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Genomic editing techniques for ensuring food security: CRISPR Cas, TALEN, ZFN, RNAi and mutagenesis

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Abstract

The agricultural sector is increasingly challenged by problems such as resource scarcity, nutrient deprivation, climate change, biotic and abiotic stress amid the increasing need of food security for ever-increasing global population. Traditional breeding methods, though applicable, are often time-consuming and ineffective in immediately addressing these issues. Genome editing technologies, such as CRISPR Cas, TALEN, ZFN, RNA interference (RNAi), and mutagenesis, offer transformative solution by enabling precise and fruitful modifications to plant genomes. These techniques facilitate rapid crop improvement, improve yields, and increase resistance to biotic and abiotic stresses, thereby fostering sustainability in agricultural systems. This review examines the principles, applications, and limitations of these genome editing techniques, emphasizing the importance of selecting appropriate techniques for attaining goals and applications. Every technique has its pros and cons. CRISPR Cas stands out for its simplicity and efficiency, while TALEN and ZFN offer higher specificity. RNAi provides a means to modulate gene expression without altering the DNA sequence, and mutagenesis generates genetic diversity through inducing random mutations by physical and chemical means. Understanding the functioning mechanisms, strengths and weaknesses of each technique is vital for its application in optimizing crop productivity and addressing global food security. This review aims to guide researchers in choosing the most suitable genomic editing tools to boost crop productivity and resilience in the face of evolving global challenges.

Keywords: CRISPR Cas9, TALEN, Zinc Finger Nucleases, RNA Interference, Mutagenesis

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Introduction

The agricultural industry faces tremendous pressure to produce more food with fewer resources while confronting climate change. Conventional breeding methods are time consuming and limited in their ability to produce desired quantities and quality of yield traits. Genome editing technologies enable precise and efficient modifications to the genetic code to for crop improvement and address pressing challenges like ensuring global food security. CRISPR Cas's simplicity and ease of design and implementation make it a highly effective and attractive option for making desired genomic alterations compared to Zinc Finger Nucleases (ZFN) as well as transcription activator like effector nucleases (TALEN) (Jw et al., 2015). This powerful technology can introduce specific mutations in diverse plant species, including potato, sweet potato, strawberry, grapes, citrus, and banana (Nadakuduti et al., 2018). Its efficacy in augmenting resistance of plants against both abiotic and biotic stresses is well-documented (Halder et al., 2022). In potatoes alone, CRISPR Cas facilitates improvements in cold-induced sweetening tolerance, effective processing, herbicide resistance, starch quality alterations, and self-incompatibility (Nadakuduti et al., 2018). ZFN and TALEN, like CRISPR Cas, induce Double strand breaks (DSBss) at targeted sites, relying on cellular repair mechanisms for correction. Their use leads to the creation of knockout mutants in many plant species (Hansen et al., 2012). RNA interference (RNAi) is another effective strategy used to target and degrade specific mRNA molecules, resulting in improved disease resistance and addressing the problem of agricultural waste caused by pests and pathogens (Halder et al., 2022). Induced mutagenesis is a functionally distinct yet another tool for creating genetic diversity and identifying primary regulatory genes for economically important traits for crop improvement (Chaudhary et al., 2019). While these genome editing techniques promise significant potential in crop improvement, comparative studies to comprehensively account for pros and cons of each technique, to identify the most suitable applications for specific crop traits and environmental conditions are limited. In this review, we will first explore five genomic editing techniques, examining their construction, mechanisms of action, and applications. Following that, we will compare and contrast these techniques—CRISPR Cas, Mutagenesis, Zinc Finger Nucleases, TALEN, and RNA Interference—with a particular emphasis on their use in enhancing crop improvement.

CRISPR Cas system

CRISPR Cas offers precise alteration of genome sequences of many crop varieties for the purpose of increasing genetic diversity and accelerating breeding efforts (Khurshid et al., 2018; Rajput et al., 2021). It facilitates the targeted mutation of

genes, gene knockouts, and gene insertion or replacement at specific sites within the DNA. This is done by activating DNA repair process such as: homologous recombination (HR), non-homologous end joining (NHJ) and generating DSBss (Zhou & Xing, 2016).

Components and working of the CRISPR Cas System

The elements of CRISPR Cas system are the Cas enzyme which act as the molecular cutter and the guide-RNA (gRNA): which direct Cas enzyme to targeted genomic location, to cleave the DNA. (Hampton, 2017). The cellular DNA repair process then take care of this cleavage, resulting into insertion, deletion, or replacement of aimed DNA sequences. The gRNA is complementary to target DNA sequence, enabling Cas enzyme and gRNA forming a complex that specifically recognizes and anneal to the targeted DNA sequence. (Sugano & Nishihama, 2018).

Pros and cons of CRISPR Cas system

CRISPR Cas offers notable advantages due to its precision and specificity, enabling targeted modifications to the genome and yielding more predictable and consistent outcomes while being a user-friendly and cheap genomic editing tool compared to other alternatives. High efficiency of CRISPR Cas in gene mutation and modification makes it a promising technique for enhancing crops (Rao et al., 2022). Its specificity in targeting specific genes reduces the risk of off-target effects, while its affordability makes it more accessible for crop improvement (Rao et al., 2022), by enabling accurate alterations to plant genome, such as gene knockout, knock-in, gene regulation, or enabling new gene functions (Zaidi et al., 2020) resulting in increased yield, grain quality, and resistance to abiotic plus biotic stressors (Jagadeesh et al., 2022). Rukavtsova et al. (2022) noted that CRISPR Cas holds potential for; increasing resistance to biotic and abiotic stress agents, altering flowering and fruit ripening times, altering plant growth traits, and bettering fruit taste. However, CRISPR Cas also has limitations. Off-target Mutations are a significant concern, leading to unintended modifications in the genome. These effects can cause uncontrolled mutations, disrupting important genes or introducing new mutations, thereby impacting the safety and efficacy of gene editing (Wei et al., 2021). The risk of Off-target Mutations is a shortcoming to the widespread application of CRISPR technology (Guo & Zhen, 2020). CRISPR Cas offers the advantage of multiplexing, allowing for targeting of multiple genes at the same time. However, it is important to acknowledge potential Off-target mutations, and the relatively low efficiency of homology directed repair (HDR) in certain plant species (Jiang et al., 2013). Many factors impact the effectiveness and precision of CRISPR Cas, such as guide RNA design, delivery method, cell type, and multiplexing. Guide RNA design plays a crucial role, requiring specific targeting of the desired genomic region while minimising unintended effects. Its length, GC content, and secondary structure can further impact efficiency (Hu et al., 2019). Virus-based delivery methods offer promising solutions to overcome the limitations of traditional genetic transformation and editing across diverse plant cultivars (Wang et al., 2022). RNA virus-based delivery of guide RNA provides significant advantages over conventional methods due to the rapid amplification of gRNAs during viral replication and movement in plants (Hu et al., 2019). While lentiviral vectors have been developed for transient nuclease expression, their *in vivo* efficacy for biomedical applications needs further investigation (Ling et al., 2021). Additionally, the specific cell type being targeted significantly impacts efficiency and precision, as some cells are more susceptible to gene editing than others (Ling et al., 2021). Multiplexing, targeting multiple genomic loci simultaneously, also presents challenges in maintaining efficiency and precision. However, pooled barley stripe mosaic virus-gRNAs can generate aimed deletions and multiple mutations which are heritable (Wang et al., 2022). Off-target Mutations occurs with the use of CRISPR Cas system leading to undesired modifications to plant genome and occur when the Cas enzyme cuts the DNA at a site which is not aimed at. Examples of these off-target Mutations include the introduced unwanted mutations that can be inherited down the generations (Khan & Ullah, 2021), depletion of unintended transcripts (Stojic et al., 2018), clonal variation in the transcriptional profile (Stojic et al., 2018) and differentially expressed genes and cellular phenotypes upon knockdown (Stojic et al., 2018). Strategies are being developed to minimise off-target effects: using more specific guide RNAs and optimising delivery methods, alternative versions of the Cas enzymes have been synthesized, such as Cas9 nickase and Cas9-HF, which confirm reduced Off-target Mutations (Chen et al., 2016). Additionally, bioinformatics algorithms are available to predict off-target scores, optimising the sequence of guide RNAs and reducing Off-target Mutations (Guo & Zhen, 2020). Integration of multi-omics approaches with CRISPR Cas improves the precision of goal oriented genomic editing in crops (Yang et al., 2021).

Practical applications of CRISPR Cas system in crop improvement

CRISPR Cas has many practical applications in crop improvement; including increased yield, betterment of nutritive quality (Liu et al., 2021), improving stress tolerance, increasing disease and pest resistance (Rasheed et al., 2021), optimising post-harvest traits, enhancing tolerance to abiotic stresses (Abdelrahman et al., 2018), improving water use efficiency, enhancing photosynthesis efficiency, optimising soil fertility and nutrient usage, improving seed quality and germination rates (Liu et al., 2021), reducing environmental impact, developing crop varieties for specific environmental conditions, establishing beneficial symbiotic relationships with microorganisms, and generating crops for alternative uses (Ricroch et al., 2017). CRISPR Cas9 created a wheat variety, resistant to powdery mildew, through altering genes associate with susceptibility to the disease, (Zhang et al., 2018). Furthermore, CRISPR Cas9 mutated the OsSWEET14 gene in rice plants, resulting in strong resistance against *Xanthomonas oryzae* without affecting yield.

The mutated rice plants also exhibited increased height without compromising reproductive growth, suggesting their potential use as improved tester lines for rice blight resistance diagnostic kits (Zeng et al., 2020). At Cold Spring Harbor Laboratory, scientists employed CRISPR Cas9 for developing a tomato variety which is resistant to *Botrytis cinerea*, a fungal disease responsible for substantial yield losses in tomato crops. By targeting a gene associated with disease susceptibility, they successfully created a highly resistant tomato variety (Perk et al., 2023). CRISPR Cas9 enhanced grain-yield related traits of maize by manipulating CLE genes, which regulate meristem size (Liu, Gallagher, et al., 2021). Additionally, CRISPR Cas9 is being utilised to target genomic regions in maize related to agronomic traits such as; yield, drought tolerance, and disease resistance (Liu et al., 2020; Liu, Gallagher, et al., 2021).

TALEN system

TALEN, is derived from proteins discovered in *Xanthomonas* bacteria, identify and anneal to precise DNA sequences, enabling precise manipulation of the genome (Zhang, Massel, et al., 2018).

Components and working of the TALEN system

A TALEN system is made up of a DNA binding domain plus a DNA cleaving domain. The DNA binding domain is constructed using an array of repetitive units complimentary to targeted nucleotide sequences. By strategically assembling these modular units, researchers can design TALEN capable of targeting virtually any desired genomic sequence. TALEN make DSBss in the DNA after annealing to their target sequence. These DSBss trigger cellular DNA repair process as in CRISPR Cas system, leading to either NHJ or HDR. Non homologous end joining often results in incorporation of insertions or deletions (collectively called indels) at the repair site, altering the gene's function. Conversely, HDR utilises a provided DNA sequence template to accurately repair the DSBs, allowing scientists to introduce specific mutations or precisely insert new genetic material (Becker & Boch, 2021).

Pros and cons of TALEN

TALEN system offers many benefits over ZFN and CRISPR Cas. A major advantage of TALEN is higher specificity for target sites and lower Off-target Mutations (Chattopadhyay et al., 2022). The repeat sequences within TALEN are the reason for specificity to specific sites in the genome, and the identification of repeat variable di-residues within the repeat regions assists in recognizing specific binding targets (Anderson et al., 2019). Manufacturing TALEN is easier than designing ZFN because it does not require the multimerization of repeat sequences to build an extended array of DNA-binding domains. (Gupta & Musunuru, 2014). TALEN system is employed to make specific gene knockouts and knock-ins (Sato et al., 2022). TALEN can also be combined with techniques like in vitro electroporation and in vitro transduction with adeno-associated viruses to introduce genetic engineering components and produce genetically engineered animals (Sato et al., 2022). However, while TALEN were once popular for genome editing, they have been largely replaced by the simpler and more efficient CRISPR Cas system (Sato et al., 2022). Its time-consuming construction and optimization process are a major shortcoming related to TALEN (Bhardwaj & Nain, 2021). Additionally, TALEN are expensive to produce and delivering them into plant cells presents challenges (Weeks et al., 2015).

TALEN is used for genome editing in many plant species such as rice, wheat, soybean, corn, and potato (Malzahn et al., 2017). TALEN offers high specificity and sensitivity toward DNA modifications, making them efficient tools for gene editing (Bhardwaj & Nain, 2021). It is also capable of multiplexing and demonstrate low toxicity (Nerys-Junior et al., 2018). Compared to CRISPR Cas9, TALEN causes fewer off-target mutations (Nejat et al., 2018). Less efficiency of HDR is also a limitation, similar to CRISPR (He et al., 2016). While TALEN have high target specificity, some off-target Mutations still occur. However, these off-target Mutations are lower than those observed with other genome editing tools (Chattopadhyay et al., 2022). Prolonged expression of TALEN can also cause off-target Mutations (Zhang, Massel, et al., 2018). TALEN system is designed to recognize specific nucleotide sequences, which helps in reducing Off-target (Zhao & Wolt, 2017). The hetero-dimerization of FokI nuclease is employed to minimise Off-target Mutations and cellular toxicity associated with TALEN (Kamburova et al., 2021).

Practical applications of TALEN in crop improvement

TALEN system is used to improve crops by enhancing stress tolerance against pests and pathogens, to increase the genetic resistance of rice to pathogens (Chattopadhyay et al., 2022) and to make desirable mutations in the genome agronomically important crops (Tussipkan & Manabayeva, 2021). In potatoes, TALEN system is also used to alter genomic regions associated with browning, lessening the accumulation of reducing sugars and making better the quality of tubers (Ahmad et al., 2022). Furthermore, TALEN system is used to make alterations in the genome of cassava, improving its nutritional content (Tussipkan & Manabayeva, 2021), and to create herbicide-resistant crops, including soybeans and maize (Bilichak et al., 2020). Disease resistant crops are created using TALEN, such as those with knockouts of genes that render rice susceptible to bacterial blight disease (Jiang et al., 2020). Moreover, TALEN system is employed to create crops with improved nutritional content, such as maize with knockouts of genes involved in phytic acid accumulation, enhancing the bioavailability of nutrients like iron and zinc (Gaikwad et al., 2020). Recent research has demonstrated the use of TALEN system with multi-omics technologies; genomics, transcriptomics, phenomics, proteomics, metabolomics, and ionomics. (Abhishek Bohra et al., 2019).

ZFN

ZFN leverage a structural motif known as the "zinc finger,"

derived from zinc finger protein (ZFP), to bind specifically to particular DNA sequences (Negi et al., 2023).

Components and functioning of the ZFN system

Zinc finger nucleases are synthetic proteins used for precise genome manipulation (Hilioti et al., 2016). As described by Xiao et al. (2012), ZFN system is made up of the ZFP and the FokI endonuclease domain. Zinc finger proteins are designed to identify discreet DNA sequences within genome. The FokI endonuclease domain, attached to the ZFP, does DNA cleavage. The FokI endonuclease domain is only active when two ZFN bind to adjacent target sequences within the genome. Upon binding, the FokI endonuclease domains dimerize and induce a DBS in DNA at the targeted target site. This mechanism allows for the incorporation of precise insertions or deletions into the genome (Hilioti et al., 2016).

Pros and cons of ZFN

ZFN offer several advantages over other genomic editing tools i.e. TALEN and CRISPR Cas9. ZFN claim high target sequence specificity and minimal off-target effects, making it a valuable technique for precise genome editing (Palpant & Dudzinski, 2012). ZFN are engineered by combining zinc-finger DNA-binding domains, which recognize specific trinucleotide sequences, to a nuclease. These zinc-finger domains provide the binding specificity for twelve to eighteen nucleotides. The FokI nuclease, which requires two zinc-finger domains to bind upstream and downstream of the cleavage site, further enhances ZFN specificity by targeting 24-36 nucleotides, significantly reducing possibility Off-target Mutations (Davies et al., 2017). Importantly, ZFN system does not leave behind foreign sequences in the final genome-edited organisms, ensuring their safety with genetically modified (GM) crop (Hilioti et al., 2016). ZFN system is relatively affordable in contrast to other gene editing techniques (Ahmar et al., 2020). While ZFN offers several advantages, it does have some drawbacks. The design and construction of ZFN can be time-consuming and costly, and delivering ZFN into plant cells presents challenges (Palpant & Dudzinski, 2012; Borman, 2011). ZFN offer higher precision and reduced Off-target Mutations compared to CRISPR-Cas9. However, synthesis of ZFN is more complex and time consuming, resulting in lower efficiency than CRISPR Cas9 and TALEN (Quazi, 2022). Although ZFN have high target specificity, off-target Mutations can still occur. However, Off-target Mutations with ZFN is generally lower than that observed with CRISPR Cas9 (Palpant & Dudzinski, 2012). Off-target Mutations arise when ZFN modify DNA sequences that are similar (but unidentical) to the target site (Borman, 2011). Additionally, incorporation of undesired foreign DNA into genome is a shortcoming associated with this system (Davies et al., 2017). To mitigate off-target mutations, researchers precisely design and synthesize ZFN, then comes utilisation of multiple ZFN to target the same gene, and screening for off-target mutations (Gaj et al., 2013).

Practical applications of ZFN in crop improvement

ZFN is being used to improve genetic resistance to pathogens in certain food crops (Paschon et al., 2019) enhancing their resistance to diseases and pests (Davies et al., 2017). Furthermore, ZFN is being employed to modify the genome of *Arabidopsis*, improving its nutritional content (Lloyd et al., 2005). ZFN are being recognized as a transformative technology for cotton improvement. enabling precise and targeted mutagenesis, gene knock out, and multisite genome editing in cotton, facilitating the inhibition of undesirable metabolic pathways. ZFN is also being used to target undesirable metabolites like gossypol in cotton seeds, leading to the development of seed-specific low-gossypol cotton (Khan et al., 2018). ZFN is employed for targeted mutagenesis and gene knockout in wheat (Miglani, 2017). Characterization revealed these ZFN-induced mutations primarily consisted of simple deletions, followed by simple insertions (Lloyd et al., 2005). ZFN targeted to promoter region of SlERF3 gene, regulates ethylene biosynthesis and plant defence responses forming tomato exhibiting enhanced resistance to *Botrytis cinerea*, a fungal pathogen.

RNAi

RNAi is the modulation of gene expression without changing DNA sequence. It refers to a molecular technique that silences gene expression (Rajput et al., 2021).

Components and working of the RNAi System for crop improvement

The RNAi system operates through the modulation of gene expression by employing small interfering RNA (siRNA) molecules that selectively target and degrade messenger RNA (mRNA) molecules, thereby instigating the downregulation of gene expression (Rajput et al., 2021). First step in the RNAi is synthesis of siRNA molecules. This is accomplished by introducing a DNA construct harbouring hairpin RNA (hpRNA) into the plant cells. Subsequently, the plant's cellular apparatus processes the hpRNA, yielding siRNA molecules that exhibit complementarity to the target genomic sequence (Bao et al., 2021). These siRNA molecules are integrated into an RNA-induced silencing complex (RISC), comprising proteins that facilitate binding of siRNA to target mRNA molecule (Bao et al., 2021). Once the siRNA associates with the target mRNA, it triggers the degradation of the mRNA, thereby impeding its translation process (Bao et al., 2021). By triggering the degradation of the target mRNA, the RNAi system effectively decreases the expression of certain genes within the crop plants. This mechanism can be employed to silence genes accountable for unfavourable traits or to amplify the expression of genes known for desirable traits (Rajput et al., 2021).

Pros and cons of RNAi

A primary advantage of RNAi is its precision and minimum off-target mutations (Hart et al., 2014). RNAi enables precise targeting of specific genes or gene families, thereby endowing it with significant potency for crop improvement (Herrera-Carrillo & Berkhout, 2017). Moreover, RNAi proves invaluable in suppressing genes that prove challenging to target using other gene editing tools, particularly those involved in intricate metabolic pathways (Alfagih et al., 2021). According to Miglani (2017), RNAi effectively targets specific genes within crop genomes, irrespective of their functions. Furthermore, RNAi stands as a relatively straightforward and uncomplicated technique when compared to alternative gene editing technologies. However, RNAi does carry certain drawbacks, such as the potential for Off-target mutations and unintended results (Ni et al., 2021). Additionally, delivering RNAi into plant cells poses challenges, and its effects are transient in nature (Mamta & Rajam, 2018). On occasions, RNAi may inadvertently impact genes that were not intended as targets, leading to unintended consequences (Negi et al., 2022). Furthermore, incomplete knockdown of the targeted gene is possible with RNAi, resulting in incomplete or unpredictable effects on crop's phenotype (Negi et al., 2022). Lastly, RNAi exhibits transient effects on gene expression, implying that these effects may not endure in the long term or be heritable (Negi et al., 2022). It offers relative ease of design and implementation and allows for multiplexing. However, RNAi does not enable precise genome editing and its effects can be transient and variable in intensity (Fire et al., 1998). RNAi demonstrates remarkable efficacy in downregulating gene expression in crops (Ashok Kumar Meena, 2017). Furthermore, RNAi can be used to target any gene within the crop genome, regardless of its function (Younis et al., 2014While RNAi exhibits high specificity in targeting individual genes, it may also exert off-target mutations on unintended genes (Alic et al., 2012).

Practical applications of RNAi in crop improvement

RNAi is used for gene silencing in many plant species, including rice, tomato, and soybean (Cason & Lord, 2023) to confer resistance against pathogens, insects/pests, nematodes, viruses, and to eliminate allergenic and poor-quality metabolites that result in substantial economic losses (Ashok Kumar Meena, 2017). Its high specificity and sensitivity make RNAi an efficient tool for crop improvement, allowing for precise and effective gene silencing (Dietz-Pfeilstetter et al., 2021). In rice, RNAi is utilised to silence the expression of OsSWEET14 gene, resulting in improved resistance against bacterial pathogen *X. oryzae* pv. *oryzae* (Xoo) (Nikolova & Toncheva, 2008). This targeted gene silencing reduces the need of chemical pesticides in rice cultivation. Similarly, in tomato, RNAi is employed to silence the expression of SlERF3 gene, leading to enhanced resistance against fungal pathogen *Botrytis cinerea* (Kumar et al., 2020). Omega-3 fatty acid desaturase (FAD3) gene family in soyabean which is responsible for synthesising α-linolenic acid (18:3), is targeted for silencing using RNAi. By reducing the levels of α-linolenic acid RNAi enables the development of soybean varieties with enhanced oil quality because it causes instability in soya bean oil (Flores et al., 2008). Such gene silencing strategies hold promise for reducing the usage of fungicides in tomato production.

Mutagenesis

Mutagenesis is a biological process that induces alterations in DNA sequence of an organism by physical or chemical agents, such as radiation or ethyl methane sulfonate (EMS) leading to genomic diversity (Sao et al., 2022) due to a vast array of genetic changes, including point mutations, deletions, insertions, and rearrangements (Chaudhary et al., 2019). These changes can subsequently alter gene expression, protein function, and overall phenotype.

Components and working of the mutagenesis system

This approach is a highly efficient for generating genetic diversity and identifying key regulatory genes associated with economically significant traits as it involves alterations in individual nucleotides, and their impact on protein synthesis can vary (Chaudhary et al., 2019). Both physical and chemical mutagenesis methods are utilised to induce mutations in seeds and other propagating materials (Oladosu et al., 2015). Selection for desirable agronomic traits is carried out in the initial generation, during which many mutant lines may be discarded. Subsequent generations are then evaluated to confirm the stability of agronomic traits through observable phenotypic characteristics, while further assessments are conducted in subsequent generations (Oladosu et al., 2015). Targeting induced local lesions in genomes (TILLING) offers a high-throughput approach for identifying induced mutations in specific genes of interest. It combines mutagenesis with isolating chromosomal DNA from each mutated line, followed by DNA-level screening of the population (Sikora et al., 2011). Plant mutation breeding or variation breeding, utilises bombardment with radiation or chemical agents to create spontaneous genomic variations in plants, enabling the development of novel crop varieties (Oladosu et al., 2015). Irradiation is employed to generate new plant varieties with better agronomic traits; increased yield, faster growth cycles, resistance to diseases, pests, and tolerance to climate change factors like extreme weather events (Oladosu et al., 2015). Induced mutagenesis, along with combined breeding techniques, offers potential to enhance both quantitative and qualitative traits in crops more rapidly than traditional breeding approaches (Oladosu et al., 2015).

Pros and cons of mutagenesis

Mutagenesis offers many edges over other gene editing tools. One is its ability to generate genetic variability without incorporating foreign DNA into genome (Zhang et al., 2023). Mutagenesis enables targeted modification of specific genes or gene families (Fujiwara et al., 2011).

This technique is also valuable for developing new crop varieties tailored to certain environmental conditions, like high-altitude or saline soils (Feldmane & Spalviņš, 2023). Mutagenesis facilitates the rapid generation of genetic diversity in crops, enabling the creation of novel traits and improved crop varieties within a relatively short timeframe (Hoffie et al., 2021). Mutagenesis is a relatively cheaper method for generating genetic diversity in crops (Negi et al., 2022). By producing genetically stable primary mutants, mutagenesis allows for immediate phenotypic analysis and efficient preselection of valuable lines for further investigations (Negi et al., 2022). Importantly, mutagenesis does not leave resides of foreign DNA sequences behind in genome modified plant, ensuring safety for crop improvement (Miglani, 2017). As a well-established technique, mutagenesis is widely employed in crop improvement for many years and has obtained regulatory approval in numerous countries (Negi et al., 2022). While mutagenesis offers several advantages, including speed, cost-effectiveness, safety, and regulatory approval, it also possesses certain limitations; the lack of precision and possibility for unintended mutations (Hoffie et al., 2021). One notable disadvantage of mutagenesis is its susceptibility to off-target mutations and unintended crop varities (Oladosu et al., 2015). Additionally, mutagenesis can generate wide range of mutations, some of which may be detrimental or have unknown effects (Chaudhary et al., 2019). Moreover, mutagenesis is very time-consuming and labour-intensive process that necessitates screening of huge populations of mutants to identify desirable traits (Rabiatul-Adawiah Zainal-Abidin et al., 2021). It offers a highly efficient and precise method for inducing mutations, making it a valuable tool for enhancing crops (Phillips, 2016). Traditional mutagenesis methods, such as chemical or radiation mutagenesis, are relatively straightforward to implement and can generate a wide range of genetic diversity. However, they lack precision and often result in numerous undesired mutations, making the identification of beneficial mutations very time consuming plus labour intensive process (Till et al., 2003). It produces genetically stable primary mutants, facilitating immediate phenotypic analysis and efficient preselection of valuable lines for further investigations (Hoffie et al., 2021). However, mutagenesis is less precise than ZFN and CRISPR Cas9 (Hoffie et al., 2021; Negi et al., 2022). Unwanted mutations can be introduced into the genomes of crops through mutagenesis, potentially limiting its utility compared to other gene editing technologies (Miglani, 2017). Previous approaches utilising random mutagenesis and conventional genetic recombination in cereal crop improvement have exhibited high risks of Off-target Mutations (Basu et al., 2023). Mutation breeding can lead to specific improvements without significantly altering the crop's phenotype, but it can also result in unintended consequences on agronomic traits; number of tillers, plant height, spike length, and days to heading (Chopra, 2005). The optimization of various factors influencing transformation efficiency in crops can also have unintended consequences on the crop's phenotype (Mishra et al., 2020). Additionally, mutagenesis can have unintended implications for the environment and human health (Datta, 2023).

Practical applications of mutagenesis in crop improvement

Mutagenesis is widely used in crop improvement across various species; rice, tomato, soybean, and durum wheat (Markvardsen et al., 1995) to improve several crop traits: yield, nutritional quality, stress tolerance and disease resistance (Penna & Jain, 2023). The resulting mutant lines exhibited reduced days to flowering, decreased plant height, and increased yield potential. Additionally, mutagenesis is utilised to modify the fatty acid levels of soybean oil by suppressing expression of the fatty acid desaturase 2 (FAD2) gene, researchers have been able to increase oleic acid and decrease the linoleic acid in soybean seed oil (Tariq et al., 2023) leading to the development of soybean varieties with better oil quality.

Regulatory frameworks for GM crops

The regulatory landscape for GM crops varies significantly between underdeveloped and developed nations. In underdeveloped countries, a lack of robust biosafety regulations and limited political support for GM crops has often led to unauthorised access to GM varieties. This unregulated access can result in the adoption of substandard or counterfeit technologies, compromising performance and productivity (Cheng-gui, 2008). While some countries have integrated socioeconomic considerations (SECs) into their domestic regulatory frameworks for biosafety and GM crop approval, many others are considering their inclusion. Real-world examples from countries that have taken this step offer valuable insights into the conceptual design, challenges, and trade-offs associated with integrating SECs into these frameworks (Falck-Zepeda et al., 2016). The Food and Drug Administration (FDA), the Environmental Protection Agency (EPA), and the Department of Agriculture (USDA) are the three federal agencies that regulate GM crops in the United States. The FDA ensures that GM crops are safe for humans to consume, the EPA assesses their impact on the environment, and the USDA oversees their field testing and commercialization (Cheng-gui, 2008). The European Union, Brazil, China, and India, have implemented regulatory frameworks for GM crops. These frameworks typically involve a rigorous evaluation of potential risks and benefits by a designated authority, such as the European Food Safety Authority (EFSA) or the National Technical Biosafety Commission (CTNBio) in Brazil. A common feature of these regulatory systems is the required labelling of GM products to increase product awareness among consumers (Bratspies, 2003). The increasing cultivation of GM crops has raised concerns related to food safety, environmental impact, and socioeconomic issues (Cheng-gui, 2008). Argentina also mandates a regulatory process for all GM plants before commercialization. Specific information is required for insect-resistant GM crops, particularly regarding insect resistance to expressed insecticidal products like Bt proteins, which are commonly used in Argentina (Ivanova, 2022).

Comparative analysis

Genomic editing methodologies are customary instruments for genetic manipulation in crop species. Among the prevalent techniques, CRISPR Cas, TALEN, Zinc Finger Nucleases, RNA Interference, and Mutagenesis stand out. Each technique possesses distinct merits and limitations, potential applications, and avenues for future investigation. Every genome editing technique operates through a distinct mechanism for instigating modifications in DNA (Table 1). Specifically, CRISPR Cas, TALEN, and Zinc Finger Nucleases function as site-specific nucleases, provoking double-strand breaks at predetermined genomic positions. Conversely, RNA Interference employs small RNA molecules to selectively target and degrade specific mRNA molecules, thereby inducing gene expression knockdown. In contrast, Mutagenesis introduces random mutations into DNA, yielding novel and enhanced crop strains. Mechanisms of these techniques are depicted in Fig. 1. Each genome editing technique possesses distinct strengths and weaknesses (Table 1).

CRISPR Cas9 exhibits high efficiency and ease of use, although it raises ethical concerns and may result in Off-target Mutations (Mashimo, 2013). TALEN and ZFN exhibit great specificity, albeit their design and assembly are laborious and expensive (Sood et al., 2013). RNAi is a potent tool for investigating and silencing functions of certain genes, yet it has limitations such as off-target silencing and incomplete gene expression knockdown (Agrawal et al., 2003). Mutagenesis eases the study of gene function, and the identification of genes implicated in specific biological processes, albeit it lacks site-specificity and introduces undesired mutations (Tadele, 2016). Moreover, the CRISPR Cas system has demonstrated successful application in various organisms, including plants, serving as a powerful tool for plant genome engineering (El-Mounadi et al., 2020). Further research is needed to address the possible risks linked to the utilisation of genomic editing techniques in crop improvement discipline.

Table 1 The comparison of structural description and functional mechanism of five genome editing techniques viz. CRISPR-Cas9, TALEN, Zinc Finger Nucleases, RNA Interference, and Mutagenesis

Genome editing technique	Description	Mechanism	Precision	Cost	Sources
CRISPR Cas	RNA-guided DNA endonucleases that are programmed to cut at targeted DNA sequences	Induces DSBs in DNA	High	Low	(Jiang & Doudna, 2017)
TALEN	Site-specific nuclease composed of a DNA binding domain and a FokI endonuclease domain	Induces DSBs in DNA	High	High	(Khan et al., 2016)
ZFN	Consist of a DNA-binding domain and a FokI endonuclease domain	Induces DSBs in DNA	Moderate	Moderate	(Gupta & Musunuru, 2014)
RNAi	Uses siRNA or miRNA to target and degrade specific mRNA molecules	Gene silencing technique that is used to knock down gene expression	Low	Low	(Boettcher & McManus, 2015)
Mutagenesis	Introduces random mutations in DNA through chemical mutagenesis or irradiation	Introduces random mutations in DNA to generate new improved strains of crops	Random	Very low	(Oladosu et al., 2015)

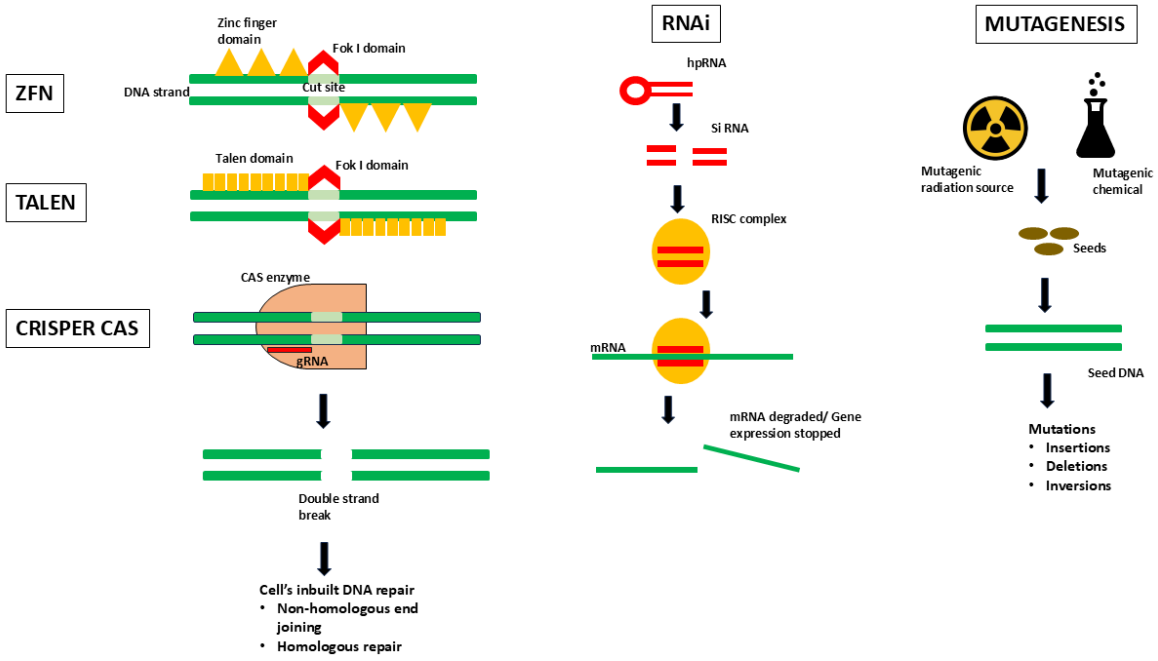

Fig. 1 Function mechanisms of CRISPR Cas, TALEN, ZFN, RNAi and mutagenesis

Conclusion

As the agriculture sector around the globe continues to face pressing challenges such as climate change, resource scarcity, and the need for food security, understanding the applications of genome editing techniques is essential. Genome editing technologies offer precise and efficient ways to modify crop genomes to better the traits like resistance to multiple stresses, crop yield, and nutritive quality. CRISPR Cas is particularly promising due to its simplicity and versatility. While other methods have their advantages, each technique has unique limitations. The choice of technology depends on aim in question and factors like precision, efficiency, cost, and potential off target mutations. Researchers can select the most appropriate technology to enhance yield, improve resilience to environmental stresses, and develop crops that meet the demands of a growing population.

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