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Review Article

Gene Editing and Its Role in Current Crop Improvement

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Abstract

Plant breeding has entered a new era with the advent of current DNA-technology tools such as genome editing. Genome editing has a lot of promise for solving some of the issues that breeders are now dealing with. The need for food and resources will continue to rise around the world, while natural resources used in food and biomass systems become scarce, and ecologically valuable natural landscapes that contribute to biodiversity are disappearing at an alarming rate. Genome editing is simple to use, inexpensive, and quick. Genome-editing tools are cutting-edge biotechnological methods that allow for accurate and efficient genome change in living organisms. It is a group of modern molecular biology techniques that allow for accurate, efficient, and targeted changes to genomic regions. For introducing desirable features in crops, genome editing is more accurate than traditional crop breeding approaches, as well as many typical genetic engineering (transgenic) procedures. Genome editing refers to a set of molecular procedures that allow organisms' genomes to be modified in specific ways. In current plant breeding, gene editing is critical for increasing yield, improving nutritional quality, and developing tolerance to biotic and abiotic conditions that impede crop development. The goal of this review is to analyze gene editing, its techniques, and its function in modern agricultural improvement in general.

Keywords: gene editing; crop improvement; plant breeding; techniques; crop

1.Introduction

Crop improvement is highly reliant on genetic variety that occurs naturally as a result of mutation (Nasti et al., 2021). When paired with the knowledge gained by mapping genes for desirable qualities on genomes, gene editing gives a precise approach for developing plant and animal lines that will flourish in new settings, boost yields, create higher quality food, and provide items for new markets (Xu et al., 2017). The method of making precise, targeted sequence alterations in the deoxyribonucleic acid of living cells and animals is known as genome editing (Rees et al., 2018).

Plant breeders respond to all of these difficulties for sustainable agriculture by developing new varieties and employing the best breeding methods available (Bohra et al., 2020). Breeding's purpose is to use genetic variances to introduce desired features. Spontaneous mutation, chemical mutagenesis, and physical mutagenesis are all examples of how genetic changes can originate (Sattar et al., 2021). Gene editing is one of a group of modern biotechnologies aimed at altering the genomes of living creatures for medical or economic reasons. Gene editing can be utilized to attain the same goals as traditional crossbreeding in agricultural applications (Comstock and Comstock, 2000). Gene editing allows for the modification of specific features in plants and animals, whereas other biotechnologies like crossbreeding are less specific (Van Eenennaam et al., 2019). Other technologies might provide the intended improvement in one feature, but they might modify other traits at the expense of health or productivity.

Gene editing gives scientists a rapid and precise approach to modify plants and animals, allowing agriculture to stay sustainable and productive in the face of a changing climate and increasing global population pressure (Qaim, 2020). Gene editing in plants is frequently achieved by changing the genome of cultured cells and then regenerating whole plants by exposing the transformed cultured cells to growth hormones (Baumann, 2020). For thousands of years, crop improvement has been a continual process (Voss-Fels et al., 2019). Natural variability, selection from closely related species, and some spontaneous mutations drew a lot of attention in the early years (Hwang et al., 2019).

Global food security is being strained by the world's growing population and changing environment (Bangira, 2018). In order to produce genetic resources with varied characteristics for breeding, both spontaneous and induced mutations have been extensively used (Jo and Kim, 2019). Genome editing tools are cutting-edge biotechnological methods that allow for accurate and efficient genome change in living organisms (Zhang et al., 2018). Plant genome editing was once thought to be a science fiction fantasy. In theory, crop breeders might introduce or delete only the required feature by editing a specific gene in the desired crop (Kim, 2020).

Genome editing has now become a part of plant breeding's innovative history (Nadeem et al., 2018). Genome editing is a collection of contemporary molecular biology tools for making precise, efficient, and focused modifications to genomic areas (Zhang et al., 2018). In a wide range of plant species, genome-editing methods have been employed to find gene functions and improve agricultural aspects. Genome-editing tools have sped up molecular breeding by allowing researchers to precisely and efficiently introduce changes in plants' genetic blueprint (Chen et al., 2019). Because of its simplicity, low cost, and flexibility, genome-editing approaches have been widely adopted by researchers (Vats et al., 2019).

Genome editing tools, particularly the CRISPR/Cas9 technology, expand the possibilities and speed with which organisms can change their genetic material (Kawall et al., 2020). It's a catch-all word for a variety of novel genetic engineering approaches. Most (e.g., ZFN, TALEN, CRISPR/Cas9) contain site-directed nucleases (SDNs), which produce double-strand breaks (DSBs) in DNA at specific, predetermined target places. Genome editing is more precise than traditional crop breeding and several typical genetic engineering (transgenic) approaches for introducing desirable features in crops (Rostoks, 2021). These technologies enable the addition, removal, or change of genetic material at particular locations throughout the genome (Goldstein et al., 2005). One of the most pressing issues regarding genome editing is whether plants developed using this technique should be classified as GM crops or treated similarly to crops created via regular plant breeding (Eriksson and Ammann, 2017). The goal of this review paper is to assess gene editing, procedures, and its role in modern crop development.

2. Literature Review

2.1 Potential uses of genome editing in agriculture

Plant breeding began with the identification of particularly desired characteristics (Brummer et al., 2011). Cross, hybrid, and mutant breeding followed, by genetic engineering and marker-assisted breeding, to mention a few breeding strategies. These advancements were required in order to supply fresh solutions to society's ever-increasing demands. Genome editing refers to a set of molecular procedures that allow organisms' genomes to be modified in specific ways (Duensing et al., 2018). The method of making precise, targeted sequence alterations in the deoxyribonucleic acid of living cells and animals is known as genome editing. Recent advancements have made genome editing widely applicable, allowing basic and applied biology to proceed more quickly (Joung et al., 2013).

DNA targeting, or our capacity to transport molecular reagents to precise places in complicated genomes, is essential for gene editing (Nastiet al., 2021). Genome editing methods can help overcome the limits of genetic linkage between distinct qualities that can occur in traditional plant breeding (Kawall et al., 2020). Many plant species have complex genomes that differ in size and organization greatly. Plant breeding is complicated by polyploidy, a large number of orthologous genes, heterozygozity, repetitive DNA, and linkage drag (Udall and Wendel, 2006).

Due to the complexity of plant genomes, which involves the targeting of many genes, traditional breeding and mutagenesis procedures that use chemicals or radiation to generate mutations in plants confront significant challenges (Podevin, et al., 2013). Genome editing has been used to test options for overcoming conventional breeding's constraints. CRISPR/Cas's genome editing techniques allow for complex alterations to genomes that were previously unattainable (Scheben et al., 2017).

2.2 Genome editing methods

The term "genome editing" encompasses a wide range of cutting-edge genetic engineering techniques. Site-directed nucleases (SDNs) produce double-strand breaks (DSBs) in DNA at specified, predetermined target loci (e.g., ZFN, TALEN, CRISPR/Cas9) in the majority of them (Kawall et al., 2020). They're all capable of precisely and precisely changing individual DNA building blocks. The new procedures can be applied in a number of different ways. Some of them can yield genetically modified plants depending on their application (Pickar and Gersbach, 2019).

2.2.1 Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas9)

CRISPR-Cas9 genome editing has been developed and used to obtain broad germplasm resources with genetic variability, thanks to the outcomes of whole genome sequencing and functional genomics investigations in crops (Wan et al., 2021). CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a novel genome editing method introduced in 2013. (Horii and colleagues, 2013). The CRISPR-Cas9 system, which uses site-directed nucleases to precisely target and modify DNA, is a plant breeding breakthrough. CRISPR has been a big part of the current explosion in genome editing research. The method has numerous uses in plant and animal breeding, as well as medicine. As a comparatively new method, it is ripe for new discoveries and advancements that will allow it to be used more effectively in a wider range of applications (Ding, et al., 2016).

Engineered CRISPR systems are rapidly improving in terms of efficiency, flexibility, and precision, allowing them to suit a wide range of needs for targeted gene alterations (Mao et al., 2019). The most powerful gene editing tool known, the CRISPR/Cas9 system, is an RNA-directed DNA endonuclease derived from the bacterial immune system. A CRISPR RNA (crRNA) molecule for target identification, a trans-activating crRNA (tracrRNA) molecule for crRNA maturation, and a CRISPR RNA (crRNA) molecule for crRNA maturation make up the Cas9 protein. CRISPR/Cas's technology allows for a variety of DNA sequence alterations (Demirci et al., 2018). Furthermore, when compared to ZFN or TALEN, this gene editing method is far less expensive, faster, more efficient, and simpler to implement. This strategy is based on the use of adaptive "immunity" mechanisms observed in bacteria, which is a specific antiviral defense of bacterial cells based on complementary binding of viral DNA and subsequent destruction of viral DNA (Gupta et al., 2017).

The CRISPR/Cas9 technique makes gene editing simpler, less expensive, more precise, and adaptable (as several genes can be edited at one time). In current years, it has resulted in a significant embrace of gene editing by researchers and biotech companies (Gupta et al., 2019). The method employs the clustered regularly interspaced short palindromic repeat (CRISPR) and the related protein Cas9. CRISPR-Cas9 has received a lot of attention in recent years due to its diverse set of applications, which include biological research, agricultural crop and animal breeding and development, and human health applications (Zhang et al., 2021). These techniques include gene silencing, DNA-free CRISPR-Cas9 gene editing, homology-directed repair (HDR), and transient gene silencing or transcriptional repression. In this technique, small guide RNAs (crRNA) are used to interfere with foreign nucleic acids in a sequence-specific manner (Arora et al., 2017).

CRISPR can be used for DNA-free gene editing without the use of DNA vectors, using only RNA or protein components. Unwanted genetic modifications induced by plasmid DNA integrating at the cut region or random vector integrations could be avoided with a DNA-free gene editing approach (Kelley et al., 2016). According to researchers, CRISPR-Cas9 has been discovered to work in almost every organism. The first CRISPR-Cas9 gene editing experiments focused on crops that are vital to agriculture. Early on, it was found that the system might be utilized in crops to increase features like yield, plant architecture, attractiveness, and disease resistance (Egelie, et al., 2016).

2.2.2 Zinc finger nucleases (ZFNs)

Several transcription factors have zinc finger motifs (Takatsuji, 1998). The C-terminal region of each finger is responsible for recognizing specific DNA sequences. Zinc finger nucleases (ZFNs) were the first artificial endonucleases developed for gene editing (Durai et al., 2005). Each ZFN is made up of the nonspecific endonuclease FokI and a DNA binding domain with a few linked zinc finger (ZF) motifs. The connection of ZF motifs promotes the production of ZF proteins (ZFP), which have a zinc-ions-chelated structure and contain roughly 30 amino acids (Kamburova et al., 2021). Combining ZFP with methylase, FokI, and a transcription activator/repressor result in ZFN (Lee et al., 2019).

By crossing with DNA and creating a -helix into the major groove of the DNA double helix, each ZF motif can bind one triplet of nucleotides (Mani et al., 2005). It's also worth noting that one ZF isn't precise enough to bind to the target genome (Filippova et al., 2002). Artificial ZFNs, on the other hand, typically have three or four ZFs, allowing the 18-24-mer site to attach after FokI dimerization, which is essential for efficient DNA restriction (Kamburova et al., 2021). During FokI dimerization, two ZFNs can bind both forward and reverse DNA strands, therefore a spacer sequence of 5 to 7 bases should be used to separate the forward and reverse target sequences (Zhang et al., 2010).

ZFNs have been successfully used for gene modification in plants since 1996, according to the first study (Van et al., 2019). Tobacco and

Arabidopsis genomes were edited using ZFN technology. To restore the function of the GUS: NPTII reporter gene in tobacco, ZFN technology was used (Wright et al., 2005). Inducing ZFN expression under the control of the heat shock protein promoter led in 106 DNA changes in Arabidopsis, including 83 (78%) 1-52-mer deletions, 14 (13%) 1–4-mer insertions, and 9 (8%) deletions with insertions. ZFNs have been utilized to change genes in tobacco, Arabidopsis, maize, soya, canola, and other plants, according to current research. Using ZFNs also allows for the introduction of mutations in the endochitinase-50 gene (CHN50) in tobacco, leading in herbicide resistance to a variety of herbicides (Kamburova et al., 2021).

Despite their success, ZFNs have not gained broad use as a gene editing tool due to a variety of drawbacks (Benabdellah et al., 2020). The essential ones are the creation of protein domains for each unique locus of the genome, the likelihood of improper target DNA cleavage due to single nucleotide alterations, and inappropriate domain interaction (Nemudryi, et al., 2014). Mutations, like ODM, are created at predetermined points. Proteins (zinc finger nucleases) with two functional regions are employed. The zinc finger portion of the protein binds to a specific gene in the plant's genetic material. The nuclease component is in charge of precise DNA cleavage (Shah et al., 2018).

2.2.3 Transcription activator-like effector nucleases (TALENs)

In 2011, the TALEN (Transcription Activator-Like Effector Nucleases) technology was created to improve genome editing efficiency, safety, and accessibility (Sun et al., 2013). The TALEN system was developed from transcription activator-like effectors (TALES) generated by phytopathogenic bacteria of the Xanthomonas genus. The restriction endonuclease FokI's catalytic domain is combined with suitable monomers of the DNA-binding domain to construct synthetic restriction enzymes that can target any nucleotide sequence in the genome (Bogdanove et al., 2011). 15–30 copies of 33–34 highly conserved amino acid sequences are found in Tale's domains (Gaj et al., 2013). The 12th and 13th amino acid residues are the exceptions, as they have a lot of variation (repeat-variable diresidues RVD). It enables the recognition code for certain nucleotides to be established utilizing a pair of such amino acids within a protein's repeating peptide chains (Brueggeman, 2013). The number of amino acids between the TALE domain and FokI, as well as the base number between binding sites, affects the activity of TALEN (Kamburova et al., 2021).

The number of mutations seen during TALEN gene editing demonstrates that deletions outnumber insertions (89 percent versus 1.6 percent). The longer TALEN spacers give more extended protruding ends for DNA fragments after DSBs, which is the cause (Campbell et al., 2013). The use of TALEN might theoretically allow for the introduction of DSB into any portion of the genome. The only constraint is the presence of thymidine upstream of the 5' end of the target sequence for the TALEN nuclease recognition sites. However, varying the length of the spacer allows for the selection of restriction sites (Kamburova et al., 2021). A protein with two functional sections (DNA-binding area and nuclease) is responsible for recognizing a specific segment in the genetic material and cleaving the DNA at that point, similar to zinc finger. There are no genes from a foreign or closely related species integrated. At predetermined points, mutations are formed (Gupta et al., 2019).

2.3 Using of genome editing approaches in plant breeding

Genome editing technologies are now being successfully employed to develop new agricultural crop types with enhanced features such as increased yield, product quality, and tolerance to biotic and abiotic challenges (Ahmar et al., 2020). Such features are frequently improved by introducing target mutations into the regulatory genes that regulate the development of undesired traits, causing their activity to be suppressed (Tang et al., 2017).

2.3.1 Crop yields increase

The most economically valuable characteristics of agricultural crops is productivity (Adeniyi et al., 2018). At the same time, using traditional breeding procedures, this feature is one of the most difficult to develop. It's because yield is frequently a quantitative multigenic trait whose development is influenced by numerous quantitative trait loci (Tester et al., 2010). Furthermore, QTL introgression between distinct varieties complicates classic yield-based selection, which is especially obvious in the case of tightly connected loci (Kamburova et al., 2021).

Genome editing methods are a promising approach for mutagenesis of target genes in a controlled and quick manner (Yin et al., 2017). Knocking off ("turning off") genes that negatively affect yield is the most effective technique to boost yields utilizing genome editing technologies (Tang et al., 2017). Rice yields improved as a result of CRISPR/Cas9-based "turning off" of the functions of yield negative regulators (Gnla, DEP1, and GS3), which manifested as more grains in panicles and larger grains, respectively (Kamburova et al., 2021). This gene loss is hereditary and can be seen in at least the T2 generation.

Furthermore, there is evidence that multiplex CRISPR/Cas9 deletion of the primary negative regulators of rice grain weight (GW2, GW5, and TGW6) allows for a considerable rise in grain weight (Fiaz et al., 2019). CRISPR/Cas9-mediated deletion of the GASR7 gene produced similar findings (a negative regulator of the wheat grain width and weight). Furthermore, CRISPR/Cas9-mediated deletion of genes involved in yield improvement allows for the modification of this economically beneficial characteristic in a variety of other crops (Bhat et al., 2020).

2.3.2 Product quality improving

Genome editing can improve the nutritional qualities of crops, resulting in healthier food. Quality of products is another economically useful feature whose selection by traditional methods is fraught with obstacles (Vaz Patto et al., 2015). As a result, selecting for this characteristic is confounded by the difficulty of acquiring targeted mutations using chemical and physical mutagenesis procedures, as well as the occurrence of negative correlations between quality and yield attributes (Roychowdhury et al., 2013). Genome editing technologies allow us to overcome the constraints of chemical and physical mutagenesis by introducing precise mutations into the genome and improving the nutritional quality of crops (Georges et al., 2017).

The use of genome editing techniques is critical for changing the chemical composition of plants (Eckerstorfer et al., 2019). For example, using TALEN and CRISPR/Cas9 systems to silence one of the major genes in phytate production, ZmlPK, corn's phytate content was reduced (Zea mays). Because phytate is regarded an anti-nutritional ingredient, it reduces the availability of proteins and minerals for digestion, the feed value of such corn grain is substantially greater (Kamburova et al., 2021). TALEN-mediated deletion of the HvPAphy gene, which plays a crucial role in phytate production, yielded similar results in barley (Matres et al., 2021).

2.3.3 Herbicide resistance improving

Herbicides are the most commonly utilized chemical compounds in agricultural operations (Jayaraj et al., 2016). This is because weeds do major damage to agriculture, diminishing output owing to resource competition with crops. However, despite the herbicides' success, their principal drawback is their non-selective action. Herbicide-resistant biotechnology cultivars were developed utilizing genetic engineering technologies to circumvent this disadvantage (Peltzer et al., 2009). Currently, transgenesis has been used to obtain all herbicide-resistant cultivars that have been approved for use. At the same time, genome editing techniques can be used to develop herbicide-resistant crop lines (Ricroch et al., 2016).

The EPSPS and ALS genes are the key genes targeted by genome editing in the creation of herbicide-resistant lines. The ALS gene codes for acetolactate synthase, which is involved in branched-chain amino acid biosynthesis, and the EPSPS gene codes for 5-enolpyruvylshikimate-3-phosphate synthase, which is involved in the production of important plant aromatic amino acids (Brasileiro et al., 2001). Thus, targeting the ALS gene for sulfonylurea herbicide resistance and the EPSPS gene for glyphosate resistance can be expected based on the genes' activities. These lines emerged through ODM-mediated targeted mutagenesis of the ALS gene in tobacco, rice, corn, and wheat (Songstad et al., 2017). Herbicide-resistant rice (Oryza sativa), wheat (Triticum aestivum), potato (Solanum tuberosum), and watermelon

(Citrullus lanatus) lines were created using single base mutations in the ALS gene. Furthermore, CRISPR/Cas9 allows for the generation of glyphosate-resistant flax (Linum usitatissimum) and herbicide-resistant rice lines by replacing two nucleotides in the EPSPS gene (Mishra et al., 2020).

2.3.4 Biotic stress resistance improvement

Biological stresses are one of the most common causes of crop losses in agriculture. The most prevalent biotic stressors that harm crops include phytopathogens (viruses, bacteria, and fungi), insects, and pests (phytophagous insects, acari, and nematodes) (Diaz, 2018). Increased self-defense systems in plants or the introduction of pathogen-targeting structures into the genome are two strategies for dealing with biotic stressors (Barea, 2015). Traditional breeding approaches for developing pathogen and pest-resistant crop lines rely on enhancing the plant's own defense systems, whereas genetic engineering is used to introduce pathogen-targeted structures into the genome. Transgenesis or RNA interference (RNAi) technologies have been used to develop the majority of biotechnological crop lines resistant to biotic stressors to date (Conner et al., 2003).

Gene editing techniques are now commonly employed to develop new resistant lineages. Plant susceptibility genes are critical for pathogen infection and growth. Resistance lines of barley, wheat, Arabidopsis, tomato, and pea can be obtained by targeting TALEN- and CRISPR/Cas9-based MLO homologues that provide resistance to powdery mildew. The production of virus-resistant crops has also relied on the targeting of disease susceptibility factors (Bisht et al., 2019). Since virus-resistant plants can be generated via CRISPR/Cas9-based silencing of eIF4e components associated to plant infection by positive sense RNA viruses. CRISPR/Cas9-mediated reduction of eIF4Es gene function enhances potyvirus resistance in Arabidopsis and cucumber. In order to generate crop lines that are resistant to biotic stress, gene editing (GE) techniques are also used to modify regulatory elements that can affect the process of pathogen growth (Kamburova et al., 2021).

2.3.5 Abiotic stress resistance improvement

Abiotic stressors are the primary variables that have a detrimental impact on most crop yields (Kumar et al., 2019). The important concern in this regard is the development of crop types that are resistant to harmful environmental influences (Snow et al., 2005). Traditional breeding approaches, on the other hand, are limited in developing such varieties because abiotic stress resistance traits are multigene regulated and have a complex inheritance pattern (Kamburova et al., 2021). Traditional breeding's drawbacks can be successfully solved with the help of GE methods. A study of the literature has revealed that the use of gene editing in several cultures increased their tolerance to abiotic stressors (Lv et al., 2020). One of the most practical targets for increasing plant stress tolerance is structural genes. Tolerance genes (T-genes) and sensitivity genes (S-genes) are two types of genes in this category (Zafar et al., 2020). T-genes code for antioxidant enzymes, whereas S genes act as negative regulators in plant defense processes. As a result, "turning off" S-genes enables for the development of drought-resistant crop varieties (Joshi, et al., 2020). CRISPR/Cas9-based targeting of ARGOS8 (a negative regulator of ethylene response) in maize confirmed this hypothesis, allowing drought-resistant lines to be obtained (Kamburova et al., 2021).

3.Conclusion

Genome editing is a cutting-edge crop improvement technology that is specific, precise, fast, and versatile. Gene editing tools are recognized as one of the most promising technologies for practical agricultural biotechnology because of their high efficiency, low cost, ease of use, and multiplexing potential. Genome editing is increasingly being employed in both plants and animals to achieve agriculturally relevant novel features and/or genetic combinations. Genome-editing tools have ushered in a new era of genome engineering, allowing for the accurate, quick, and effective engineering of plant genomes. Finally, gene editing technology offers the potential to develop novel types of crops that are resistant to biotic and abiotic challenges, as well as increased food value and production. But, in order to efficiently use these technologies, concerns relating to biosafety evaluation, including regulatory framework change, must be resolved.

Conflict of interest

There are no conflicts of interest declared by the authors.

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