Li, Li, Zhou, et al., 2020). Harnessing the knowledge of the promoter database of SWEET, transcripts, and protein profile of SWEET in engineered reporter lines and knockout lines of rice, a valuable diagnostic kit has been devised for effector binding element mutations efficacy assessment and software for prediction of suitable resistance gene-set of blight resistance in the particular geographic region in rice (Eom et al., 2019). The blast disease has a devastating effect on rice productivity and a report showed that mutating *OsERF922* enabled rice to cope better against blast disease (Wang et al., 2016). Likewise, a study demonstrated that tungro disease susceptible rice variety (IR64) can be converted into tolerant cultivar through gene edits in the *OseIF4G* gene associated with rice tungro spherical virus (Macovei et al., 2018).

The crop productivity and yield are decided by complex signal networks composed of intersection of transcription factors, genes, and plant hormones. Among major determinants of yield, poor grain filling in hybrid *japonica* rice is thought to be the main bottleneck restricting the wide cultivation of hybrid rice in grower countries. The characterization of an allele for grain filling rate (GFR1) by using CRISPR/Cas9 gene editing can be a molecular asset for improving the grain-filling rate in the hybrid *japonica* rice (Liu, Zeng, et al., 2019). A similar example of gene editing is seen in the case of AH2 locus characterization, which encodes a MYB transcription factor playing an important role in the determination of hull development, palea identity, and grain size in rice (Ren et al., 2019). Moreover, CRISPR/Cas9 gene editing is widely used to identify the causal genes in major quantitative trait loci. Employing CRISPR/Cas9, a novel rice grain size gene *OsSNB* was identified in a genome-wide association study in the natural population of rice. It has been proposed that *OsSNB* will serve as a key target for yield improvement in rice (Ma et al., 2019). In a detail investigation, Huang et al. (2018) have identified 129 putative genes related to high yield from 30 rice cultivars using IR8 (as a landmark), its ancestors and descendants. Among these, 57 genes were mutated through the CRISPR/Cas9 system to know their function. The analysis suggested that a locus Os10g0555100, encoding glucosyltransferase,
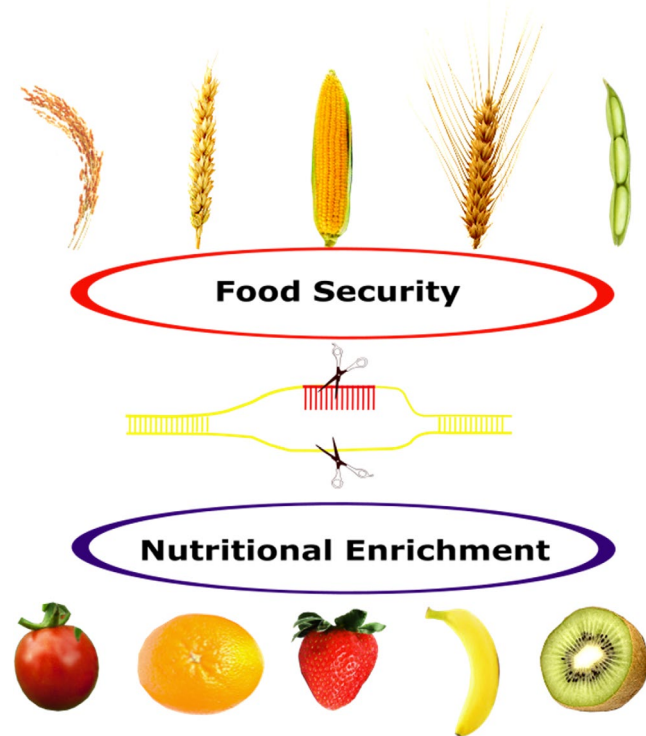
TABLE 1 Major accomplishments of CRISPR/Cas system-based gene editing in important crops

S.N.	Crop	Target	Mutation	Trait modification	References
1.	Rice	*OsSWEETs*	*cis*-regulatory alleles	Blight resistance caused by *Xanthomonas oryzae* pv. *Oryzae*	Oliva et al. (2019)
2.	Rice	*OsSWEETs*	*cis*-regulatory alleles	Bacterial blight resistance and diagnostic kit to check bacterial blight resistance	Eom et al. (2019)
3.	Rice	*OsXa13*	*cis*-regulatory alleles	Bacterial blight resistance	Li, Li, Zhou, et al. (2020)
4.	Rice	*OsERF922*	Loss of function	Blast resistance	Wang et al. (2016)
5.	Rice	*OseIF4G*	Loss of function	Tolerance for tungro spherical virus	Macovei et al. (2018)
6.	Rice	*OsGFR1*	Loss of function	High grain filling in japonica hybrid	Liu, Zeng et al. (2019)
7.	Rice	*OsAH2*	Loss of function	Improved hull development and grain size	Ren et al. (2019)
8.	Rice	*OsSNB*	Loss of function	Novel allele for high yield	Ma et al. (2019)
9.	Rice	*Os10g0555100*	Loss of function	Growth and panicle architecture	Huang et al. (2018)
10.	Rice	*OsAAP5*	Loss of function	High yield and tiller number	Wang, Wu, et al. (2019)
11.	Rice	Golden rice 1 & golden rice 2	Targeted gene insertion	High β-carotene in grains	Dong et al. (2020)
12.	Rice	*Cytochrome P450s* and *OsBADH2*	Loss of function	Improved fragrance in grains	Usman et al. (2020)
13.	Rice	*OsC3'H*	Loss of function	Improved biomass saccharification	Takeda et al. (2018)
14.	Rice	*OsMYB101*	Loss of function	Enhanced lignin accumulation	Miyamoto et al. (2019)
15.	Rice	*OsWaxy*	Loss of function	Low amylose content	Zhang et al. (2018)
16.	Rice	*OsWaxy*	*cis*-regulatory alleles	Low amylose content	Huang et al. (2020); Xu et al. (2020)
17.	Rice	*OsRR22*	Loss of function	High tolerance for salinity stress	Zhang, Liu, et al. (2019)
18.	Rice	*OsSAPK2*	Loss of function	High tolerance for drought stress	Lou et al. (2017)
19.	Rice	*OsNRAMP5*	Loss of function	High tolerance for cadmium stress	Tang et al. (2017)
20.	Rice	*OsALS1*	Loss of function	Herbicide tolerance	Kuang et al. (2020)
21.	Maize	*ZmPhytoene synthase*	Loss of function	Gene-editing protocol optimization	Zhu et al. (2016)
22.	Maize		DMC1 promoter-driven Cas9	Gene-editing protocol optimization	Feng et al. (2018)
23.	Maize		Biolistic delivery of RNP complex	Gene-editing protocol optimization	Svitashev et al. (2016)
24.	Maize	*GOS2* promoter	GOS2 promoter inserted within 5'UTR region of *ZmARGOS8*	High yield under drought condition	Shi et al. (2017)
25.	Maize	*ZmSH2* & *WX*	Loss of function	Super sweet and waxy corn	Dong et al. (2019)

(Continues)

TABLE 1 (Continued)

S.N.	Crop	Target	Mutation	Trait modification	References
26.	Maize		Haploid induction	Protocol optimization for one step genome editing in crops	Kelliher et al. (2019)
27.	Maize	Multiple genes	Loss of function	Characterized novel genes for agronomic and nutritional importance in maize	Liu et al. (2020)
28.	Wheat	*TaPDS*	Loss of function	Gene-editing protocol optimization	Upadhyay et al. (2013)
29.	Wheat	*TaMLO*	Loss of function	Resistance to powdery mildew	Wang et al. (2014)
30.	Wheat	*TaEDR1*	Loss of function	Resistance to powdery mildew	Zhang et al. (2017)
31.	Wheat	*Taα-gliadin*	Loss of function	Less gluten content in wheat grain	Sanchez-Leon et al. (2018)
32.	Wheat	*TaDREB2 & TaERF3*	Loss of function	Successful desired mutation attained in protoplast	Kim et al. (2018)
33.	Wheat	*TaMS1*	Frameshift mutation	Generated male-sterile lines useful in hybrid production	Okada et al. (2019)
34.	Wheat			Protocol optimization for delivery of RNP complex	Hayta et al. (2019)
35.	Wheat	*TaALS*	Loss of function	Herbicide (sulfonylurea and imidazolinone) tolerance	Zhang, Liu, et al. (2019)
36.	Wheat	*TaGW7*	Loss of function	Enhanced weight and shape of wheat grain	Wang, Pan, et al. (2019)
37.	Barley	*HvCKX1 & HvCKX3*	Loss of function	Altered root morphology and cytokinin metabolism	Gasparis et al. (2019)
38.	Barley	*Hv d-Hordein*	Loss of function	Increased starch content, amylose content, and beta-glucan content	Yang et al. (2020)
39.	Barley	*HvHPT & HvHGGT*	Loss of function	Significant reduction content of both tocopherols and tocotrienols	Zeng et al. (2020)
40.	Banana	*MaRAS-PDS1,2 & MaPDS*	Loss of function	Protocol optimization for gene editing	Kaur et al. (2018); Naim et al. (2018)
41.	Banana	*MaGA20ox2*	Loss of function	Development of semi-dwarf banana cultivar	Shao et al. (2020)
42.	Banana	*MaeBSV*	Loss of function	Resistance to banana streak virus	Tripathi et al. (2019)
43.	Banana	*MaLCYepsilon*	Loss of function	Fortification of β-carotene in banana fruit	Kaur et al. (2020)
44.	Tomato	Multiple genes	*cis*-regulatory alleles	Fruit size, inflorescence branching and plant architecture	Rodriguez-Leal et al. (2017)
45.	Tomato	*SlCLV3 &Arabinosyl-transferase*	*cis*-regulatory alleles	Extra flower and increased fruit size	Xu et al. (2015)
46.	Tomato	*Pectate lyase, Polygalacturonase 2a & β-galactanase*	Loss of function	Longer fruit shelf life	Wang, Samsulrizal, et al. (2019)
47.	Tomato	*SlFUL1 & FUL2*	Loss of function	Evaluated role in fruit ripening	Wang, Tavano, et al. (2019)
48.	Tomato	*SlALC*	Gene replacement	Longer fruit shelf life	Yu et al. (2017)

TABLE 1 (Continued)

S.N.	Crop	Target	Mutation	Trait modification	References
49.	Tomato	*SP, Ovate, Fasciated, Fruit Weight 2.2, Multiflora, Lycopene Beta Cyclase*	*cis*-regulatory alleles	Domestication of wild relative of tomato with increase fruit size, number and lycopene	Zsogon et al. (2018)
50.	Tomato	*SP, SP5G, SlCLV3, SlWUS, & SlGGP1*	*cis*-regulatory alleles	Increase fruit production, ascorbic acid synthesis and salt resistance	Li et al. (2018)
51.	Tomato	*SlENO*	Loss of function	Enhanced fruit number and size	Yuste-Lisbona et al. (2020)
52.	Tomato	Multiple genes	*cis*-regulatory alleles	Developed cultivars suitable for urban farming	Kwon et al. (2020)
53.	Citrus	*CsLOBs*	*cis*-regulatory alleles and loss of function	Resistance to citrus canker disease	Jia et al. (2016, 2017)
54.	Citrus	*CsLOB1*	*cis*-regulatory alleles	Resistance to citrus canker disease	Peng et al. (2017)
55.	Apple	*MdPDS*	Loss of function	Protocol optimization for efficient gene editing	Nishitani et al. (2016)
56.	Apple	*MdDIPM4*	Loss of function	Resistance to fire blight disease	Pompili et al. (2020)
57.	Grapes		Loss of function	Protocol optimization for efficient gene editing	Sunitha and Rock (2020); Wang, Tu, et al. (2018); Osakabe et al. (2018)
58.	Kiwi fruit	*AcPDS*	Loss of function	Efficient protocol optimization for gene editing	Wang, Wang, et al. (2018)
59.	Kiwi fruit	*AcCENs*	Loss of function	Transformation from perennial to annual	Varkonyi-Gasic et al. (2019)
60.	Soybean	*GmPRR3b*	Loss of function	Early flowering and high growth	Li, Li, Li, et al. (2020)
61.	Soybean	*GmPRR37*	Loss of function	Development of cultivar suitable to grow at high altitudes	Wang, Sun, et al. (2020)
62.	Soybean	*GmSPLs*	Loss of function	Change in plant architecture, increased node and branch number	Bao et al. (2019)
63.	Soybean	*GmFT2a*	Loss of function	Change in photoperiodism and delay in flowering time	Cai, Chen, Liu, et al. (2018)
64.	Potato	*StPPO*	Loss of function	Reduction in enzymatic browning of tubers	Gonzalez et al. (2019)
65.	Potato			Establishment of efficient protocol for gene editing	Andersson et al. (2017); Butler et al. (2020)
66.	Strawberry	*FaTM6*	Loss of function	Improvement in the quality of berry	Martin-Pizarro et al. (2019)
67.	Oilseed rape	*BnITPK*	Loss of function	Low phytic acid and increase in protein level in oil	Sashidhar et al. (2020)
68.	Oilseed rape	*BnSFAR4 & BnSFAR5*	Loss of function	Increased seed oil content	Karunarathna et al. (2020)

controls growth, and panicle architecture have much important agronomic potential (Huang et al., 2018). Thus, gene editing greatly helped to discover novel genes or alleles responsible for more productivity for instance, amino acid permease (OsAAP5) was found to regulate tiller number, grain quality, and yield (Wang, Wu, et al., 2019).

In addition to increasing crop yield, gene editing *via* CRISPR/Cas9 is largely used for improving desirable traits in rice. In an elegant study, carotenoid enriched rice variety was made, which accumulates high β-carotene content in the endosperm (Dong et al., 2020). In this study, a cassette containing golden rice 1 and golden rice 2, consisting of SSU-crtI and ZmPsy30 driven by the endosperm-specific glutelin promoter, was precisely inserted through CRISPR/Cas9 based targeted insertion and engineered plant have high β-carotene without any penalty in yield and growth. Developing golden rice via genome editing is a welcome step to combat vitamin A deficiency-related diseases among large populations inhabiting rice grower countries. Very recently, researchers succeeded in generating high yielding fragrance rice variety by a targeted mutation in cytochrome P450s and *OsBADH2* (Usman et al., 2020). Further, lignin represents a source of some aromatic compounds as well as determining the plant biomass. The mutation in p-coumaroyl ester 3-hydroxylase, a key enzyme involved in the lignin biosynthesis, caused variation in lignin content, composition, cross-linking of the cell wall and improves biomass saccharification in rice (Takeda et al., 2018).

Nevertheless, CRISPR/Cas9 induced knockout mutation in *OsMYB101*, a transcriptional repressor of lignin biosynthesis, enhanced lignin accumulation in culm cell walls, which is a much-needed character (Miyamoto et al., 2019). Moreover, the presence of amylose content (AC) in rice is not considered good because it creates problems during cooking. Therefore, breeders need to develop a variety with low AC in rice grain. Keeping this fact, a study demonstrated that editing the waxy (WX) gene by CRISPR/Cas9 significantly reduced the AC level, improving the quality of rice (Zhang et al., 2018). The targeting of the motif in the promoter region is a bit different strategy to alter gene expressions over conventional mutagenesis. This strategy was adopted to lower AC in rice grains by using CRISPR/Cas9 (Huang et al., 2020) and base editors (Xu et al., 2020) in which they have created multiple alleles of *Wx* with a range of AC in grain. Moreover, several research groups have attempted mutagenesis in genes involved in salinity stress (*OsRR22*), drought stress (*OsSAPK2*) and cadmium stress (OsNramp5), resulting edited lines exhibited better tolerance to respective stresses (Lou et al., 2017; Tang et al., 2017; Zhang, Liu, et al., 2019). One of the notable accomplishments of current gene-editing efforts in rice is the generation of rice germplasm with herbicide-tolerant features, which minimize the overall cost of rice cultivation (Kuang et al., 2020).

6.1.2 | Maize

Maize, a member of the grass family Poaceae, is one of the major cereals producing worldwide. Besides serving as a staple crop in some parts of the world, maize can also be used in some additional applications including corn ethanol, production of corn syrup, and fodder for livestock. The genetic improvement of maize is largely relying on traditional and mutation breeding programs before the era of transgenesis and precision gene editing. Like crops, Agrobacterium-mediated transformation is the most reliable way of delivering the CRISPR/Cas9 system for any gene-editing attempt in maize. However, the recalcitrant nature of calli and low transformation efficiency is thought to be a major bottleneck limiting the success of large editing events in maize. Overcoming this limitation, Zhang, Zhang, et al. (2019) developed a novel ternary vector module in which they have introduced the morphogenic regulator gene in the CRISPR/Cas9 system. The *Agrobacterium*-mediated delivery of this ternary vector system is promising and showed an additive effect in terms of improved transformation efficiency of recalcitrant maize (Zhang, Zhang, et al., 2019). Moreover, several studies have been conducted to devise a strategy for getting gene-editing events using CRISPR/Cas9 in maize for instance, maize codon-optimized Cas9 protein proved to be very effective for targeting mutation in the phytoene synthase gene (Zhu et al., 2016). The use of *dmc1* promoter-driven *Cas9* (Feng et al., 2018) and direct delivery of CRISPR/Cas9 ribonucleoprotein complexes (Svitashev et al., 2016) were further improvements for robust genome editing in maize. The development of maize variety with better grain yield under drought conditions is a notable achievement of gene editing. Based on previous information on *ARGOS8*, a report has shown that precise insertion of maize *GOS2* promoter within 5′-UTR of *ARGOS8* increased *ARGOS8* transcripts and thereby more grain yield per acre obtained under drought condition at the flowering stage (Shi et al., 2017). In a consumer-driven market, the demand for sweet, waxy, and baby corn variety is increasing day by day. Keeping this fact in mind, Dong et al. (2019) have developed super sweet and waxy corn variety by mutating *SH2* and *WX* genes involved in starch and sugar metabolism (Dong et al., 2019). An exciting study opens a new horizon of trait modifications in crops such as maize by showing one step genome editing can be possible during haploid induction, suggesting that gene-editing attempts will rise in maize in the near future (Kelliher et al., 2019). In a comprehensive study, Liu et al. (2020) have used large scale CRISPR/Cas9 mutagenesis with genetic mapping to validate the function of underlying genes in QTL. By integrating gene-editing tools with high-throughput genomic approaches, the role of 743 candidate genes was explored determining the traits for agronomic and nutritional importance in maize (Liu et al., 2020). The collective knowledge of this study and generated mutants will serve as useful resources for accelerating high precision breeding program in maize.

6.1.3 | Wheat and barley

Wheat is the second most staple food crop in the world. Like other crops, natural available genetic diversity is conventionally being used for introgression of a specific trait in suitable/

cultivated wheat variety. Nevertheless, a number of reports were published during the recent past years showing the usefulness of the CRISPR/Cas9 system to generate the allelic mutation in wheat. Despite the difficulty in the *Agrobacterium*-mediated efficient transformation method, the presence of three genomes in wheat (*Triticum aestivum* L., $2n = 42$, AABBDD) is another great challenge to deal with during gene-editing efforts in wheat (Slade et al., 2005). However, Upadhyay et al. (2013) have shown that the CRISPR/Cas9 system can be applied for gene editing in wheat by obtaining target gene mutations in cell suspension culture of wheat (Upadhyay et al., 2013). Thereafter, researchers were succeeded in targeting three putative *MLO* loci, which encode proteins to repress defense against powdery mildew disease and developed resistant wheat variety (Wang et al., 2014). The resulted lines displayed notable disease resistance against powdery mildew and providing a methodological framework to change alleles in hexaploid wheat (Wang et al., 2014).

Similarly, a study showing that targeting *TaEDR1,* a negative regulator of defense response against powdery mildew, with CRISPR/Cas9 exhibited resistance to powdery mildew disease, which provides a new allele for molecular breeding in wheat (Zhang et al., 2017). It is noteworthy to mention here that powdery mildew disease is prevalent in wheat and causes significant yield loss (approaches to 40% reduction in yield under severe condition). Another big issue of wheat is the presence of gluten causing celiac disease, an autoimmune disorder, in some individuals. The following research illustrated that casual immunogenic peptide in patients with celiac disease is synthesized by a member of the gliadin gene family of wheat. The CRISPR/Cas9-based targeting of α-gliadins resulted in a strong reduction in α-gliadins level in wheat grain, which makes it safe to consume by patients facing celiac disease (Sanchez-Leon et al., 2018). Numerous successful attempts have been made to edit the drought and other stress-related genes, namely *TaDREB2* and *TaERF3,* in a transient systems like in protoplasts, suggesting that it can be used as a rapid system to know the actual targets and off-targets of designed gRNAs in wheat (Kim et al., 2018). Interestingly, a study developed a platform for producing male-sterile wheat variety using the CRISPR/Cas9 system. It should be noted there is a high demand for male-sterile plant in the hybrid seed technology industry, which is considered a difficult task due to the strong inbreeding habit of wheat. Okada et al. (2019) generated a male-sterile wheat by targeting *Ms1* gene consequence biallelic frameshift mutation into *Ms1* caused complete male sterility in wheat cultivars such as Fielder and Gladius (Okada et al., 2019). This study provides a significant step to capture the concept of heterosis to improve the yield and fitness in elite wheat cultivars. In the same connection, some recent reports describing the protocol of efficient development of an Agrobacterium-mediated delivery of CRISPR/Cas9 RNP for wheat genome editing are now available (Hayta et al., 2019).

In an exciting report, the herbicide tolerance was developed in wheat by introducing a mutation in *acetolactate synthase* and *acetyl-coenzyme A carboxylase* genes using base editors (Zhang, Liu, Chai et al., 2019). The presence of weeds in the field greatly reduces wheat grain yield and such mutant variety is tolerant to sulfonylurea, imidazolinone and aryl-oxyphenoxy propionate type herbicides providing an edge for weed management in wheat farming. In addition to protecting crops from pathogens and weed, genome editing helped to raise mutants with high grain productivity per plant in wheat. Wang, Pan, et al. (2019) reported mutation in *TaGW7* homoeologs in the B and D genome of hexaploid wheat increased the shape and weight of wheat grains in a dosage-dependent manner suggesting an important role of *TaGW7* for deciding yield component during domestication of wheat (Wang, Pan, et al., 2019). Considering the vast application of the CRISPR/Cas9 system and speeding the traits improvement in wheat, researchers have devised a web-based tool, WheatCRISPR, for designing precise sgRNAs. We anticipate exciting and novel implications of gene editing will coming soon in wheat (Cram et al., 2019). Similar to wheat, gene-editing tools, especially CRISPR/Cas9 has been applied in important cereal crop barley (*Hordeum vulgare*) to change cytokinin metabolism (Gasparis et al., 2019) and grain composition (Yang et al., 2020). Using the CRISPR/Cas9 system, Zeng et al. (2020) created mutants of *HvHPT* and *HvHGGT* for tocopherol (vitamin E) enrichment in barley grain; however, loss of function of these genes reduced both tocopherols and tocotrienols level (Zeng et al., 2020).

6.2 | Horticultural crops

6.2.1 | Banana

Banana, the most economically important tropical fruit, is growing in more than 130 countries of the world. In banana plantations, most of the high yielding varieties are longer than 2 m and facing the challenge of weak lodging. Thus, a short height of the plant is thought to be a favorable trait in banana farming, which is also suitable for machine harvesting of fruits and plant maintenance. It should also be noted that the scope of traditional breeding for the traits improvement is considered as difficult in banana as it has a triploid genome that leads to plant sterility; thus, gene editing can be a viable alternative for genetic modification (Dash & Rai, 2016). In the beginning, genome editing studies in banana using CRISPR/Cas9 were reported after getting success in mutations of two RAS-PDS genes in banana cv. Rasthali (Kaur et al., 2018) and *MaPDS* gene in the Cavendish cultivar (Naim et al., 2018). Further, the gibberellin biosynthesis gene, *MaGA20ox2*, was successfully edited by using the CRISPR/Cas9 system in "Gros Michel" cultivar resulting in a semi-dwarf mutant cultivar (Shao et al., 2020). Similarly,

researchers were able to edit sequences of endogenous banana streak virus in the B genome of banana to inactivate virus transfer during breeding events (Tripathi et al., 2019). These initial reports open a new avenue for gene-editing attempts in banana. In a recent report, the β-carotene-enriched banana was developed by CRISPR/Cas9-based editing of lycopene epsilon-cyclase gene in the Cavendish banana cultivar (Kaur et al., 2020). In addition to generating material for breeding in banana, our group is trying to develop banana cultivar using the CRISPR/Cas9 tool with the core aim for the nutritional enhancement of banana fruit in high yielding and lower nutrient containing popular cultivar in India.

6.2.2 | Tomato

Tomato is an essential edible crop, and fruit of the plant is widely consumed in various ways. Like other crops, there is also a need to improve the traits like high productivity, large fruit size, better quality with least postharvest loss, and flavor in tomato. In an elegant study, Rodriguez-Leal et al. (2017) have developed a number of alleles that differed in fruit size, branching pattern, and inflorescence in tomato by targeting promoter regions of the crucial genes regulating fruit size, inflorescence branching, and plant architecture. The authors created a wide range of *cis*-regulatory alleles that differed by alteration of gene expression that eventually determined large quantitative phenotypic variation in tomato (Rodriguez-Leal et al., 2017). In addition, CRISPR/Cas9 technology was used to investigate the arabinosyl-transferase gene function as new components of the *CLAVATA* signaling pathway essential to control meristem size in tomato (Xu et al., 2015). The shelf life of a fruit is a very important trait and studies have shown that remodeling of cell wall plays a bigger role in ripening and shelf life that determine the life spawn of tomato fruit, which ultimately reduces the postharvest loss of crop. In the same line, substantial work has been performed to decipher the role of cell wall-modifying enzymes such as pectate lyase, polygalacturonase 2a, and β-galactanase using CRISPR/Cas9 to illustrate their role in fruit ripening (Wang, Samsulrizal, et al., 2019). A similar attempt was made to know and confirm the previously investigated ripening-related transcription factors, Fruitful 1 and 2, provide an insight about the independent and overlapping function of these regulators in controlling the ripening process in Alisa-craig variety (Wang, Tavano, et al., 2019). Similarly, Yu et al. (2017) have shown that CRISPR/Cas9-induced mutagenesis and gene replacement of normal ALC allele generated the long-shelf-life tomato (Yu et al., 2017). An interesting report came out recently in which Zsogon et al. (2018) were able to develop a new tomato variety from wild landrace *S. pimpinellifolium* by editing six loci important for yield and productivity using the CRISPR/Cas9 tool. This study demonstrated

that engineered lines contain very high amounts of lycopene (500%), ten times more fruit number and three times increase in fruit size compared to wild-type *S. pimpinellifolium* (Zsogon et al., 2018). This report opens a new avenue for a molecular breeding programs to exploit the available genetic diversity of wild relatives of cultivated crops. The inbreeding is widely used for crop improvement; however, is often associated with fitness penalties and loss of genetic diversity. It has been found that wild relative of tomato (*S. pimpinellifolium*) harbors significant salt and disease tolerance trait and introgression of such characters in cultivated tomato (*Solanum lycopersicum*) via conventional breeding is a slow process. Hence, using multiplex CRISPR/Cas9 based editing of coding sequences, promoters or upstream open reading frames of putative genes linked with fruit size, architecture, and ascorbic acid synthesis, Li et al. (2018) successfully modified desired phenotypes along with parental disease resistance and salt tolerance in *S. pimpinellifolium*. The edited lines displayed a change in day-length sensitivity, shoot architecture, fruit production, and nutrient composition which finally accelerated the domestication of *S. pimpinellifolium* (Li et al., 2018). During the domestication of tomato, fruit size and locule number are the major traits. With the help of CRISPR/Cas9, characterization of *ENO* gene in determining fruit locule number and size seems to be a milestone in the molecular breeding program of improving tomato varieties (Yuste-Lisbona et al., 2020). In an excellent study, Kwon et al. (2020) have shown the potential use of CRISPR/Cas9 to generate a repertoire of solanaceous crops for urban agriculture. Authors demonstrated that a vine-like wild tomato variety can be converted into compact architecture and early yielding variety using single-step CRISPR/Cas9, which is suitable for urban farming (Kwon et al., 2020).

6.2.3 | Citrus

Citrus is an important fruit representative of the family Rutaceae, and one of the rich sources of vitamin C. In the plantations, citrus canker (caused by *Xanthomonas citri* subsp. citri) is a major disease affecting the production and quality of citrus fruits. The genetic modification for the improvement of citrus through breeding or gene manipulation has several challenges. Studies have identified CsLOB1 as a candidate gene for controlling susceptibility to citrus canker of sweet orange by bacterium *Xanthomonas axonopodis* (*Citrus X sinensis*) and editing of *CsLOB1* gene resulted in varying resistance responses among stably transformed edited lines (Jia et al., 2016, 2017). Similarly, another report showed that it could be possible to obtain edited lines having resistance to citrus canker by targeting the promoter region of *CsLOB1* (Peng et al., 2017). These studies illustrate that the devastating effect of citrus canker can be managed through

CRISPR/Cas9-based editing of responsible genes associated with this trait.

6.2.4 | Apple and grapes

The successful application of gene-editing tools in important crops encourages researchers to attempt for improving apple, grapes and commercial fruits. As we know, sequence information and delivery of CRISPR/Cas complex is a prerequisite for any gene-editing efforts and later is thought to be the most difficult task. Considering this limitation, studies have first established the protocols for direct delivery of CRISPR/Cas9 ribonucleoproteins as well as by *agrobacterium*-mediated transformation to achieve efficient DNA-free targeted mutations in both apple and grapevine (Nakajima et al., 2017; Nishitani et al., 2016; Osakabe et al., 2018). Afterward, a fire blight disease tolerant edited cultivar of apple was developed by making knockout of *MdDIPM4* which interacted with the effector protein of bacteria (Pompili et al., 2020). Regarding the grapes, some studies reported the highly efficient CRISPR/Cas9 gene-editing events in grapes which mostly focussed on method development and functional analysis of genes (Sunitha & Rock, 2020; Wang, Tu, et al., 2018); however, using this tool result related to the improved variety of grapes is yet to come.

6.2.5 | Kiwifruit

Kiwifruit (*Actinidia chinensis*), an edible berry with sweet and unique flavor, is commonly cultivated in China, Italy, New Zealand, and Iran. The gene-editing attempts have been carried out once the optimization of the conditions was succeeded in kiwifruit. A robust system for gene-editing using polycistronic tRNA-sgRNA cassette rather than traditional CRISPR/Cas9 have been devised which increased mutagenesis frequency nearly 10-fold higher in the case of phytoene desaturase gene in kiwifruit (Wang, Wang, et al., 2018). A year later, a study reported that mutation of CEN-like genes *AcCEN4* and *AcCEN* through CRISPR/Cas9 transformed a climbing woody perennial kiwifruit, which takes many years to develop axillary inflorescences and fruit, into a compact plant with terminal flower and fruit development within a year (Varkonyi-Gasic et al., 2019). The annualization of perennial horticultural crops is indeed a revolutionary accomplishment of gene editing in recent years.

6.3 | Other crops

Soybean is an important legume crop that is widely consumed as a raw vegetable, processed food, and soy milk

products. Several studies demonstrated the effectiveness of CRISPR/Cas9 to rapidly modify the crucial traits compared to conventional breeding. The genome-wide association study and analysis of a large number of QTLs mapping 279 landraces of soybean responsible for flowering and maturity time indicated possible loci shaping these traits. With the help of CRISPR/Cas9 gene editing, Li, Li, Li, et al. (2020) characterized a locus encoding *GmPRR3b* is the causal gene for regulating early flowering and vigorous growth. These characters are considered to be promising for developing a variety that can grow at high altitudes (Li, Li, Li, et al., 2020). However, another study demonstrated that it is *GmPRR37*, which controls the flowering time and photoperiodism, using gene editing and thus enabling soybean variety to grow in hill region (Wang, Sun, et al., 2020). Moreover, researchers have mutated four gene members of *SPL* family, which resulted in a significant change in plant architecture showing increased node number and branch number per plant of soybean (Bao et al., 2019). In the same context, editing of *GmFT2a* through CRISPR/Cas9-mediated mutagenesis resulted in a delay in flowering time and photoperiod responses in soybean (Cai, Chen, Liu, et al., 2018). All of these reports clearly indicated that fine-tuning of photoperiodism and flowering time is the key research focus for improving soybean. However, reducing bitterness caused by saponins and isoflavones and nutritional improvement by editing crucial genes will be a prime goal for soybean research in the near future.

The enzymatic browning, catalyzed by polyphenol oxidases leading to the formation of dark-colored precipitates, causes undesirable changes in taste and nutritional deterioration in potato tuber (*Solanum tuberosum*). A study reported that mutation in a member of the *StPPO* gene family is sufficient to reduce enzymatic browning of tubers and improved the nutritional quality of tubers (Gonzalez et al., 2019). In general, potato and some diploid relative of potato are normally recalcitrant to *Agrobacterium tumefaciens*-based transformation. Nevertheless, few recent studies have established a refined protocol of gene editing by the hairy root and protoplast transformation, which will increase the gene-editing efforts in potato (Andersson et al., 2017; Butler et al., 2020). The CRISPR/Cas9 system has also been implicated in strawberries for increasing the quality enhancement berries. Recently, researchers have used the CRISPR/Cas9 system for editing gene controlling the development of the floral parts for instance, petals, anthers, and pollen grains and demonstrated that *FaTM6* is involved in stamen development and berries size as well (Martin-Pizarro et al., 2019).

Being as a very important oilseed crop, *Brassica napus* is also widely used as a fodder for livestock. The CRISPR/Cas9 gene editing has been applied to improve the nutritional value of brassica in recent years. The phytic acid in oilseed rape is considered antinutritive, which hinders the availability of free phosphorus and adversely affects the mineral absorption

in the human gut. Sashidhar et al. (2020) developed a mutant of oilseed rape using CRISPR/Cas9, which contained low phytic acid and an increase in protein level in oil (Sashidhar et al., 2020). In another exciting report, researchers have generated CRISPR mutants of *BnSFAR4* and *BnSFAR5,* causing increased seed oil content by altering oil biosynthesis genes expression in oilseed rape (Karunarathna et al., 2020). These studies establish the ability of the gene-editing tools and extend a new perspective of improving yield and quality in major oilseed crops.

7 | GLOBAL POLICY FOR REGULATION OF EDITED CROPS

The advent of the CRISPR/Cas9 system resulted in unprecedented applications in diverse areas, thus boosting crop biotechnology by developing a number of genome-edited crops in the agriculture sector in recent years. Moreover, many of gene-edited varieties also serve as important genetic resources for introgression of a particular trait in the breeding program to make a superior variety. Considering that gene-edited crops are mostly free from any foreign DNA molecules developed to date, there are differences in a global consensus of regulation and acceptance of edited crops among policymakers. The release of edited crops is regulated under the policies framed for Genetically Modified Organisms (GMOs) in the European Union countries at present (Table 2). In contrast, regulators in

the USA agreed that there is no need for special oversight of the edited crops as genetic alterations have been brought in crops itself (Table 2). There is no foreign DNA introduced in the plant. Such a step was welcomed by agro-industries as well as in academia in the USA. By changing the existing policy, US Department of Agriculture (USDA) decided to deregulate a common white button (*Agaricus bisporus*) mushroom, which was created by the CRISPR/Cas9 tool under the framework devised for regulating GMOs. This mushroom was developed by targeting polyphenol oxidase gene which imparts resistance to browning after cutting and bruising (Waltz, 2016). In the same manner, Canada also considered gene editing as a fast version of traditional breeding. The policy in Japan strictly is known for regulating GM crops but Japan is now ready to adopt gene-edited crops and also promoting the scientific awareness programs among the public about gene-edited crops. Recently, regulators in Japan were convinced that since a particular gene is altered or disabled in CRISPR/Cas-based edited organisms, those are deferred from transgenic crops, thus allowing edited foodstuffs to be used by consumers without additional safety evaluations. However, the Japanese regulators directed that provided recommendations always have a possibility of further safety evaluations when sufficient details of edited crops or organisms are not given. Australian policymakers decided that it is unnecessary to regulate the gene-edited plants when NHEJ machinery repairs the break naturally. It was debated that there is no introduction of new genetic material; however, if editing tools

TABLE 2   Policies for regulation of gene-edited crops throughout the world

S.N.	Country	Regulated/ deregulated	Details	Remark, if any
1.	USA	Deregulated	There is no foreign DNA in edited crops hence edited crops cannot be considered as GM crops	
2.	Europe	Regulated like GMO	Gene-edited crops must have to go through assessment rules designed for the release GM crops	
3.	Australia	Deregulated	Edited crops allowed when NHEJ machinery repair the break naturally	Edited crops are regulated, if donor template or foreign genetic material inserted using editing tools
4.	Argentina, Brazil and Chile	Under existing GMO rules	Edited crops are deregulated if they have not any transgene	Case to case assessment of edited crops
5.	Japan	Deregulated		Edited crops can be subject of reassessment, if sufficient information is not provided
6.	Canada	Deregulated		Edited crops are considered as fast version of traditional breeding
7.	New Zealand	Regulated		
8.	India	Not defined		India has defined policy and framework to regulate GM crops, however, there is no guideline for the release of edited crops yet

using a donor template or insert other genetic material into the cell will continue to be regulated by the Office of the Gene Technology Regulator. Some countries of Latin America incorporated new additional rules for regulating gene-editing organisms in their current adopted biosafety rules and regulations. The regulatory framework of Argentina devised a procedure for a case to case assessment to determine whether edited crop will be a subject of regulation under GMO or not. The gene-edited crops will not be subject to GMO regulation if transgene is not inserted in the genome. The same rules are also followed by governments of Brazil and Chile. However, the European Court of Justice passed a judgment defining that since CRISPR/Cas mutagenesis brought alterations in genetic material that satisfies the definition of a GMO and thus should follow regulations of the GMO (Callaway, 2018). This decision led to the surge of protest among the scientific community in many European Union countries as there is growing consensus throughout the world that CRISPR-modified crops are at least as safe as traditionally made mutants. Having witnessed the CRISPR bonanza, the Russian government recently decided to spend huge investment on gene-editing programs aiming for the development of 10 new varieties of crops. A large number of research programs and activities are undergoing in government-funded research institutes as well as sponsored centers for the improvement of various crops in India at present. When new crop varieties come up by employing gene-editing tools under ongoing programs, there will be a need for policies and regulatory framework for the release of edited crops in India.

It is a need of an hour for the general public and policy-makers to understand the differences in fundamentals of gene editing and GM crops. The country like the United States is always known to adopt new technology including GM crops in a proactive manner, and readily allows the adoption of gene-edited crops in the agriculture sector. In a recent decision, Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) put gene-edited soybean, which has a high oleic low linolenic (HOLL), under the nonregulated article. After the verdict of the regulatory body, this gene-edited soybean will reach to consumer's hand very soon. This soybean is developed using the TALEN-based gene-editing technology by Calyxt Inc. Hence, it is amenable that countries supporting and funding the research programs aimed to develop better crop varieties by using gene-editing technology should also encourage educational and awareness campaigns to change public perception for the social acceptability of edited crops.

8 | CONCLUSIONS AND PROSPECTS

The CRISPR/Cas-driven genome editing opens a new dimension in plant research and leads to phenomenal success in

Box 2 Delivery of CRISPR/Cas module and RNPs in plant cell

The delivery of the CRISPR/Cas system relies mostly on Agrobacterium-mediated transformation in crops, which integrates entire transgene cassette into the host genome. A well-established transformation protocol is a prerequisite for Agrobacterium-mediated transformation, which is devised for a limited number of crops yet. Further, the chance of getting more off-targets is very high in case of stable integration of the CRISPR/Cas module in the genome. Moreover, the Agrobacterium method is unsuitable for vegetative propagating crops such as grapes, potato and banana. Woo et al. (2015) developed a novel alternative method in which preassembled Cas9 protein-gRNA RNPs was directly delivered into protoplast without inserting recombinant DNA in the host genome. In addition, the protoplast method can also serve as a promising strategy to assess the mutation rate and efficiency of the pool of sgRNAs. This method was applied to create gene editing in Arabidopsis, tobacco, rice, and lettuce, and reported that mutant alleles were successfully inherited to the next generation (Woo et al., 2015). Numerous reports illustrated a complete knockout mutation can be attained in various crops using the protoplast delivery method (Andersson et al., 2018; Park & Choe, 2019). However, it is challenging to obtain plantlets from the regeneration of protoplast in the case of recalcitrant monocots cereals such as maize, sorghum, wheat, and barley. Overcoming this, embryo cells of maize were targeted for delivering RNP complex using the particle bombardment method, which created DNA-free genome editing in maize (Svitashev et al., 2016). In further improvements, a novel ternary vector system has been made by integrating a morphogenic regulator in CRISPR/Cas9 modules, enhancing transformation efficiency in maize (Zhang, Zhang, et al., 2019). Recently, a plant negative-strand RNA virus-based vector has been engineered to make DNA-free *in planta* delivery of the CRISPR/Cas9 system (Ma et al., 2020). The viral vector is capable of inserting single and multiple mutations simultaneously and remains stable after the transmission of RNPs in tobacco cells. Concerning gene-editing efforts in crops, the development of haploid induction editing technology (HI-Edit) is a remarkable advancement in the precision mutagenesis enabling direct editing of elite inbred lines by a single cross (Kelliher et al., 2019). HI-Edit is faster and more efficient for creating edits in germplasm to be used as breeding materials than conventional trait introgression methods for crop improvement.

crop biotechnology quickly. The use of gene-editing allows efficient, precise, and targeted mutagenesis for increasing yield and nutritional value in crops comprising cereals, fruits, and horticultural crops. Such applications provide an edge to the current undergoing genetic engineering efforts for food security and fighting against the undernourishment of nutrients, vitamins, and nutraceuticals to ever-increasing global population. The availability of versatile CRISPR/Cas tools enabled to make any possible change in the genome. The discovery of additional bacterial enzymes, similar to Cas9 protein such as Ca12a, Cas13a, CasX, and CasY, offers much flexibilities of PAM sequence and minimal off-targets further widening applications in plant. The information of gene and genome sequence are prerequisites for gene-editing attempts in any organism. Progression of Next-Gen Sequencing technologies helped establish a whole-genome database of fruits and members of the Solanaceae, including tomato, potato, pepper, and eggplant in recent past. The vast use of gene-editing tools for improving the quality of these crops will be a next the step in the coming years to ensure their full potential for benefiting the society. Using gene editing, the targeted mutation can be achieved without an introduction of any foreign DNA thus edited crops may bypass legislative GMO regulations in many countries including, USA, Argentina, and Australia. However, unintended targets and genome integrity seem to be the main concern of a broad use of the CRISPR/Cas-based gene editing for improving crops in sustainable manner. The lack of transformation method and delivery of RNP complex in many crops often limit the pace of the gene-editing attempts in major crops (Box 2). Refinement in editing ability, development of novel tools, innovation in the delivery of CRISPR/Cas system, establishing transformation protocol, and easy identification of heritable mutants will be major prospects further advancing gene editing efforts in more commercial crops. Integrating these improvements, CRISPR/Cas gene editing will be the lucrative tool for developing new stress resilient varieties, alleles in precision breeding, better yielding crop, protection against pathogens, and enhancing nutritional value in future.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST

All authors declare no conflict of interest.

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