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Using CRISPR/Cas9 Technology to Improve Crops and Address the Global Food Crisis: A Review

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This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

By developing a revolutionary method for modifying the genomes of living things, genome editing (GE) has completely changed the biological sciences. Recent years have seen the development of several technologies that make altering complex genomes possible. For agricultural crop production to be sustained and contribute to global food security, a fast and dependable method of raising yield and resilience to different environmental pressures is therefore required. The GE instruments for

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crop enhancement are explained in detail in this analytical research. Zinc-finger nucleases (ZFNs), transcriptional activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR) are a few examples of these tools. This category also includes mega-nucleases (MNs). In particular, the most recent advancements in the use of CRISPR/Cas9 for genome editing for major agricultural improvements - like the creation of crops that are more yielding and of higher quality - are covered this paper. When this approach is put into practice, non-transgene crops with desired traits will be produced, which might lead to increased yield capacity under different environmental challenges. To increase agricultural production and guarantee food security, the CRISPR/Cas9 methodology may be used in conjunction with existing and future breeding techniques (such as omics-assisted breeding and speed breeding). The difficulties and restrictions associated with CRISPR/Cas9 have also been covered. Plant breeders and scientists doing a detailed analysis of the use of CRISPR/Cas9 to enhance crops by focusing on the desired gene will find this material to be helpful.

Keywords: Yield; biotic; abiotic; CRISPR/Cas9 stressors in crops.

1. INTRODUCTION

The amount of food resources available is increasing arithmetically, despite the worrying pace at which the world's population is growing. By 2050, it is predicted that there will be 10 billion people on the planet [1]. The increasing number of people on the planet combined with the adverse effects of climate change might ultimately lead to problems with food security. A decrease in the productive regions and a lower yield might make the problem worse. If the pace of acceleration of global temperatures continues, this impact may be more noticeable. Crop production capacity has to almost double in order to ensure food security, and in order to accomplish this, highly tolerant cultivars against a variety of stressors need to be produced [2,3]

The main issue with relation to global food security is an improvement in agricultural crop productivity employing the most recent breeding methods. Nonetheless, food safety and optimal food production cannot be guaranteed by traditional breeding methods. Traditional breeding methods, such hybridization, have been used to improve variabilities and produce crops, which has somewhat increased agricultural productivity [4,5]. The study of gene function has centred on genetic engineering for a number of years. This entails identifying biological tricks and mutating cells both physically and biologically to increase agricultural yield. It is not possible to have a 100% success rate due to many problems. The actual yield of the main cereal crops, such wheat in Northwest Europe and rice in East Asia, has been documented in recent years. Furthermore, certain features of the insertion of transgenes into the genome of the plant host cause public worry about edible crops and are not always recognised since the techniques and advantages are not always clear. Thus, the key to overcoming these constraints is the use of biotechnological technologies for crop development. Secondly, TALENs cause double strand breaks in the structures they target, which triggers the DNA response pathway that ultimately destroys the genome. Despite the fact that ZFNs and TALENs are often employed to modify the genomes of plant and animal cells, there are some restrictions that make these techniques less effective. ZFNs have a low specificity, which often causes off-target alterations. For ZFNs and TALENs, vector construction is a labor-intensive and time-consuming process [6]. Therefore, in order to accomplish these goals and grow food crops, ongoing advancements in crop breeding programmes are essential. The ability to precisely and deliberately modify a crop's genetic makeup has been made feasible by recent advancements in CRISPR/Cas9 technologies, which has accelerated the shift to improved crops. This method has only been used to a small number of species thus far. Since 2013, the application of CRISPR/Cas9 and its variations has taken centre stage. A DNA fragment known as CRISPR is made up of tiny, non-contiguous DNA repeats separated apart by spacers or variable sequence pieces. Thus, this discovery suggested that CRISPR/Cas9 may be in charge of inducing adaptive immunity in prokaryotes. Diseases may be cured by ISPR/Cas9, and plant biology is greatly impacted by its use [7]. The bacterial phenotypic was thought to be phage-resistant in the 2007 experiment that first disclosed the use of CRISPR/Cas9, an adaptive immune system whose phenotype could be changed by adding or removing particular spacers. In the future, genetically modifying plants to boost harvests will be simple. The use of Cas9, a protein also

referred to as "biological scissors," revolutionises genome editing and establishes new standards, opening up new avenues for the enhancement of agricultural products. Here, we took a quick look at the potential uses of CRISPR/Cas9 in crop development, as well as their drawbacks and future opportunities. Nowadays, CRISPR/Cas9 is being employed in plant breeding for a variety of purposes, including disease resistance, drought resistance, salt tolerance, and genetic crop enhancement. Cas9 also enhances the grain production and quality of crops. Using Cas9 has also boosted the production of biomass. Similarly, Cas9 has been used to improve morpho-physiological features and increase the efficiency with which nutrients are used [8].

2. TOOLS FOR EDITING GENOMES

With its goal of revolutionising plant breeding, genome editing may be able to protect the world's food supply. First-generation genome editing instruments, known as meganucleases, are distinguished by the presence of a recognition site ranging in size from 12 to 40 base pairs [9]. Because of their specificity and wide recognition site, they are often referred to as homing endonuclease enzymes and are frequently referred to as the most characterised restriction enzymes. Studies have shown that non-homologous end joining (NHEJ), which is in charge of knocking out genes found in tobacco and Arabidopsis, is the mechanism that causes double strand breaks to be repaired. Since the catalytic region of meganucleases is connected to DNA binding domains, dissociation is not feasible, making it almost impossible to modify the meganucleases using other genome editing methods. Meganucleases (MNs) in cotton and maize have been used in a number of investigations employing alerted MNs; still, further research is necessary to improve the approach.Because ZFNs produce double-stranded breaks (DSBs), they are a more dependable and effective gene-editing tool than other options [10]. The first method of manipulating the genome via the use of engineered nucleases was ZFNs. This method was made feasible by the discovery of the Cys2-His2 Zinc-finger domain. The two primary domains that make up the structure of zinc finger repeats are as follows: (1) the DNA-binding domain, which has 300–600 zinc finger repeats. Between 9 and 18 bp, zinc-finger repeats may be seen individually; (2) the DNA slice domain, which is thought to be a nonspecific domain of type 2 restriction endonucleases. ZFNs are made up of two monomers that are assigned to specific object sequences that are reversely adjacent to 5- and 6-bp DNA [11]. Dimer enzymes with the FokI domain cut DNA. A zinc-finger domain that comprises specified or sporadic guiding sites in the genome reads the precise order of 24 to 30 bp. The area of genome editing has shown progress and success in gaining the ability to manipulate and modify the fundamental genetic markers. TALENs have been in widespread usage for a long time [12]. By consolidating the FokI slicing domain to the DNA-binding domains of the TALE proteins, TALENs were created. The effective modification of a single base pair is achieved by the complicated duplication of 34 amino acids seen in these TALEs. In addition to causing alterations, TALENs also initiate pathways where editing takes place by generating DSBs. The TALEN system contains both the nuclear localization sequence and the central domain. The ability of proteins to modify DNA was first investigated in 2007. In terms of genome editing programmes, TALENs are more dependable and efficient (Zhao et al. 2023).

2.1 An Overview of CRISPR/Cas9

The CRISPR/Cas9 genome editing approach is characterised by short, repeating genetic variations called clustered regularly interspaced short palindromic repeats, which are present in the majority of bacterial and archaeal species. Plants are protected against external agents such as bacteria, viruses, and other elements by a robust defence mechanism developed by CRISPR/Cas9 and associated proteins [13]. The Nobel Prize committee's recent acknowledgement of the researchers for their work on a CRISPR/Cas9 genome editing technique brings us one step closer to creating and growing new crop kinds [14]. The single guide RNA (sgRNA), a target-specific RNA, and endonuclease DNA (Cas9) make up the two components of the system. Because different spacer sequences are required to use Cas9 as a target, CRISPR/Cas9 is a quick, efficient, affordable, and flexible technology. Plant cells are given portions of CRISPR/Cas9, either in DNA or RNA, to manipulate their genomes in a predefined pattern by cutting plant DNA. Plant cells therefore start mechanisms to “patch” the break in order to preserve genomic integrity, and this suggests the creation of many types of changes in the guided sequence. Little deletions or insertions that may place during the NHEJ/homology-directed repair (HDR) process to mend the break may result in gene mutations.

During the process of characterising spacer absorption, DNA patterns associated with bacteriophage invaders known as spacer pre-cursors, or protospacers, were discovered [15]. These motifs, which are brief trinucleotide extensions that come just after the protospacers, are important for both identifying certain protospacers and directing the placement of spacers that are inserted into the protospacer repeat arrays. Within the repeating Cas protein chain, the CRISPR RNA precursor that was transcribed from a CRISPR site divided into mature RNA molecules (to crRNA). Each crRNA is made up of a spacer that surrounds brief DNA duplications and functions as a little RNA guide to assist proteins in triggering an antiviral response [16]. It has been shown that Streptococcus thermophiles use CRISPR/Cas9 to cut plasmid DNA. These findings bolster the idea that the CRISPR/Cas9 system assisted the molecular genesis of adaptive immunity. As shown in Fig. 1, each genome-editing method offers benefits and downsides [17].

2.2 The Mechanism and Function of CRISPR/Cas9

Three phases of the immune response are aided by the CRISPR/Cas9 system: interference, expression, and adaptability [18]. A combination of many Cas proteins is utilised as indicator proteins in Type I and III systems. The Type II system can be developed more effectively to serve as a mechanism of genome editing since its design is simpler than that of the other systems [19]. Concerning the Type II system, research has demonstrated that a brief trans-noncoding RNA known as transactivating CRISPR/Cas9 RNA, discovered in S. pyogenes, regulates the synthesis of crRNAs by matching bases with regions of pre-crRNA transcripts that are duplicated through the combined action of RNase III and Cas9. identified the potential usage of a Type II system and fused tracrRNA and crRNA to produce a synthetic sgRNA. From the target binding DNA sequence located at the 5′ end of the 20-bp sgRNA sequence, any sequence may be generated [20].

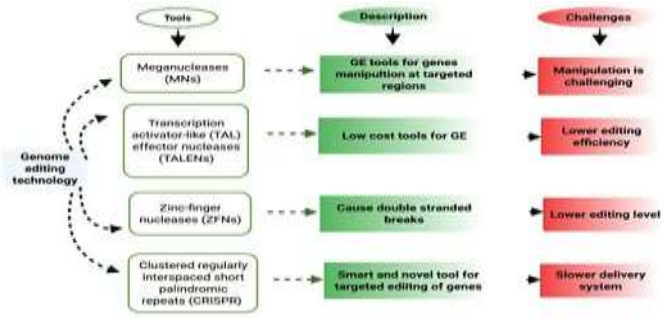

Fig. 1. A comparison of instruments for genome editing. A comparison of these technologies reveals both their limits and their effectiveness in modifying the genome. Thus, compared to other technologies, the CRISPR/Cas9 approach is more precise

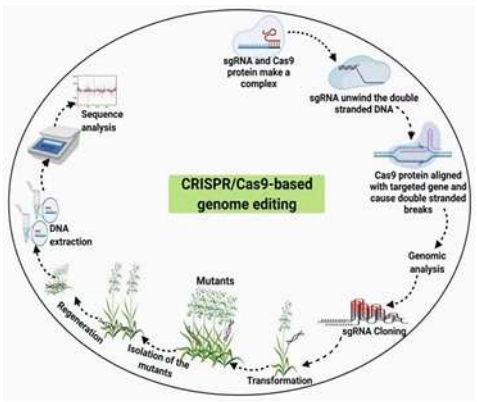

Fig. 2. Cas9-based genome editing mechanism. The Cas9 gene-editing system includes the sgRNA and Cas9 protein complex, sgRNA-induced DNA unwinding, Cas9-induced gene cutting, analytic tools, cloning, transformation, and more. This editing technique does not need any external elements

3. USING CRISPR/CAS9 TO IMPROVE CROP QUALITY

The most popular technology now available is CRISPR/Cas9, which offers a number of benefits including time efficiency, cheap editing costs, remarkable versatility, and the capacity to instantly control the replication of numerous genes [21]. This method of genome editing is more effective and adaptable than previous ones [22]. Compared to TALENS, ZFNs, and MNs, this method - CRISPR/Cas9 - is easy to use, effective, and less expensive. It can modify several genomes at once. The CRISPR/Cas9 system has been used to several plant species because of its advantageous characteristics, and it offers the greatest resolution to a number of problems that arise in plant breeding. Crops that are monocots or dicots have benefited from the use of CRISPR/Cas9 in terms of yield, quality, disease resistance, and climate variability resilience [23]. The genomes of cereal crops like wheat, corn, rice, and cotton, as well as vegetables like potatoes and tomatoes and fruits like bananas and apples, have all had their genomes altered using the CRISPR/Cas9 technology. The most popular use is the deletion of target genes acquired by the induction of frameshift mutation-causing indels. Cas9 has been used to introduce a great deal of significant features into crops, which have been covered in several reviews [24].

Crop species undergo radical transformations thanks to CRISPR/Cas9. Because of its promise, this potent approach has been implemented in many labs worldwide [25]. Here, we've included a few of its applications that have been thought to enhance crop quality and output. Utilising CRISPR/Cas9, cultivars with excellent nutritional values and resistance traits are created, marking the biggest advancement in genome editing in contemporary technology [26]. The crop seeds of Camelina sativa, which is frequently grown due to its greater proportion of fatty acids, were processed to produce a commercial oil. When Cas9 was used to modify a flowering locus and a squamosal promotor binding protein in the same species, the plants displayed a 90% mutation frequency in late flowering. A multiple CRISPR/Cas9 system was used in concert with other tools to produce and modify six modified PYL genes of ABO receptors, which had a mutation frequency ranging from 13% to 93% in the first generation [27]. *Nicotiana benthamiana* has a green fluorescent gene that was modified by RNA-facilitated endonucleases. Subsequently, the instructions to the plant genes to produce and modify transcriptional characteristics were modulated by means of the tobacco rattle virus [28]. Inherent genetic changes in subsequent progenies were then investigated. Genes involved for grain dormancy were upregulated due to an ABA-inducible protein produced by the barley gene HvPM19. 10% of HvPM19's mutation frequency was produced by CRISPR/Cas9-induced mutations [29].

3.1 CRISPR/Cas9-Assisted Development of Disease Resistant Varieties

Plant diseases have a negative impact on crop quality and output. Bacteria, nematodes, fungus, viruses, and insects are the main causes of illness, and they significantly lower crop yields. A serious problem now is the emergence of these insects' lethal epidemics together with other biotic stressors [30]. In order to protect plants from these assaults, it is crucial to comprehend the links or interactions between plants and pathogens. Demand for wheat-based goods safe for people with celiac disease is rising. Wheat that is safe for celiacs lowers the chance of developing chronic illnesses. There is no way to increase these features using conventional breeding techniques. Lately, cultivars safe for celiac disease have been created by editing the glutenin genes using CRISPR/Cas9. These methods produce children with deleted and silenced gliadins, potentially reducing the patient's exposure to epitopes associated with celiac disease (CD). Similarly, CRISPR/Cas9's contribution to the development of strict-gluten-free wheat, which lowers the risk of celiac disease (CD), was examined in another research. For example, the OsERF922 gene was knocked out using Cas9, which led to more resistance to blast production. Similarly, Cas9 has been used to accomplish a targeted mutation of SWEET1E, resulting in plants that are resistant to blight. As a result, genome editing has been effectively used to study the interaction between pathogens and plants, and CRISPR/Cas9 is an effective technique for creating cultivars that are resistance to illness by changing the gene that causes the disease [31]. Cas9 modification of the effector binding element in the CsLOBI gene promoter region was done to strengthen citrus's resistance to *Xanthomonas*. Wheat protoplasts have had the TaMLO gene edited using the Cas9 method, which has increased the plants resistance to powdery mildew. Similar to this, Cas9 was used to target

EDRI homologs in order to build wheat resistance to powdery mildew [32]. Similarly, tomato MLO gene variants were produced, increasing resistance to powdery mildew. It was estimated that viruses represent the root cause of half of plant illnesses, which result in significant losses in crop quality and output. DNA viral amplicons significantly improved the genome's targeting efficiency. When geminivirus replicons were employed for Cas9 transient expression against dwarf virus in hexaploid wheat, gene expression was shown to be upregulated by around 12 times. CRISPR/Cas9 has been used to target the genome of the geminivirus and stop its proliferation. Rather of treating the illnesses that viruses cause, Cas9 may be utilised to alter the DNA of the viruses [33]. The production of sgRNA cassata may be regulated by virus promoters, which can improve Cas9-mediated modification of the viral genome. Francisella novicida was recently discovered to have a unique ortholog of Cas9, a gene-editing tool for RNA-based viruses. These new paralogs provide resistance to illnesses. Because gene Ntab0942120 is susceptible to potato virus Y, tobacco, a significant cash crop, is negatively impacted [34]. This gene was knocked out using the CRISPR/Cas9 method, resulting in transgene-free homozygous altered plants that were resistant to PVY. around brassica. Five genes were removed from potatoes in an effort to make them resistant to Phytophthora. As a result, Cas9 is an effective tool for improving crops' genetic composition and boosting their resilience to viruses and other sporadic agents [35].

3.2 Production of CRISPR/Cas9-Based Climate-Smart Crops

Cas9 has been used to address a number of abiotic stressors in important crops, including rice, wheat, maize, cotton, and potatoes. By creating crops that are resistant to abiotic stressors or climate change, plant breeding has modernised plant breeding. Any sequence may be changed, and any character can be introduced into crops using CRISPR/Cas9. Numerous genes linked to abiotic stress tolerance were found by molecular breeders, who then incorporated them into crops. Mutants of the slmapk3 protein gene produced by CRISPR/Cas9 enhanced tomatoes' defence response to drought stress [36]. Cas9 has been used to investigate two genes, TaDREB3 and TaDREB2, associated with abiotic stress tolerance in wheat protoplasts. When compared to plants of the natural type, all mutant plants demonstrated drought tolerance. A thorough analysis of how to use CRISPR to increase plant tolerance to drought stress. Two rice genes, OsMKP2 and betaine aldehyde dehydrogenase OsBADH2, were modified using the Cas9 technique. The host genome was combined with these genes, which conferred resistance against various stresses, by the use of protoplast transformation and particle bombardment method. The function of the rice OsAnn3 gene, which was altered to withstand cold stress, was investigated in plants with altered genomes. To investigate the stress mechanism in rice, the gene SAPK2 was modified. This gene improved rice's resistance to salt and drought, according to a prior research. Targeting the ARGOS8 gene to produce novel variations has led to the development of drought tolerance, and this is very significant for maize [37]. By modifying the pattern of protein expression, Cas9-induced mutation of Leaf 1 and 2 gave drought tolerance. Additionally, rice's ability to scavenge reactive oxygen species (ROS) was improved by targeted mutagenesis of the OsRR22 gene. One significant legume crop that is heavily impacted by drought stress is chickpea. Up until now, CRISPR has only been used in a single research to introduce gene mutations. Cas9 targeted two genes, 4CL and RVE7, to improve chickpea resistance to drought stress. In the future, drought-tolerant chickpea varieties will be developed thanks to the innovative method of Cas9 deletion of these genes. Compared to wild-type plants, AREB-1 activated Arabidopsis by CRISPR exhibited improved resistance to drought [38]. Herbicide tolerance has also been introduced into crops via the use of CRISPR. Base manipulation made it possible to artificially evolve OsALS1 in plants in order to create novel rice germplasm that is resistant to herbicides [39].

4. CROP DOMESTICATION THROUGH THE USE OF CRISPR/CAS9

Plant breeding and crop domestication have produced high-yielding crops that are suited to their natural growth environments. However, a growing variety of agricultural issues are brought about by the growing human population, including changes in the climate, fluctuations in abiotic and biotic pressures, and damage to arable land, in addition to the need for more definite and sustainable farming practices [40]. Neighbours of contemporary farmed and orphan crops are regarded as a significant source of new

diversity. Nevertheless, their unattractive appearance and poor yield hinder their commercial cultivation. Recently, the concept of de novo subjugation by gene modification has been proposed as a way to quickly domesticate wild and orphan crops, allowing them to benefit from inherited genetic differences and domesticated plant traits [41]. This is mostly encouraging; in the meanwhile, a large number of domesticated genes from the past are ideal candidates for Cas base editing since they are easily categorised, have a straightforward genomic structure, and are monogenetic. Generally speaking, ground cherries have many undesirable traits, such as little fruit and a strong stem that lead to fruit falling. The gene SP5G was mutated using the CRISPR base gene-editing technology, leading to an abundance of fruits [42].

Nowadays, wild plants are domesticated for human purpose using CRISPR/Cas9. All major crops, such as rice, wheat, and maize, were first domesticated by ancient farmers. However, during domestication, our ancestors used a limited number of original species and just chose plants with superior traits, such high yield and ease of breeding, which led to a decrease in the natural variety of plants. Remembering that one of the primary considerations in the selection process is genetic variety, domesticating wild plants or crops may help to maintain it [43]. It has been possible to domesticate wild tomatoes—which have various defaults in fruit output but are stress-tolerant - by using the CRISPR/Cas9 technology. In one research, six QTLs that were relevant for yield were altered; all lines showed increased fruit quantity and size. Numerous other crops, including quinoa, potatoes, and bananas, are significant regionally, have high nutritional value, and are well suited to their particular environments. Their poor yield and fruit loss hinder their larger-scale production despite these characteristics. CRISPR/Cas9 is an effective method for modifying genes and generating desired traits in crops [44]. Recently, ground cherries have been grown with more flowers and larger fruits thanks to the use of this technology. We are optimistic that we can modify the genome and improve global food security now that the genes controlling the domestication process have been identified [45]. These cutting-edge methods will help us increase the effectiveness of genome editing using CRISPR/Cas9. The first step is to effectively screen for the required qualities that need editing. Whether genetic editing is done by polygenic or monogenetic means, understanding the genetic information pertaining to desired qualities is a must. Choosing an effective tool is essential to achieving high-quality editing outcomes. It is important to identify off-target effects, and one way to achieve this is by constructing sequences that are very affinistic. Using knockout techniques, it is possible to investigate and use a variety of genetic variation sources to provide desired modifications that will better suit the needs of agricultural crops. The greater the effectiveness of CRISPR/Cas9, the higher the likelihood of effective editing and the higher the desired outcomes. Fig. 3 shows new approaches to enhance genome editing using CRISPR/Cas9 [46].

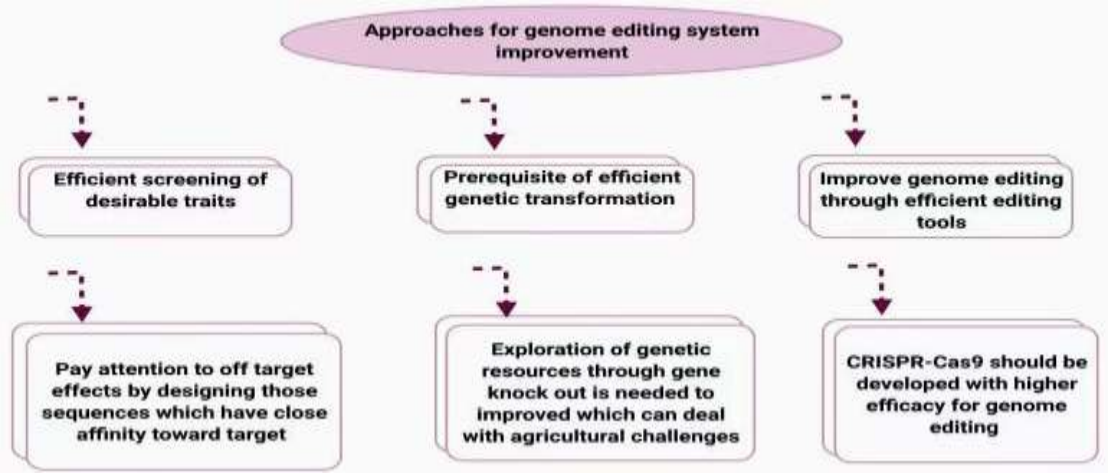

Fig. 3. Innovative techniques to enhance genome editing using Cas9. A perfect gene transformation method, effective screening of the intended features, and genetic material discovery via gene knockouts might all improve the editing efficiency of Cas9

High-yielding crops that are adapted to their natural growing conditions have been developed as a result of crop domestication and plant breeding. However, the growing human population brings with it a host of agricultural challenges, including changing climatic patterns, fluctuations in biotic and abiotic stressors, and degradation of arable land, in addition to the need for more standardised and sustainable farming methods. Neighbours of contemporary farmed and orphan crops are regarded as a significant source of new diversity. Nevertheless, their unattractive appearance and poor yield hinder their commercial cultivation. Recently, the concept of de novo subjugation by gene modification has been proposed as a way to quickly domesticate wild and orphan crops, allowing them to benefit from recollected genetic differences and characteristics of domesticated plants. This is mostly encouraging; in the meanwhile, a large number of domesticated genes from the past are ideal candidates for Cas base editing since they fall into clear categories, have straightforward genomic structures, and are monogenetic [47].

5. OBSTACLES AND RESTRICTIONS IN THE USE OF CRISPR/CAS9

There are a number of limitations with CRISPR/Cas9 uses in plant breeding. Gene availability is crucial for this tool since the primary obstacle is the limited gene pool of significant features. Deciphering the data from genomic sequences and exploring priceless genetic resources are thus essential if we are to enhance important crops. It's crucial to keep in mind that there are still issues with this technology, such as the dearth of effective transformation methods and the difficult and drawn-out procedures involved in plant regeneration from cultures. Concerns about biosafety prevent its use in crop development. Thanks to advancements in identification techniques, plant mutants exhibiting off-target characteristics may now be identified and isolated via further crossings. In the future, sgRNAs with strong attraction for directed sequences and the selection of Cas9 with high fidelity, encompassing appropriate experimental methods, may be able to overcome off-target consequences using these strategies. These unfavourable circumstances make the problems associated with the commercialization of crops modified by genetic engineering the other main source of worry. According to a recent ruling by the European Union's Court of Justice, crops with altered genomes need to adhere to the same guidelines as other genetically modified crops.

Further advancements in this genome editing technique and more thorough study might make the CRISPR/Cas9 system a valuable tool for breeding new crop plants and advancing the establishment of a sustainable agricultural system that could feed the world's fast growing population. Due to its size, this technique's efficacy is limited, making it unsuitable for packaging into viral vectors and delivering them into somatic tissues. The optimal outcome for effective genome editing would be to use CRISPR/Cas9. Using CRISPR/Cas9 may result in a lot of unintentional off-target modifications to the genome. New CRISPR/Cas9 variants have, however, improved the editing effectiveness of target bases in the targeted sequence by detecting distinct PAMs. Issues with transgenic crop commercialization develop, in large part because of the costs and restrictions imposed by the legal framework for the field release of genetically modified organisms [48]. This articulation of barriers with the deployment of CRISPR/Cas9 in various nations follows. A few of the primary issues with the CRISPR/Cas9 delivery method are its poor efficiency or lack of effectiveness, which prevents it from being used for larger-scale genome editing. By stander mutations based on CRISPR/Cas9 may result in a variety of dysregulations in plants, making this a major issue that requires attention. The occurrence of off-target effects may be reduced with careful off-target sequence selection which can impair our editing efficiency. Unplanned point mutations, deletions, insertions, inversions, and translocations are among the off-target impacts. It was shown that more than half of the mutations caused by RNA-guided endonuclease did not materialise on-target in many investigations using early CRISPR/Cas9 agents. Numerous methods, such as prime editing, shortened gRNAs, cytosine adenine base manipulators, biased and unbiased off-target detection, and others, have been developed to lessen these off-target impacts. It is challenging to shorten the lifespan of genomic DNA because to the ineffective Cas9 protein delivery mechanism.

6. CONCLUSIONS AND FUTURE PROSPECTS

Using a variety of techniques, the aim of growing affordable and safe crops to satisfy the growing worldwide need for food may present difficulties. Utilising contemporary methods to increase crop

variety will be a crucial component. Compared to traditional breeding techniques, the adoption of sophisticated breeding procedures enables scientists to quickly modify genes and introduce the desired gene into the genome. A very important and ground-breaking technique for gene editing is CRISPR/Cas9. Thus, using this method to improve crop quality, productivity, and resistance to disease might be a major area of study in the future. It has been dynamically implemented in many plant systems during the last five years in order to carry out real-world research, counteract stress-induced reactions, and improve notable agronomic characteristics. Still, a number of adjustments to this instrument ought to raise the intended efficacy, and the majority of the research is preliminary and needs more work. However, CRISPR/Cas9-based genome editing will gain recognition and prove to be an essential technique for producing "suitably manipulated" plants, which will aid in achieving the goal of eradicating hunger and ensuring sustainable food supply for the world's growing population. The development of contemporary breeding techniques has been widely acknowledged as a fresh approach to our power to modify genomes, which has challenged our understanding of current regulatory guidelines. Given the widespread use of GE instruments in plants, the topic of GE plant protection deserves international attention. It is essential that the regulations governing innovative crops be multifaceted, accurate, and able to distinguish between genetically engineered (GE) and genetically modified (GM) crops. With the use of cutting-edge CRISPR/Cas9 techniques, next-generation sequencing, functional genomics research, and innovative systems biology, smart crop creation with increased yield and improved characteristics will be possible. Global food security may be achieved via the use of speed breeding programmes and the CRISPR/Cas9 technology. The benefit of combining genome editing with next-generation sequencing is the CRISPR/Cas9 technology. Researchers may now carry out thorough mutational screening. At every stage of the process, optimising and carefully constructing gRNAs is crucial to prevent or lessen the negative impacts of off-target gene editing. As a result, there are several benefits to using the CRISPR/CAS9 library, including high multiplexing, specificity, and high targeting efficiency of a gene. It is crucial to do a quality check of the CRISPR library at every stage of the screening process in order to minimise or eliminate unwanted findings. To understand the function of genes, analysis of gene function using the aforementioned technique is essential. The ongoing descriptions of recently disclosed CRISPR/Cas9 techniques and the creation of new tools indicate that the CRISPR/Cas9 toolkit for plant engineering will continue to grow in the future. With the use of this collection of technologies, it will be possible to do specified genome editing in plants that have had their genomes modified without leaving any transgenic residue.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mostafa K, et al. Abiotic and biotic stress tolerance in plants via genome-editing tools. Genome Editing and Global Food Security, Routledge. 2023; 133-154.
2. Aman Mohammadi M, et al. CRISPR-Cas engineering in food science and sustainable agriculture: Recent advancements and applications." Bioprocess and Biosystems Engineering 2023;46(4):483-497.
3. Guo Y, et al. CRISPR/Cas9 gene editing technology: a precise and efficient tool for crop quality improvement. Planta. 2023; 258(2):36.
4. Abdallah NA, et al. Genome editing techniques in plants: a comprehensive review and future prospects toward zero hunger. GM Crops & Food. 2021;12(2): 601-615.
5. Abdul Aziz M, et al. Genetically engineered crops for sustainably enhanced food production systems." Frontiers in Plant Science. 2022;13:1027828.
6. Asmamaw M, Zawdie B. Mechanism and applications of CRISPR/Cas-9-mediated genome editing. Biologics: Targets and Therapy. 2021;353-361.
7. Bao A, et al. The CRISPR/Cas9 system and its applications in crop genome editing. Critical reviews in biotechnology. 2019;39(3):321-336.
8. Chaudhry A, et al. The changing landscape of agriculture: Role of precision breeding in developing smart crops. Functional & Integrative Genomics. 2023; 23(2):167.
9. Elsharawy H, Refat M. CRISPR/Cas9 genome editing in wheat: Enhancing

quality and productivity for global food security—A review. Functional & Integrative Genomics. 2023;23(3): 265.
10. Eş I, et al. The application of the CRISPR-Cas9 genome editing machinery in food and agricultural science: Current status, future perspectives, and associated challenges. Biotechnology advances. 2019;37(3):410-421.
11. Giordano A. From the lab to the field: CRISPR/Cas addressing challenges in agriculture. Journal of Experimental Botany. 2023;74(12):3399-3401.
12. Gupta B, Shrestha J. Abiotic stress adaptation and tolerance mechanisms in crop plants; 2023.
13. Gurunathan S, et al. CRISPR/Cas9 genome editing tool for rice crop improvement. Cornous Biology. 2023;1(1): 1-6.
14. Hafeez U, et al. Advances in Breeding and Engineering Climate-Resilient Crops: A Comprehensive Review. International Journal of Research and Advances in Agricultural Sciences. 2023; 2(2):85-99.
15. Hamdan MF, et al. Genome editing and biotechnological advances for crop improvement and future agriculture. Frontiers in Plant Science. 2023;14: 1132821.
16. Hamza A., et al. CRISPR/cas9: an approach to meet global food demand. Plant Cell Biotechnology and Molecular Biology. 2021;220-233.
17. Hemalatha P, et al. Multi-faceted CRISPR-Cas9 strategy to reduce plant based food loss and waste for sustainable bio-economy–A review. Journal of Environmental Management. 2023;332: 117382.
18. Imran M, et al. CRISPR-Cas9-mediated editing of BADH2 gene triggered fragrance revolution in rice. Physiol. Plant. 2023;175: e13871.
19. Jang G, Joung YH. CRISPR/Cas-mediated genome editing for crop improvement: Current applications and future prospects. Plant Biotechnology Reports. 2019;13:1-10.
20. Jinhua S, et al. Trehalose induced drought tolerance in plants: Physiological and molecular responses. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 2022; 50(1):12584-12584.
21. Karkute SG. et al. CRISPR/Cas9 mediated genome engineering for improvement of horticultural crops. Frontiers in Plant Science. 2017;8:1635.
22. Khuntia R. CRISPR-Cas9 Directed Genome Modification for Abiotic Stress Tolerance Rice; a Review. TESS Res Med Clin. 2022;1(1):104.
23. Kobayashi K, et al. Genetically Modified Rice Is Associated with Hunger, Health, and Climate Resilience. Foods. 2023; 12(14):2776.
24. Lacchini E, et al. CRISPR-mediated accelerated domestication of African rice landraces. PLoS One. 2020;15(3): e0229782.
25. Lakhawat SS, et al. Implications of CRISPR-Cas9 in Developing Next Generation Biofuel: A Mini-review. Current Protein and Peptide Science. 2022;23(9): 574-584.
26. Li Q, Li CH. CRISPR/Cas9 from bench to bedside: What clinicians need to know before application? Military Medical Research. 20207;(1):1-3.
27. Lio A. Assessing the Use of CRISPR-Cas9 in Agriculture. University of Toronto's Journal of Scientific Innovation. 2023;45-56.
28. Liu H, et al. CRISPR/Cas9 technology and its utility for crop improvement. International Journal of Molecular Sciences. 2022;23(18):10442.
29. Mahrukh et al. Tweaking CRISPR/Cas for developing salt and drought tolerant crop plants. Advanced Crop Improvement, Volume 2: Case Studies of Economically Important Crops, Springer. 2023;513-527.
30. Mariyam S., et al. Review on nitric oxide at the forefront of rapid systemic signaling in mitigation of salinity stress in plants: Crosstalk with calcium and hydrogen peroxide. Plant Science. 2023; 111835.
31. Nazir R, et al. Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated genome-editing toolkit to enhance salt stress tolerance in rice and wheat. Physiologia Plantarum. 2022;174(2): e13642.
32. Park JR, et al. Applications of CRISPR/Cas9 as new strategies for short breeding to drought gene in rice. Frontiers in Plant Science. 2022;13:850441.
33. Pirzadah TB, Malik B. Pseudocereals as super foods of 21st century: Recent technological interventions. Journal of Agriculture and Food Research. 2020;2: 100052.

34. Rasheed A, et al. CRISPR/Cas9 is a powerful tool for precise genome editing of legume crops: A review. Molecular Biology Reports. 2022;49(6):5595-5609.
35. Rasheed A, et al. A critical review: recent advancements in the use of CRISPR/Cas9 technology to enhance crops and alleviate global food crises. Current Issues in Molecular Biology. 2021;43(3):1950-1976.
36. Razzaq A, et al. Modern trends in plant genome editing: an inclusive review of the CRISPR/Cas9 toolbox. International Journal of Molecular Sciences. 2019; 20(16):4045.
37. Saini H, et al. CRISPR/Cas9-gene editing approaches in plant breeding. GM Crops & Food. 2023;14(1):1-17.
38. Shaniware YA, et al. The roleof geneticsand plant breeding for crop improvement: Current progress and future prospects. Int. J. Plant Soil Sci. 2023; 35(20):190-202.
39. Son S, Park SR. Challenges facing CRISPR/Cas9-based genome editing in plants. Frontiers in Plant Science. 2022;13: 902413.
40. Straiton J. Biohacking the food chain: Using CRISPR to combat the global food crisis, Future Science; 2022.
41. Tuncel A, et al. Genome-edited foods. Nature Reviews Bioengineering. 2023; 1-18.
42. Valdés E, Lecaros JA. Ethical issues in genetically modified foods: From transgenesis to CRISPR-Cas9 Genome Editing Technology. Handbook of Bioethical Decisions. Decisions at the Bench, Springer. 2023;I:723-736.
43. Verma NV, et al. Emerging extraction and diagnostic tools for detection of plant pathogens: recent trends, challenges, and future scope. ACS Agricultural Science & Technology. 2022;2(5): 858-881.
44. Yigider E, et al. Advances in bread wheat production through CRISPR/Cas9 technology: A comprehensive review of quality and other aspects. Planta. 2023; 258(3):55.
45. Yijun G, et al. The intervention of classical and molecular breeding approaches to enhance flooding stress tolerance in soybean–An review. Frontiers in Plant Science. 2022;13:1085368.
46. You S, et al. The development and application of CRISPR/Cas9 genome editing platform. Journal of Taiwan Agricultural Research. 2019;68(4):274-292.
47. Zaib M, et al. Drought stress and plants production: A review with future prospects.
48. Zhang D, et al. CRISPR/Cas: A powerful tool for gene function study and crop improvement." Journal of Advanced Research. 2021;29:207-221.

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