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A Review on Revolutionary Strategy for Crop Improvement: Genome Editing

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Authors' contributions

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Review Article

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ABSTRACT

Genome editing technology revolutionized crop improvement technology through sequence-specific, precise, site-directed, safe genetic manipulation and combat the major 21st century challenge such as achieving world food security meeting rising global food demand and improving food nutrition in the face of rapidly changing climate conditions. Crop improvement using conventional and molecular breeding approaches takes time, causing biosafety concerns and cannot equipoise with raising demand. Genome editing system like zinc finger nuclease (ZFN), transcription activator-like effector nucleases (TALEN), and clustered regularly interspaced short palindromic repeats (CRISPR) made a desirable targeted modification in crops for improving crop yield, nutraceutical quality and also enhance tolerance to environmental stress (biotic or abiotic) through add the desirable trait(s) and remove the undesirable. Genome manipulation tools progression creates new breakthroughs and speeds up crop improvement through site-directed mutagenesis efficiently for crop improvements to meet the ever-increasing global demand for food and produce more resilient crop with great flexibility to combat climate change.

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1. INTRODUCTION

In the 21st century world facing a major issue of the growing population their food requirement, malnutrition, climatic changes, and loss of biodiversity, that are pushing agricultural output [1]. "According to the estimate, the world population is expected to exceed 9 billion by 2050, and simultaneously agriculture will face huge challenges, requiring crops with higher yields and of improved quality, and needing fewer inputs" [2]. "Accordingly, the effective production of staple crops, such as rice, maize, wheat, and soybean will increase by just 38-67%" [3,4]. "Although traditional breeding and molecular breeding are the most widely used approaches recently in crop improvement it is labour intensive and it usually takes several years to develop from the early stages of screening phenotypes and genotypes to the first crosses into commercial varieties. In addition, genetic engineering (GE) has been the most common technology for the genetic improvement of crops since the early 1970s" [5]. But some unwanted effect restricts the use of genetically modified (GM) crops such as random insertion of foreign DNA, a new gene is placed instinctively anywhere in the genome, and may deactivate or disrupt the functioning of other genes or even cause severe undesirable effects. Additionally, GE technologies offer no degree of reproducibility, as there is no assurance that the new sequence will be introduced at the same place in two different cells because these insertions are random.

"Recently, genome editing has developed to overcome limitations of traditional and molecular breeding that use sequence-specific nucleases (SSNs) to introduce targeted mutations in crops with high precision and efficiency" [6]. "Genome editing is the process of making deliberate changes to specific DNA sequences using programmable nucleases. The engineered programmable nucleases in this case target and cleave DNA with greater specificity, precision, and efficiently" [7]. "This technology has been recognized for its precision and potential to remove undesirable traits while adding desirable traits by generating double-strand breaks (DSBs) to cleave the DNA sequence and repair the DSBs. These DSBs can be repaired in cells by homology-directed recombination (HDR) or non-homologous end joining (NHEJ) repair mechanisms, resulting in mutant lines with precise stable targeted mutagenesis. Genome editing produces modification through targeted point mutation that alters the reading frame and causes gene knockout, or site-directed insertion/deletion/substitution and chromosomal rearrangement, at specific sites in the genome of organisms" [8]. "Genome editing system mainly relies on DSB and repaired mechanism NHEJ or HDR in which various enzymatic mechanism has been used to join break end. In NHEJ, directly join the end of DSB without the prerequisite for a homologous repair template [9] and it happens in higher eukaryotes with low fidelity in the repair" [10]. "Error-prone nature, in NHEJ, causes the addition or deletion of nucleotides and that leads to DNA sequence changes at the targeted DSB sites. Sometimes NHEJ can cause complete gene function loss in many cases, as indels introduced into exons can result in missense or nonsense mutations. In most genome editing works NHEJ pathway is extensively used to knock out genes. While HDR has a lower efficiency as compared to NHEJ of genome editing because a homologous sequence serves as a template to repair the DSBs allowing an accurate repair, and this system is also used for the accurate gene replacement/insertion at target loci in the presence of an exogenously supplied donor DNA as a repair template".

"Genome editing efficiently use the intrinsic DNA repair machinery very precisely to edit the genome. All genome editing tools depend on the SSNs which make double-stranded breaks at the specific target locus" [11]. Basically, four site-specific epigenetic modifications tools are used to produce site-specific DSBs such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), clustered regularly interspaced short palindromic repeats/ CRISPR-associated protein 9 (CRISPR/Cas9) and CRISPR/ CRISPR from Prevotella and Francisella 1 (CRISPR/Cpf1) system that use sequence-specific nucleases (SSNs) composed of a DNA-binding domain to offer sequence specificity linked to a nuclease domain to introduce DNA strand breaks at the targeted sequence. Among them, CRISPR-Cas, in combination with modern breeding methods, will play a significant role in the remodelling of crops as the next-generation plant breeding technique.

2. MEGANUCLEASES

"Meganucleases (MegaN) are naturally occurring first class of endonucleases which and categorized by the presence of a broad recognition site of about 12–40 bp, which were discovered in the late 1980s and first to be used for genome editing" [12]. MegaN also known as homing endonucleases (HEs) as creating DSB at a specific site in a very precise manner [13]. "The nomenclature of the HEs is patterned after that of restriction enzymes" [14]. "Due to the large recognition site meganucleases are perfect tools for genome engineering, but the limited number of naturally occurring meganucleases is not sufficient to conceal all interesting loci. However, the construction of sequence-specific meganuclease for all possible sequences is costly, time-consuming, and problematic and additional efforts should be required to remodify meganucleases along with other genome-targeting techniques in different crops and it seems to be problematic, because other domains like DNA-binding domains are often combined with the catalytic domain and cannot be separated from one another".[9] Therefore, scientists concentrated on other gene editing techniques like ZFNs, TALENs, and CRISPR that were more effective, precise, and straightforward.

3. GENOME EDITING TOOLS AND PRINCIPLES

3.1 ZFNs

"ZFNs were one of the most effective first-generation genome editing tool which were first exploited to edit plant genomes in 2005" [15]. "ZFNs were chimerically engineered nucleases and generated by combining two domains 1) sequence-specific zinc finger DNA-binding domain and 2) nonspecific DNA cleavage domain derived from the type II restriction endonuclease *FokI"* [16]. "Each sequence-specific zinc finger DNA-binding domain recognizes 3-base pair (bp) DNA motif sequence [17,18], and four to six zinc fingers (ZFs) are used to make a single ZFN subunit that binds to the DNA sequences of 9–18 bp". "While the DNA cleavage domain is a nonspecific cleavage domain that induces a targeted DNA break cleaves 9/13 nucleotide (nt) downstream of the recognition site. It does not recognize any specific sequence at the site of cleavage" [19]. "Among two domain one DNA-binding domain recognized a specific site and bind then transfer signal to the cleavage domain through allosteric interactions, and cleavage occurs. To create DSB in DNA *FokI* nuclease must be dimerized so, it is compulsory to design a pair of ZFNs" [20]. For the dimerization of *FokI* required to attach to forward and reverse strands respectively and the two target sequences, forward and reverse, must be separated by a 5 to 7 bp spacer sequence.

Nevertheless, the assembly and design of ZFNs are complicated and technically challenging. While the outsourcing of the modules is expensive and commercially not viable [21], so these are the limitation that greatly hampered the use of ZFNs for genome editing.

3.2 TALENs

TALENs are a second-generation genome editing platform that was first used for plant genome editing in 2011 [22,23] and secreted protein produced by the plant pathogenic bacterial genus *Xanthomonas* that proteins, after binding to DNA, mimic transcription factors and can modulate the activation of the target gene(s). TALENs, like ZFNs, are engineered nucleases that are created by fusing a transcription activator-like effector (TALE) DNA binding protein with the non-specific DNA endonuclease *FokI* [24]. TALE proteins have a central DNA-binding domain (DBD), N-terminal nuclear localization signal, and C-terminal activation domain. The DBD has 34 amino acid residues in tandem repeats, with two highly variable residues at the 12^{th} and 13^{th} positions. Two variable amino acid residues are known as repeat variable diresidues (RVD) and function as DNA binding codes that identify particular nucleotides. The recognition and binding of DNA by TALE proteins are simple.

The DNA is bound by this chimeric nuclease, which causes DSBs. The majority of these DSBs are repaired by NHEJ machinery through indels, which lead to an edited genome. Model plants such as arabidopsis, rice, and tobacco have been edited by TALENs [22,25,26]. But some restrictions, like non-specific binding and off-target effects, consult for more optimization [23].

3.3 CRISPR/Cas9

The most recent second-generation genome editing tool, the type II CRISPR/Cas9 system from *Streptococcus pyogenes*, has gained widespread acceptance for its ease of use,

effectiveness, easiness, and amicability. A bacterial defence mechanism against bacteriophages is called CRISPR [27-29]. The history of CRISPR began in the 1980s when Ishino and colleagues studying the *iap* gene found 29-nt repeat sequences in the *E. coli* genome [30]. The Cas9 endonuclease, a crRNA (CRISPR RNA), and a tracrRNA are the main components of the CRISPR/Cas9 system (trans-activating crRNA). Cas9 endonuclease's main role is to cut entering phage DNA into small pieces that are then integrated into the CRISPR array as a spacer. Following that, crRNA and tracrRNA are transcribed from the CRISPR array, forming a double-stranded RNA structure that recruits Cas proteins for cleavage [28,31]. The binding and cleavage of target DNA require the presence of the protospacer adjacent motif (PAM) sequence (5′-NGG-3′) in downstream of the target DNA [28]. The Cas9 endonuclease has two nucleic acid binding grooves called the REC lobe and NUC lobe [28,32]. While the NUC lobe is made up of three components- RuvC, HNH, and PAM-interacting domains- the REC lobe is a functional domain that is specific to Cas9 [33]. The nuclease domain structures of RuvC and HNH can be predicted using their homology. When it comes into contact with sgRNA at its REC lobe, the Cas9 enzyme turns active. When the correct PAM is found, the HNH nuclease domain of the Cas9-sgRNA complex cleaves the RNA-DNA hybrid while RuvC cleaves the other strand. The DSBs are then fixed either by the unreliable NHEJ or the extremely accurate HR pathways. NHEJ typically causes indel mutations, whereas HR precisely fixes the DSBs through gene insertion or replacement techniques [34]. The construction of CRISPR/Cas system doesn't need any complex protein engineering steps. The CRISPR/Cas platform enables us to edit multiple genes simultaneously by introducing DSBs at multiple sites [35].

3.4 CRISPR/Cpf1

In mammalian systems, CRISPR-Cpf1 (CRISPR from *Prevotella* and *Francisella 1*) has been discovered as a new system for targeted genome editing [36]. Cpf1 is a class II type V endonuclease that recognizes a T-rich PAM (5′- TTTN-3′) present in the 5′ end of the target site and cleaves it with a single 44-nt crRNA. In contrast to SpCas9, which produces blunt ends, Cpf1 produces sticky or cohesive ends, which improves the effectiveness of integrating a gene into a specific region of the genome. Additionally, it has been demonstrated that Cpf1 enzymes exhibit less off-target activity than Cas9 nucleases [37]. Compared to SpCas9, these advanced features make it a more interesting editing system in plants [38]. Numerous studies have shown that this new system is a useful DNA-free genome-editing tool for editing plants [39,40]. System also used for multiplexed gene editing, targeted gene insertion, indel mutation, gene knock out, transcriptional repression using catalytically inactivated Cpf1 (dCpf1), or transcriptional activation using dCpf1, and epigenomic editing suggests that widespread adoption of Cpf1 genome editing technology could have a significant impact on plant biotechnology and that improve crop yield and quality.

4. GENERAL PROCEDURE FOR GENOME EDITING

Genome-editing tools are widely used for gene knockout, gene insertion, and gene replacement in crop plants to perform multiplex editing, mutant libraries, and gene regulation for a spectrum of uses. The direction to conducting genome editing necessitates selecting an appropriate target gene for cassette delivery, the efficient method for cassette delivery, to achieve temporary or stable transformation, precise and efficient gene expression regulation, and avoidance of side effects associated. Mutations at off-target sites are affecting the efficiency of nucleases such as ZFNs, TALENs, and CRISPR/ Cas9. Many computer-based programmable nucleases are eventually designed to assist highly précised site-specific genome editing and widen the scope e.g., the use of Cpf1 nucleases in CRISPR [41].

Plant transformation and an effective regeneration method are required for successful genome editing to revolutionize agricultural productivity. The techniques for inserting DNA into plant cells (transient transformation), integrating it into the plant genome (stable transformation), and regeneration is important in genome editing for crop improvement [42,43]. To raise genome-edited plants, the simplified transformation and regeneration strategy must be optimized e.g., the floral transformation method for *Arabidopsis thaliana.* Cells, calluses, somatic embryos, and other tissues are typically transformed using Agrobacterium, PEG, protoplast, electroporation, and biolistic methods. Among them, *Agrobacterium tumefaciens* mediated transformation strategy is commonly used for manipulation. As a result of the

transformation strategy, some vector fragments may be transferred and integrated with the desired fragment at random loci [44]. Off-target editing [45-47] might even increase the risk of unintended changes. These could be lowered by successfully transferring *in vitro* pre-engineered RNPs (ribonucleoproteins containing sgRNA and Cas9) for genome editing. Arabidopsis, tobacco, rice, lettuce [48], petunia [49], maize [50], wheat [45,46,51], grapevine, and apple are among the plant varieties that have been reported to be produced by the RNPs with mutated alleles [52]. However, there are restrictions related to the use of RNPs, necessitating additional design and delivery changes.

General procedure for genome editing in plants has to be followed for eliminating chances of off-target and target and undesirable mutation: (1) selection of an appropriate target; (2) select the appropriate nuclease based on the target sequence; (3) construction of the vectors for genome editing; (4) validation of vector activity through protoplasts; (5) delivery of genome editing construct into plant cells; (6) regenerate genome-edited cells into plantlets *via* tissue culture; (7) screen and genotype the resulting genome-edited plants [53].

5. DIFFERENT STRATEGY OF GENOME EDITING

5.1 Gene Knockout

Knocking out target genes is currently the most popular and significant application of genome editing. The primary pathway for DSB repair in plants is NHEJ, which can result in indels (small deletions or insertions) for one gene [9] or for multiple gene e.g., Arabidopsis, rice, maize, soybean and tobacco [54-60] or large fragment of chromosome deletion. The majority of indels introduced in the relevant region result in frameshift mutations, which impair gene function. The mutations are stable and passable to succeeding generations, which is crucial. CRISPR/Cas9 is currently the most popular tool for deleting genes due to its ease of use and high efficacy.

Genome editing has been used for simultaneous targeting of multiple genes in many plant species, including Arabidopsis, rice, maize, soybean, and tobacco [54-60]. For example, three negative regulators of grain size in rice, GW2, GW5, and TGW6, were simultaneously knocked out using CRISPR/Cas9, and the new varieties showed 20-30% significant increase in grain size and weight compared to the wild type [61]. For the deletion of large fragment, creation of DSB at two sites on the same chromosome and rejoin *via* NHEJ pathway results in the deletion being useful for studying gene clusters. Mostly, TALEN and CRISPR have been used for this purpose in various crop species such as rice, up to 245 kb has been removed from the genome with a high frequency using CRSIPR/Cas9 [62], and our group successfully deleted a large genomic fragment in Arabidopsis containing the *CBF1, CBF2* and *CBF3* genes [63].

Gene knockout mutants are quite challenging to produce using conventional genetic methods due to their complex genetic makeup, which makes it difficult to conduct gene function research on them. Tools for genome editing are particularly helpful for polyploid crops that lack mutant resources. The tool of genome editing is helpful for gene knockout in polyploid crops such as (citrus and apple), tetraploids (cotton, pasta, wheat, and potato), hexaploids(Camelina and bread wheat), and octoploid (sugarcane) [64,65].

5.2 Targeted Genome Editing

Generating a one-for-one replacement of a DNA fragment (gene replacement) or inserting a new sequence into a specific genomic locus (gene knock-in) is known as gene targeting. Functional genomics research uses gene targeting for a variety of purposes, including precise gene modifications and epitope tagging of endogenous proteins. Gene targeting is helpful for crop improvement because many traits that are significant in agriculture can be conferred by point mutations or indels at specific loci in the gene's coding region or promoter region. Research on homologous recombination-based gene targeting has been the main focus [66,67].

5.3 Transcriptional Regulation

In transcriptional regulation, deactivated Cas (dCas9/dCpf1) and TAL effectors can upregulate or downregulate the expression of specific sequences but do not cut the target DNA [68,69]. Combining a TAL effector or dCas9/dCpf1 to proteins or domains with different activities, such as transcriptional activators, DNA demethylases, DNA methyltransferases or repressors can effectively control gene expression patterns and methylation status [70]. In order to change the expression of certain genes in Arabidopsis and tobacco, the VP64 transcriptional activator and

the SRDX transcriptional repressor have been fused to TAL effectors, dCas9 or dCpf1 [71,72].

5.4 CRISPR System in Metabolic Engineering

Genome editing includes extensive research in metabolic engineering through CRISPR/Cas9 in plant cells that produce specific metabolites. In *Papaver somniferum,* [73] edited the pathway of biosynthesis of benzylisoquinoline alkaloids (BIAs) for next-generation metabolic engineering by knocking out 30 OMT2 genes though NHEJ DNA repair CRISPR/Cas9 mechanism. A regulatory gene called 40 OMT2 (40-O-methyltransferase) participates in the synthesis pathway of codeine, noscapine, papaverine, and morphine via various BIA pathways. By introducing breaks with related gene sequencing, such techniques can be used to transform valuable medicinal plants into biofactories for the mass production of particular metabolites.

6. APPLICATIONS OF GENOME EDITING TECHNOLOGIES IN CROP IMPROVEMENT

6.1 Yield Improvement

Yield is one of the utmost important quantitative traits in crop production. In rice, the weight of grain elevates the production of rice. CRISPR/Cas9-mediated multiplex gene editing was used for rapid pyramiding to improve grain weight in LH422, by targeting three major genes that negatively regulate rice grain weight (GW2, GW5, and TGW6) [61]. For the high yielding objective, gene pyramiding through conventional breeding is complicated and time-consuming so pyramiding of major genes of yield contributing traits through CRSIPR/Cas9 has not only enhanced the grain weight in rice but also helps the study of quantitative traits [74] efficiently used the CRISPR/Cas9 mediated multiplex regulate the heading date of rice through CRISPR/Cas9 mediated multiplex to target three major negatively regulate heading date genes (Hd2, Hd4 and Hd5) for early maturity in rice.

6.2 Genome Modification for Nutrition Improvement

The genome editing tools also improve quality traits of crop plants such as in rice grain amylose content increase by editing starch-associated genes (SBEII b and SBEI) *via* CRISPR/Cas9 [75]; in maize reduction of anti-nutritional substance Phytic acid (PA), myo-inositol 1, 2, 3, 4, 5, 6-hexakisphosphate via gRNAs targeting the ZmIPK (inositol phosphate kinase) gene that catalyzes a key step in PA biosynthetic pathway by two gRNAs [76]. In oil seed plants shelf-life improve by the increase in oleic acid content that improves oxidative stability such as in soybean, by targeting FAD2-1A and FAD2-1B genes that convert oleic acid to linoleic acid using TALENs [77] improve shelf life.

6.3 Biofortification

A considerable portion of the population suffers from nutrient deficiencies. Developing a strategy for crop biofortification using gene-editing methods could also be used to address this. With the help of other gene-editing tools, the CRISPR/Cas9 tool has been used to produce improved crops with increased nutritional value and fewer undesirable compounds.

6.4 Biotic and Abiotic Stress Resistance

The genome editing techniques results showed excellent potential in improving crop resistance to a variety of biotic and abiotic stresses by targeting traits that are primarily controlled by negatively regulatory genes [78].

The CRISPR-Cas9 technique has been successfully used by DuPont scientists to produce novel variants of ARGOS8, a negative regulator of ethylene responses in maize [79]. The 5′-untranslated region of the native ARGOS8 gene has been replaced by the native maize GOS2 promoter or by homology-directed DNA repair for the development of novel ARGOS8 variants for the breeding of drought-tolerant maize crops. Three homeoalleles (TaMLO-A, TaMLO-B, and TaMLO-D) of the MLO gene that provides resistance to powdery mildew in bread wheat were successfully edited using the CRISPR/Cas system [65].

6.5 Induction of Haploid and Artificial Apomixis

To generate highly homozygous, stable varieties, conventional plant breeding requires six to seven generations of self-pollination. As compared to conventional breeding, recombinant haploid is easily fixed within two generations, and that speedup breeding program with lower cost. An effective method for creating haploid inducer

lines is direct editing of endogenous plant genes. Knockout in the gene MTL/PLA1/NLD, which codes for a phospholipase specific to sperm cells, resulted in the production of male gametophytes with defects and maternal haploid induction traits in maize, rice, and wheat [80]. CRISPR-mediated mutagenesis was used to modify DMP gene, and similar outcomes were obtained in maize [81]. *A. thaliana* haploid inducer line generated after CENH3's N-terminal a-helix was deleted using CRISPR-Cas9 [82].

Apomixis multiplied seeds asexually that are genetically identical to their mother cells [83]. More than 400 species naturally exhibit apomixis. However, this phenomenon does not occur in major crops, and conventional breeding makes it very challenging to engineer. Apomixis involves the development of unreduced female gametophytes (apomeiosis), the development of embryos from gametophytes without fertilization of the egg cell (parthenogenesis), and endosperm fertilization. Apomeiosis, or "mitosis instead of meiosis" (MiMe), can be induced apomixis in rice by using CRISPR-Cas9 to knock out the meiotic genes REC8, PAIR1, and OSD1 [84]. Another strategy is to stimulate female gamete's embryonic development without fertilization. In unfertilized egg cells, BBM1 was misexpressed, which led to rice embryogenesis. Synthetic apomixis was produced by combining this procedure with MiMe mutations created by editing [84].

7. CHALLENGES

"For the wider application of genome editing in biology, medicine, and agriculture high reliability is critical for economically important traits in crop plants; sometimes any off-target mutation could create major undesirable changes in the plant and elevation several concerns. Genome editing is more accurate as compared to traditional breeding and GE. In CRISPR–Cas9 system off-target activity is a major concern in Cas9 [85] due to improper concentration in the Cas9: sgRNA ratio, insufficient Cas9 codon optimization, and the presence of promiscuous PAM sites are some of the reasons for off-target cleavage of the DNA regions" [86]. "Off-target effects can be removed by repeated backcrossing but backcrossing is time-consuming and hampered the advancement in crop improvement. Recently, high-fidelity Cas9 variants have been engineered by substituting 3-4 amino acids that variants have been rather effective in addressing off-target issues in plants" [87]. "Although genome editing has many benefits over traditional crop breeding, there are still some obstacles to overcome before it can be applied to horticultural crops. Because molecular and genetic studies are difficult in horticulture crops, they ultimately, hinder the study of functional genomics for the trait of interest. For genome editing, delivering editing reagents and the procedure for regenerating the edited mutants are prerequisites for the success of a system. In most case editing reagents delivered *via* Agrobacteria or virus systems, and the edited plants are regenerated via *in vitro* tissue culture. But in many crops, the protocol for transformation and regeneration from tissue culture is not readily available. Additionally, regeneration by tissue culture may be problematic or inadequate to a few model genotypes for that *in planta* transformation is the best substitute for *in vitro* tissue culture technique in which the stigma, apical meristems, pollen or inflorescences are targeted for infection" [88]. "This method has been effectively used to transform tomato [89] and *Brassica* species [90] and should be additionally explored to be recalcitrant to traditional genetic transformation".

8. CONCLUSION

Over the past numerous decades, conventional breeding relies upon plant populations with enough variability, however this variability is specifically derived from spontaneous mutations or from artificial mutations and such types of variability are normally and occur at random, and derived useful variability is time-consuming process. In contrast to an advanced molecular biology technique, genome editing can produce precisely site-specific targeted modifications in any crop [91,92]. For improvement of crop and functional genomics, the genome editing technique proved the most useful and versatile tool because of its efficiency, safety, simplicity, stability as well as highly specific nature. These tools provide significant opportunities for future plant science progression and crop accelerated remodelling in view of global food security and sustainable agriculture.

Among the diversified tools like targeted oligonucleotide mutagenesis, meganucleases, zinc fingers, TALENS, and CRISPR/Cas9 nowadays CRISPR/Cas9 discovery paved way for revolutionary crop improvement due to their simplicity, versatileness, low cost, and stood to democratize the field of genome editing as compared to other methods. The main

advantage of genome editing is that genome-edited cultivars are completely indistinguishable from those generated using conventional breeding methods because initially used transgenes to produce the genetic changes can be easily excised from the genome by genetic segregation and they do not contain foreign DNA like GMO crops [93]. Clear road map and allowed genome-edited plants without the cumbersome GMO regulation provide more way forward for researchers and breeders to harness the power of the genome editing for the farming community and also further alleviate public concerns. Thus, traits developed through genome editing strategies are considered nature-identical traits because they resemble traits derived through conventional breeding without the use of transgene transfer. So, the US Department of Agriculture (USDA) has approved commercial cultivation and market access without any regulation for genome-edited crops such as anti-browning mushroom, waxy corn, green bristlegrass, stress-tolerant soybean, and some nutritious crops (alfalfa, false flax, and soybean) [93]. India has allowed genome-edited plants without the cumbersome GMO regulation at the Genetic Engineering Appraisal Committee (GEAC) and the Union Ministry of Environment, Forest and Climate Change issued a notification regarding the issue, which has exempted Site-Directed Nuclease (SDN) 1 and 2 genomes and the Institutional BioSafety Committee (IBSC) under the Environment Protection Act would now be entrusted to certify that the genome-edited crop is devoid of any foreign DNA. Still, genome-edited crops are raising concerns in many countries which may hinder their further development. So, overcoming the possible threats due to genome-edited crops needs debate involving scientific, public, and government personnel in drafting global regulations (exclusively for genome-edited crops) for uniformity across the world.

9. FUTURE PERSPECTIVES

9.1 Epigenomics and Regulatory Function

Knowledge of genomic functions, as well as their effects on gene activation and repression, is required for efficient genome editing. As a result, it also paves the way for the exploration of epigenomic regulation through the use of genome editing tools to manipulate histone modifications or DNA methylation patterns, which is promising for basic research and crop improvement because altered epigenetic marks may be passed down to future generations without changing the sequence of the genome itself. Despite the fact that it has not yet been demonstrated in plants, epigenome editing tools are expected to be developed in the near future due to their potential value. Limited genetic resources and knowledge of genes responsible for trait of interest also hampered the growth of genome editing.

9.2 High-Throughput Mutant Libraries

In genome editing, gene knock-down strategy is frequently used for that knowledge of genome sequence and gene functions of different crops are a prerequisite and as most of the genes sequenced to date have unknown functions that may control important traits and for functional genomics, large-scale mutant libraries at the whole-genome level construction are also helpful [94].

9.3 Gene Multiplexing

In plants, many biochemical pathways are controlled by complex genetic systems, and the manipulation of single desirable traits depends on the editing of multiple genes of complicated metabolic pathways, therefor system with the capacity of manipulating multiple genes simultaneously provides great opportunities for the field of crop improvement [94]. So, a better understanding of biochemical pathways and their regulations for several metabolites should be required for harnessing the full potential of genome editing.

9.4 Designer Crop

In the 21st century, genome editing crops have incredible possibilities to address food deficiency and create the healthy hunger-free world by increasing crop yield, overcoming malnutrition, and increasing nutritional value through biofortified crops enriched with good fatty acids, amino acids, essential vitamins, and minerals, reduce agriculture losses through developing stress-tolerant crop plants which will address biotic and abiotic stress [95] through the development of the designer crop.

9.5 Off-Target Delivery

Revolutionized genome editing technology changes the scenario of crop improvement

but certain issues such as DNA delivery methods, the balance between HR/NHEJ pathways, and off-target effects must be addressed to harness the full potential of technology [96].

9.6 Another Technology

Integration of genome editing with other existing technology like nanomaterials-based delivery provides significant advances. Some other non-invasive technology like infrared or other wavelength-based emissions could provide a new way for early screening of edited events [97].

9.7 Regulatory Framework

Restrictive regulatory approach and treatment genome edited plants as GMO delay progress and application to agriculture. For the safety purpose, science-based regulation has been anticipated [98] but regulation must be reasonable for transgene-free edited products [99]. Additional effort is required to ensure regulatory transparency and relaxation especially for the developing countries where food production demand is high with nutrition enrichment [100].

CONFERENCE DISCLAIMER

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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