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The impact of genome editing to improve abiotic stress tolerance in crop

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Abstract

The availability of genome sequences for various crops, as well as developments in genome editing techniques, have made it possible to breed for practically any desirable characteristic. Advances in genome editing tools such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) have allowed molecular scientists to target any gene of interest more precisely. CRISPR/Cas9 genome editing, in contrast to first-generation genome editing techniques, incorporates straightforward designing and cloning techniques, with the same Cas9 potentially available for usage with different guide RNAs targeting various locations in the genome. Furthermore, the availability of Cas9 enzymes from other bacterial species has increased the specificity and efficiency of gene editing techniques. This review summarises the possibilities useful to plant biotechnologists for crop improvement utilizing CRISPR/Cas9-based genome editing technologies, as well as papers demonstrating how CRISPR/Cas9 has been utilized to improve biotic and abiotic stress tolerance. The primary elements that affect plant growth and reduce agricultural productivity are biotic stress like disease and insect pests as well as abiotic stress like cold, drought, and saline-alkali stress. The application of genome editing techniques in creating resistant variants has huge potential. These methods have produced outstanding results in the resistance breeding of significant vegetable, fruit, and cereal crops like maize, rice, wheat, etc.

Keywords: Gene editing, CRISPR/Cas, TALEN, ZFNs, abiotic stress, crop breeding

Introduction

Genome editing, refers to performing precise alterations at particular sites in the genome to modify the DNA sequence. The discovery of site-broken DNA technology was a major breakthrough in genome editing methods. Recent years have seen a tremendous advancement in genome editing techniques because of the creation of synthetic sequence-specific nuclease (SSN). zinc finger nuclease (ZFN), transcription activator-like effector nuclease (TALEN), and CRISPR/Cas are the three main types of genome editing tools used by researchers (Li, Wu *et al*. 2022). Because of their difficult maintenance and many unsuccessful attempts rate, ZFN and TALEN have not been commonly used. The CRISPR/Cas system realizes the recognition process by the straightforward and adaptable base complementation of the guide RNA and target sequence, and the choice of the target site simply needs to be made in agreement with the requirements of the PAM (protospacer-adjacent motif) of various systems.

The CRISPR/Cas system is easily understood, adaptable, stable, effective, and simple to modify in comparison to the first two generations of genome editing methods. A defence mechanism called CRISPR/Cas prevents bacteriophages and mobile genetic elements from invading bacteria and archaea (Hille, Richter *et al*. 2018) [16] Since 2013, the CRISPR/Cas system has been effectively used to edit and regulate the genomes of several organisms (Ding, Chen *et al*. 2018). However CRISPR/Cas has only recently overtaken other genetic modification tools in plants, it has already proven to have significant potential for genetic crop improvement (Wolt, Wang *et al*. 2016) [44].

Nowadays, the CRISPR/Cas system is frequently utilised to increase crop quality and quantity, boost biotic stress resistance and abiotic stress resistance (Ma and Liang 2021) [32], give crops herbicide resistance, and domesticate new crops (Zsögön, Čermák *et al*. 2018) [49]. CRISPR/Cas9 was utilised by Wang *et al*. (2020a) to modify 25 conserved amino acid sequences at the C-terminal of rice cytokinin-activation enzyme-like gene LONELY GUY (OsLOGL5). The modified lines produced under various environmental circumstances considerably higher grain yield. A prominent source of scent is 2-acetyl-1-pyrroline (2AP), and BADH2, a protein that boosts aroma, can have its activity hampered or be defective, which can greatly raise the level of 2AP. Researchers developed new aroma-producing germplasm for *Oryzasativa*(rice), *Zea mays*(maize), and*Sorghum bicolour*(sorghum) by using CRISPR/Cas9 to impair BADH2 activity. With the growth of industry and the frequent occurrence of extreme weather conditions, the natural environment has gradually changed in a way that makes it unsuitable for the growing of crops. The production and quality of crops are affected by biotic pressures such as fungal, bacterial, viral diseases and insect pests as well as abiotic factors such as high temperature, low temperature, drought, saline-alkali, and heavy metals (Li, Wu *et al*. 2022) [29]

The needs of resistance breeding cannot be addressed by conventional crossbreeding, mutagenesis breeding, and other breeding techniques. The current standard technique for resistance breeding, CRISPR/Cas, can be utilised to develop crops in a specific direction and drastically reduce the breeding life. This essay examines the use of CRISPR/Cas in crop resistance gene modification and outlines potential issues and difficulties (Liu, Zhang *et al*. 2017) [30].

Overview of genome editing

Genetic engineering has advanced significantly and had great success since recombinant DNA technology first appeared in Paul Berg's lab in 1972. I gave a brief explanation of the techniques of various genome editing systems and how they can be used to improve crops. I also pointed out the various benefits and uses of engineered nucleases, as well as the biosafety and regulatory aspects of plants created using such technologies. The successful modification of a cell's genetic makeup is made possible by new genome editing tools, commonly known as genome editing with engineered nuclease (GEEN) technologies. These tools allow DNA molecules to be cut and re-joined at specific locations.

However, it is very challenging to edit the huge and complicated genomes of higher organisms, especially plant genomes, utilising restriction endonucleases and ligases. The restriction endonucleases limited ability to "target" shorter DNA sequences is an issue (Cong, Ran *et al*. 2013) [5, 35]. While this level of specificity works with short DNA bacteria and viruses, it is insufficient for working with huge plant genomes. The development of "artificial enzymes" in the form of short nucleotide sequences (oligonucleotides), which could specifically link to specific sequences in the structure of the target DNA and have chemical groups capable of cleaving DNA, was one of the earliest attempts to develop methods for editing complex genomes.

Several innovative genome editing methods have been created in recent years

1. CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/Cas9)
2. ZFNs (zinc finger nucleases)
3. TALENs (transcription activator-like effector nucleases)

Table 1: Comparative features of genome editing tools.

Feature	CRISPR/Cas	ZFNs	TALENs
Target site length	20-22 bp	18-36 bp	28-40 bp
Nuclease protein	Cas	FokI	FokI
Repair events	NHEJ	NHEJ	HDR
Efficiency	High	Low	Moderate
Specificity	Low	Moderate	High
Cost	Low	High	Moderate

1. CRISPR/Cas9

The CRISPR/Cas9 gene editing method, whose effectiveness in genome editing was initially proven in 2012 in mammalian cells, has revolutionised research in animal and plant biology (Jinek, Chylinski *et al*. 2012) [22]. The two components of the CRISPR cleavage mechanism are the Cas9 nuclease enzyme, which cleaves three to four bases after the protospacer neighbouring motif, and a brief synthetic gRNA sequence of 20 nucleotides that binds to the target DNA. The CRISPR/Cas9 system has three major components:

1.2 Cas9 protein

The CRISPR (clustered regularly interspaced short palindromic repeats)/CRISPR associated (Cas) protein system, of which the most well-known version is CRISPR/Cas9, is a new and frequently used method for editing Genome. This technique makes use of the adaptive bacterial and archaeal immune systems, whose mechanism depends on the existence of particular sites known as CRISPR loci in the bacterial genome. These loci are made up of repeat spacer sequences and operons that code for the Cas9 protein. Short pieces of foreign DNA (plasmid or viral) that have undergone recombination and been integrated into the bacterial genome make up the spacers in the repeat array (Deng, Huang *et al*. 2019) [6, 17]

1.3 Guide RNA

Contrary to chimeric TALEN proteins, the CRISPR/Cas9 system recognises a target site through a complementary sequence-based interaction between the target site's DNA and guide RNA (noncoding) (Jinek, Chylinski *et al*. 2012) [22]. The guide RNA and Cas protein complex also has nuclease activity for precise cleavage of double-stranded DNA using Cas9 endonuclease (Ran, Hsu *et al*. 2013) [35]

The CRISPR/Cas type II-A system, which is made up of three genes encoding CRISPR RNA (crRNA), trans-activating crRNA (tracrRNA), and Cas9 protein, is the most "well-known" system. It is present in the bacteria *Streptococcus pyogenes*. On the basis of this technology, universal genetic constructs have been developed that encode synthetic CRISPR/Cas "genome editor" components (Doudna and Charpentier 2014) [8]. A simplified form of the system was also developed, consisting of the Cas9 protein and a single guide RNA made up of short, mature crRNA and tracrRNA from the CRISPR system. Based on complementarity, the guide sequence locates the target DNA site, attaches to it, and Cas9 cleaves the DNA at the target location (Graham and Root 2015) [13].

1.4 DNA repair mechanisms

The repair of the DNA break generated by the nucleases is an essential stage in the genome editing process. Endogenous biological methods, such as NHEJ (nonhomologous end-joining) or HDR (homology-dependentor directed repair), are used to fix DNA breaks (Hsu, Scott *et al*. 2013) [18]. The simplest mechanism for joining the ends of cleaved DNA is called NHEJ. This process frequently results in the deletion or insertion of nucleotides (indels), which changes the reading frame of the gene and causes a "knockout" of that gene.

If no indels are found, the DNA is retrieved with no observable alterations. The HDR method, on the other hand, uses a sequence with homology to the target as a template for repairing the break or DNA damage. A homologous recombination is performed during HDR to allow for the recovery or insertion of new gene sequences. This approach is straightforward, gives the precise effect on the DNA target, and may be applied in practically any current molecular biology lab (Lowder, Malzahn *et al*. 2016) [31].

2. ZFNs

ZFNs were the first generation of chimerically built nuclease-based genome editing tools that were created after the functioning Cys2-His2 zinc finger (ZF) domain was discovered. The zinc-finger nucleases (ZFNs), which contain different DNA-binding and DNA-cleavage domains, are the class of targeting reagents that have emerged as the most adaptable and successful in recent years (Kamburova, Nikitina *et al*. 2017) [23].

The most beneficial of these were a group of zinc fingers (ZFs) called Cys2His2 that each had 30 amino acids and one zinc atom. The crystal structure of a group of fingers attached to DNA demonstrated that each finger makes remarkable modular contact with 3 bp of DNA on average (Pavletich and Pabo 1991) [34]. The modular construction, assembly, and optimisation of zinc fingers against particular target DNA sequences occurs during the design and implementation of ZFNs. Individual ZFs are then linked together to target bigger sequences. Zinc finger domains have been developed over time to identify a variety of triplet nucleotides. This made it possible to choose and connect zinc fingers in a way that would make it possible to recognise the target sequence of interest.

Zinc fingers have been effectively utilised in numerous organisms, including plants, since the first article on them was published in 1996(Gaj, Gersbach *et al*. 2013) [11]. Examples include precise targeted addition of a herbicide-tolerance gene as well as insertional disruption of a target locus in maize. Other examples include targeted inactivation of endogenous genes in Arabidopsis, high frequency alteration of tobacco genes, and precise targeted addition of a herbicide-tolerance gene.

Zinc finger nucleases have demonstrated the ability to modify genomic areas of interest, revolutionising the field of genome editing and paving the way for both fundamental and applied research. With regard to effectiveness, high specificity, and minimum nontarget effects, ZFNs are superior to other techniques, and current efforts are concentrated on further enhancing design and delivery as well as broadening their uses in various crops of interest.

3. TALENs

The development of a novel genome editing system using chimeric nucleases known as TALE nucleases (TALENs) was made possible by the discovery of new transcription activator-like effector (TALE) proteins that recognise and activate particular plant promoters through a set of tandem repeats. This search for efficient and particular manipulation of target genomic DNA led to the discovery of these TALE proteins (Jankele and Svoboda 2014) [21].

TALE proteins are made up of three domains: a signal for nuclear localization, a domain that binds to DNA, and a domain that activates the target gene's transcription. The capacity of these proteins to bind DNA was initially disclosed in 2007, and a year later, two research teams were able to decipher the recognition code used by TALE proteins to recognise their target DNA sequence.

It is demonstrated that the CRD (central repeat domain), which gives DNA binding and host specificity, is a component of the DNA-binding domain in TALE monomers. Each 34-amino acid long tandem repeat in the CRD binds to one nucleotide in the target nucleotide sequence. The CRD is made up of tandem repetitions of 34 amino acid residues. The final tandem repeat, known as a half-repeat, only has 20 amino acid residues and binds to a nucleotide at the 3′ end of the recognition site. While studies have shown that TALE proteins can generally be designed to bind any DNA sequence of interest, the 5′-most nucleotide bases of the DNA sequence that a TALE protein bind must always be a thymidine. Studies have also shown that if this requirement is not met, TALE transcription factors (TALE-TF), TALE recombinases (TALE-R), and TALENs will not function as effectively (Lamb, Mercer *et al*. 2013) [26].

The first attempt made was the development of chimeric TALEN nucleases once the DNA code recognition requirements by TALE proteins had been figured out. A plasmid vector that had previously been used to produce ZFN was modified for this purpose by inserting the sequence encoding the DNA-binding TALE domain (Christian, Cermak *et al*. 2010) [4].

According to the mode of action and specificity of TALENs, double strand breaks should be able to be introduced into any region of the genome, provided that region has the recognition sequence that corresponds to the DNA-binding domains of TALENs. Given the simplicity of site-directed manipulation using the TALEN system, the genes modified by this system have been successfully used in a number of animal and plant species, with examples from plants including rice, wheat, Arabidopsis, potato, and tomato. This is a short time after the unravelling of the TALEN mode of action (Xiong, Ding *et al*. 2015) [46].

Off-Target Effects

In bacteria and archaea, the CRISPR/Cas9 system serves as an RNA-based adaptive immune system. When a bacterial colony is attacked for the second time, transcripts of these repeats drive a nuclease to the complementary DNA of the invading virus, destroying it. The CRISPR/Cas9 system can be rebuilt in mammalian cells to enable its gene-targeting capacity in eukaryotic cells by using three minimum components: Cas9, a specificity-determining CRISPR RNA (crRNA), and an auxiliary trans-activating RNA (tracr RNA). The first 20 nucleotides of the sgRNA are complementary to the target DNA sequence, and are followed by a sequence known as the protospacer adjacent motif (PAM), which is usually NGG (Horvath and Barrangou 2010) [17].

Although Cas9 targeting specificity is thought to be tightly controlled by the sgRNA's 20-nt guide sequence and the presence of a PAM adjacent to the target sequence in the genome, off-target cleavage activity could still occur on DNA sequences with three to five base pair mismatches in the PAM-distal part of the sgRNA-guiding sequence (Gasiunas, Barrangou *et al*. 2012) [12]

Off-target site detection in a highly sensitive and comprehensive method remains a significant difficulty in the field of gene editing. The T7 endonuclease I test was first employed to detect off-target mutations; however, it has poor sensitivity it cannot detect off-target mutations with frequency of less than 1% and is neither feasible nor cost-effective for large-scale screening. Because the tools used by Web-based algorithms presume that off-target sequences are strongly linked to the on-target site, they may miss harmful off-target sites with less sequence similarity. This is an inherent drawback of these algorithms (Hsu, Scott *et al*. 2013) [18].

Genome Editing to Improve Stress Tolerance in Crops

Genome editing can be used to increase crop stress tolerance against a variety of conditions. There are some of the major types of stress that can be targeted for improvement: Abiotic stress and Biotic stress.

1. Abiotic stress

Abiotic stresses occur as a result of global climate change over time (Bhat, Mir *et al*. 2021) [3]. Abiotic stresses reduce germination, reduce photosynthetic activity and carbon assimilation, suppress flowering and pollen sterility, and limit crop productivity. Stress, which affects various elements of plant growth, poses a substantial threat to plant survival and accounts for a significant loss in agricultural output.

Drought, salt, severe temperatures, chemical toxicity, and oxidative stress are all harmful to agriculture and contribute to environmental degradation. Which can result in a 50% drop in crop yield (Liu, Iketani *et al*. 2022). In such cases, it is critical to develop crop types that are more adaptable to a wide range of environmental conditions. Although traditional breeding improves result significantly, it has the disadvantage of reducing genetic variation and fitness. Salinization had been reported to damage 7% of the Earth's surface and 20% of agricultural land, with the issue only expected to worsen (Al Murad, Khan *et al*. 2020) [1]. Salt stress causes osmotic stress, ion stress, and secondary stress in plants, reducing agricultural yield and quality (Siddiqui, Al Othman *et al*. 2017).

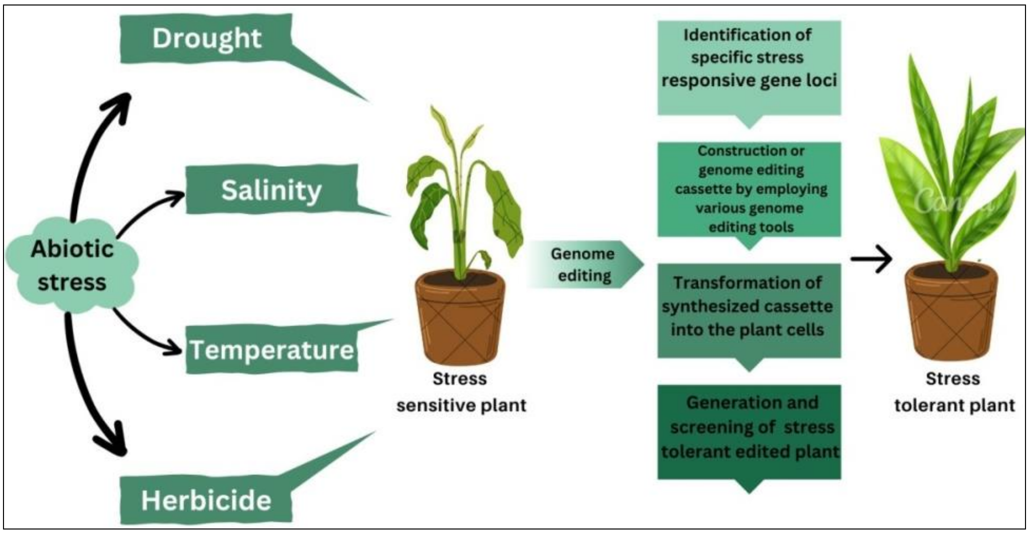

Fig 1: An overview of Biotic and Abiotic stress tolerance There are some stresses about using genome editing to improve plant abiotic stress:

1.1 Drought stress

Drought is a major abiotic stress that has a global impact on the growth of crops. To improve drought tolerance in crops, genome editing may be utilised to change genes involved in water consumption productivity, stomatal regulation, osmotic adaptation, and stress signalling pathways.

Drought stress is the leading source of productivity and yield losses in important crops, representing the greatest risk to global food security. The natural ARGOS8 promoter sequence of maize was changed by GOS2 promoter using the CRISPR/Cas system to increase maize yield under drought stress in the field. CRISPR/Cas modification of wheat TaDREB2 and TaERF3 increased drought tolerance. OsERA1 mutagenesis using CRISPR/Cas9 produced in high drought stress resistance in rice. Santosh Kumar *et al*. (2020) [38] employed CRISPR/Cas9 to modify the OsDST gene to produce a mutant of the indica mega rice cultivar MTU1010 with larger leaves, decreased stomatal density, and improved leaf water retention during drought stress (Santosh Kumar *et al*., 2020) [38].

CRISPR/Cas9 produced SRL1 and SRL2 gene alterations in rice to create the twisted leaves phenotype and resistance to drought by altering protein expression patterns and reactive oxygen species collecting. Under drought conditions, tomato plants with high water content in the leaves were obtained using CRISPR/Cas9 to change GID1, and tomato drought tolerance was effectively improved.

Furthermore, CRISPR/Cas9-mediated SlLBD40 gene mutation dramatically improved tomato drought tolerance.

1.2 Salinity Stress

Salt stress is a primary abiotic stress that is responsible for a considerable decrease in global agricultural productivity (Sevanthi, Sinha *et al*. 2021) [40]. It impacts almost 80 million hectares of global agricultural land, and this area is predicted to grow as the global climate changes (Kumar, Kumar *et al*. 2013) [24].

Although the fact that the prospective of genome editing in plant development is well recognised, there are few studies on the successful application of this strategy for establishing salinity stress resistance in crops.

Rice's OsRR22 gene, which encodes for a TF (transcription factor) involved in the modulation of signalling and cytokinin metabolism, has been altered to impart salt stress resistance. Under salinity stress, rice mutants of OsRR22 generated using CRISPR/Cas9 fared better. Furthermore, genome editing has been employed to gain a better understanding of the molecular control of several genes related to abiotic stress adaption. RAV2 is an AP2/ERF family transcription factor that plays an important role in abiotic stress tolerance (Duan, Li *et al*. 2016) [9, 27].

Recently, the rice drought and salt tolerance (DST) gene was modified using CRISPR/Cas to improve salt/drought stress resistance in plants. The resulting dst184-305 mutant plants have lower stomatal density and higher levels of water retention in the leaves. In mutant plants, the decrease in stomatal density was connected to the downregulation of several genes associated with stomatal development, especially MUTE, ICE1, and SPCH1 (Santosh Kumar, Verma *et al*. 2020) [38].

Additionally, with the introduction of CRISPR/Cas, the 8CM and PRD domains of HyPRP1 (hybrid proline-rich protein 1) changed in tomatoes, resulting in increased salinity stress resistance (Santosh Kumar, Verma *et al*. 2020) [38].

1.3 Cold Stress

Plants grow at their optimum temperature, thus any change in this temperature can strongly inhibit their growth and yield. As the earth's temperature increases because of global warming, developing creative techniques to fight the impacts of temperature extremes in plants is an essential issue (Raza, Razzaq *et al*. 2019) [36]. Using genome editing, researchers targeted to increase the survival of various plants in response to temperature stress.

Cold stress, which includes temperatures as low as 20°C and as high as 0°C, inhibits plant growth and development and extremely restricts plant geographical distribution and crop production. Low temperature limits plant metabolic response and causes osmotic stress, oxidative stress, and other stress.

Zeng *et al*. discovered that CRISPR/Cas9-created ospin5b mutants, gs3 mutants, and osmyb30 mutants improved spike length, grain size, and cold tolerance. The CRISPR/Cas9-mediated slmapk3 mutant sustained reactive oxygen species homeostasis by modulating the expression of antioxidant enzymes and HSPs/HSFs genes, improving tomato plant high temperature tolerance. High temperatures have an impact on the entire crop growth cycle, particularly during heat sensitive periods such as early establishment, flowering, and gametophytogenesis.

Pyl1/4/6 triple knockout rice was created by CRISPR/Cas9 editing. The mutant outperformed the wild type in terms of yield, temperature tolerance, and germination before harvest.

Table 2: Genes targeted by CRISPR/Cas system for improving tolerance against abiotic stress.

Plant	Stress	Targeted genes/ Tolerence	Reference
Arabidopsis	Drought	OST2	(Salehi-Lisar and Bakhshayeshan-Agdam 2016) [37]
Arabidopsis	Drought	Trehalose	(Wu, Shao *et al*. 2017) [45]
Rice	Drought/salinity	miR535	(Santosh Kumar, Verma *et al*. 2020) [25, 38]
Rice	Drought/salinity	DST	(Duan, Li *et al*. 2016) [9, 27]
Rice	Drought/salinity	SAPK2	(Liu, Lin *et al*. 2019)
Maize	Drought	ARG0S8	(Guo, Rupe *et al*. 2014) [14]
Wheat	Drought	TaDREB2	(Shi, Habben *et al*. 2015) [41]
Rice	RR22	Tolerance	(Zhang, Liu *et al*. 2019) [47]; (Han, Chen *et al*. 2022) [15]
Barley	HVP10	Sensitive	(Fu, Wu *et al*. 2022) [10]
Cotton	AITR genes	Tolerance	(Wang, Xun *et al*. 2021) [43]
Tomato	HyPRP1	Tolerance	(Tran, Doan *et al*. 2021) [42]
Wheat	Two HAG homologs	Sensitive	(Zheng, Lin *et al*. 2021) [48]
Rice	TIFY1a, TIFY2b	Cold stress-responsive transcription factor	(Raza, Razzaq *et al*. 2019) [36]
Rice	Ann3	Ca2+-dependent phospholipid-binding proteins involved in plant development stress responses	(Bertier, Ron *et al*. 2018) [2]
Tomato	CBF1	Cold stress responsible gene	(Huang, Zeng *et al*. 2017) [19]

Application of Genome Editing Systems in Crop Improvement

One of the most significant goals of contemporary biotechnology is the development of new types of crops that are high-yielding, tolerant to biotic and abiotic stresses, and have high nutritional value.

Genome editing technologies offer a wide range of practical uses in this regard (Kamburova, Nikitina *et al*. 2017) [23]. In order to achieve this, genome editing systems have been used in plant breeding to: (1) insert point mutations similar to natural SNPs (Illa-Berenguer, LaFayette *et al*. 2023) [20], (2) make minor changes to gene function (Mao, Zhang *et al*. 2013) [33], (3) integrate foreign genes, (4) perform gene pyramiding and knockout, (5) repress or activate gene expression, and (6) perform epigenetic editing (Kumar and Jain 2015) [25].

For example, the application of ZFN in Arabidopsis thaliana (Li, Liu *et al*. 2016) and Zea mays allowed for the effective insertion of herbicide-resistance genes into specified places in the genome (Shukla, Doyon *et al*. 2009), resulting in the production of genotypes that were herbicide tolerant.

Through Cibus Rapid Traits Development System (RTDS) (Sauer, Narváez-Vásquez *et al*. 2016) [39], ODM method has considerably advanced, and this technology has been effectively used in a number of crops. Applications involve, but are not limited to, the improvement of herbicide tolerance, insect resistance, increased disease resistance (bacterial and viral), improved nutritional value, and enhanced yield without the introduction of foreign genes as has been used in traditional genetic engineering approaches for crop development (Sauer, Narváez-Vásquez *et al*. 2016) [39].

The creation of species resistant to various infections or pests is a popular application of genome editing technologies in plant breeding. These techniques have been utilised to modify essential plant immune phases at various levels in multiple crops (Li, Liu *et al*. 2012).

Conclusion

New breeding techniques allow scientists to introduce desired features more precisely and quickly than conventional breeding. CRISPR/Cas9-based genome editing is a game-changing approach. The use of genome editing techniques in agricultural crops for improving production, nutritional value, disease resistance, and other qualities will be a major focus in the future. It has been used extensively in many plant systems during the last five years for functional investigations, decreasing biotic and abiotic stresses, and improving other essential agronomic features. Though various adjustments to this technology must result in increased on-target efficiency, the majority of work completed is preliminary and requires improvement. Traditional plant breeding methods, such as intergeneric crosses and chemical/physical mutagenesis, are non-specific. Furthermore, due to linkage drag, introduction of advantageous characteristics into an elite variety is frequently followed by introduction of non-target features.

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