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Modifying plant genomes to increase resilience to abiotic stress

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Abstract :

If we want to ensure that future generations have enough to eat, we must triple our current agricultural yields. Because present crop varieties and crop growth methods may not be resilient enough to resist the increasing abiotic stresses caused by climate change, crop production is projected to become more difficult in the future. The majority of agricultural crops have their average yields reduced by more than 50% due to abiotic stress, making it the leading cause of crop loss globally. Drought, salt, excessive heat, and cold are the primary environmental factors that negatively impact agricultural output and efficiency. Sustainable food production relies on improved crops, and current crop improvement techniques are great at making plants more resistant to abiotic stress. Genome editing is one of the foremost cutting-edge approaches to agricultural enhancement. Agricultural experts in particular are ecstatic about the new possibilities presented by genome editing, which might lead to the development of better crop types via the targeted introduction of desirable features. It is feasible to develop crop types with better abiotic stress tolerance and produce targeted genome modifications by genome editing, which is similar to mutational breeding. In order to better protect crops from abiotic stress, this review will quickly go over the following topics: abiotic stress, genome editing, mechanisms, various kinds, and applications.

Key words: CRISPR/Cas9, crop improvement, abiotic stress, tolerance, genome editing, rice

Introduction

Reduced food grain yield and productivity due to abiotic stressors poses a danger to global food security. A number of factors, including heat, salt, cold, and drought, have a detrimental impact on agricultural output. Both of these pressures have a negative impact on crop yields when they work together. Climate change increases the impact of both individual and interdependent stresses on agricultural yields. Recent research have shown that abiotic stress is already a problem in this area,

and there is concern that climate change will make it worse in the future. Stresses related to water, heat, cold, and salt are more common than beneficial ones for plant development. Drought and salinity are two of these factors that are worsening in many areas. (Kundzewicz et al., 2005; Fedoroff et al., 2010). According to Cramer et al. (2011), abiotic stress may impact over 90% of crops grown on agricultural land in rural settings at some time during the growing season.

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In order to create cultivars that are resistant to abiotic stresses and produce more food to sustain a growing population, understanding how plants respond to stress is essential. Plants produce stress signals as a defense mechanism against harmful environmental stresses. Similar to how biotic stress causes changes in plant physiology, abiotic stress signals the activation of certain metabolic pathways in response to the stress. As an example, salinity and dryness primarily cause hyperosmotic stress, which is also called osmotic stress. Plants experience salinity-related toxicity due to both osmotic stress and ion toxicity. Salinity and drought have more complicated secondary consequences, such as metabolic dysfunction, oxidative stress, and pressure and harm to biological components like nucleic acids, proteins, and lipids in the cell membrane. Therefore, there are unique and complementary signals for salinity and drought. At the cellular and whole-plant levels, drought and salinity both interfere with osmotic equilibrium and ionic homeostasis, respectively (Mathivanan, 2021).

Molecular damage, growth stop, and plant death are all outcomes of sudden shifts in water and ion balance. While main stress signals do trigger certain cellular responses, secondary signals are responsible for developing the vast majority of responses. A hyperosmotic signal is a key indicator of salt and drought because it causes plants to accumulate the phytohormone abscisic acid (ABA), which triggers many adaptive responses (Zhu et al., 2006). According to Orvar et al. (2000), chilling stress affects plant growth and development by changing the shape and stability of cell membranes. Cold stress changes enzyme function, including ROS scavenging enzymes, and alters the structure of proteins or protein complexes. These actions cause photo inhibition, which in turn hinders photosynthesis. According to Siddiqui et al. (2006) and Ruelland et al. (2009), these actions lead to substantial damage to the membrane, suppression of photosynthesis, and decreased photosynthesis. There is currently no technique that can alter plant genetics with pinpoint accuracy, despite the

abundance of sophisticated crop enhancement tools available. It is now much easier to create a variety with great specificity and accuracy because to genome editing. It is possible to modify a plant's characteristics by inserting targeted genetic modifications into its DNA using these techniques. The magnitude of these changes might range from a single base change to the addition or removal of an entire gene or genes. Researchers have made extensive use of genome-editing techniques because they are simple, economically feasible, and flexible; they have also accelerated molecular breeding and made it possible to incorporate mutations into the genetic code of plants with extreme efficiency and precision (Shakespeare et al., 2022).

Genome editing

Genome editing is a process in which new DNA can be inserted, existing DNA can be removed or replaced by using artificially engineered nucleases, or "molecular scissors". The nucleases make the specific cut at desired locations in double-stranded DNA and this will be repaired by using endogenous repairing mechanisms such as Homologous recombination & Non-homologous end joining (Deriano & Roth, 2013; Osakabe & Osakabe, 2015). Genome editing is mediated through two different systems, one is SSR (site-specific recombinase) and SSNs (site-specific nucleases) cleaving the target sequence by SSNs is followed by a cellular DNA repair mechanism. At present, there are four families of engineered nucleases are used in genome editing: those are Engineered Meganuclease (MegaN), Zinc Finger Nuclease (ZFN), Transcription Activator like Effector Nuclease (TALENs), and Clustered Regularly Interspaced Short Palindromic Repeats /CRISPR-cas9 nuclease system (Osakabe & Osakabe, 2015). Meganucleases are one of the endonucleases occurred naturally and were first identified in the late 1980s. According to Suzuki et al. (2020), they can recognize and cut long DNA sequences (12 to 40 base pairs) that are either unique in most genomes or very nearly so. Zinc finger nucleases (ZFNs) are site-specific nucleases that cleave DNA *in vitro* in precisely specified locations. This was initially demonstrated in 1996 using protein domains such as "zinc fingers" in combination with FokI endonuclease domains. This chimeric protein contains a modular structure in which each "zinc finger" domain can recognize a single triplet of nucleotides (Joung &



Sander, 2013). Continuous research efforts of various scientists led to finding new genome editing technologies like TALENs and CRISPR/Cas with more precision and simple manner when compared to the Meganuclease and Zinc finger-based genome editing. It has been demonstrated that the TALEN and CRISPR systems function in plant, animal, and human cells. By effectively manipulating genomes, such editing techniques have the potential to address a variety of challenging issues, such as the development of mutants with more precision and the production of plants with desirable traits.

Clustered regularly interspaced short palindromic repeats (CRISPR)

CRISPR/Cas is one of the most powerful genome editing technology and the most notable CRISPR system is the CRISPR/Cas9 (based on Cas9 protein), which has clustered regularly interspaced short palindromic repeats. This technique makes use of the adaptive bacterial immune system, whose workings depend on the existence of unique bacterial genome regions known as CRISPR loci. Operons encoding the Cas9 protein and a repeating array of repetitive spacer sequences make up these loci. Short segments 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. The CRISPR system mainly depends on RNA-DNA binding to achieve sequence specificity not like ZFN and TALEN systems, which rely on protein-DNA binding specificity (Rath et al., 2015). The functional characterization of the CRISPR/Cas showed viral resistance mechanism along with components which are inevitable for this system such as crRNA, PAM motif, and tracrRNA (Gasiunas et al., 2012; Jinek et al., 2012). The DNA sequence that follows the protospacer adjacent motif (PAM) DNA sequence is the target of the Cas9 nuclease in the CRISPR system. PAM is a part of the plasmid or virus causing the invasion, but it is not a part of the bacterial CRISPR locus. If the PAM sequence is not immediately after the target DNA sequence, Cas9 will not successfully connect to or cleave it. Its own CRISPR locus of the bacterial genome is being protected since the PAM is a crucial targeting element not present in bacteria. RNA-guided CRISPR/Cas9 could be used for the gene editing of other species than bacteria that cause double-stranded DNA breaks (DSBs) in a site-specific manner. These DSBs was proven in plant, human, and animal cells. In this approach, RNA-DNA pairing of a 20-nt region in the chimeric single-guide RNA (sgRNA)

binding on its target DNA sequence with specificity. Consequently, a RNA:DNA heteroduplex is forming and Cas9 domains cause the double strand breakage. CRISPR is quicker, less expensive, more precise, and more effective than previous genome editing technologies (Rath et al., 2015; Jinek et al., 2012; Gasiunas et al., 2012).

Crop plants and abiotic stresses

Crops grown under challenging environments often encounter simultaneous occurrences of multiple abiotic stresses rather than individual stress, negatively affecting crop growth and productivity. Some abiotic stresses often co-exist together and are referred to as companion stress, for instance, drought and heat. The negative effect of the interaction of stress on crop production is more adverse than the effect of individual stress. Similarly, crop response to the combination of stress differs from the response to individual abiotic stress. Unique and overlapping pathways exist in tolerant crop plants to encounter the negative effect of abiotic stress. Ultimately the tolerant crop plants compromise with the regular source-sink relationship which affects the crop growth and productivity (Xiong et al., 2012).

Effect of drought, salt and cold stress in plant physiological modifications

All three stresses predominantly create a loss of cell water leading to a decrease of cell osmotic potential, but the factor of water loss from cells varies among stresses: i) In drought, water loss from cells is due to water shortage in the soil. ii) whereas in salt stress, osmotic or water potential of the surrounding root zone is decreased by ions which prevents the uptake of water by roots. iii) In cold stress, cell water loss is mainly due to the inability of the plant to transport water inside the plant cells causing physiological drought. An increase in osmotic stress induces abscisic acid (ABA) biosynthesis, activating various drought, salt and cold stress-responsive genes in the plant system (Boudsoq & Lauriere, 2005). These three stresses reduce osmotic potential in the plant system by increasing the solute concentration (particularly Na^+), which deleteriously impacts proteins and enzymes. High-volume production of low molecular weight osmolytes is a typical method of reducing the stress caused by salt, cold, and drought. Production of more low molecular weight osmolytes, such as proline (Hesham & Fahad, 2020), betain (Aziz et al., 2017), and carbohydrates (Marques & Arrabaca, 2004), can counteract this effect



by preventing cellular dehydration and turgor loss (Beck et al., 2007),

Regulation of gene expression in response to abiotic stress

Stresses such as drought, salinity, and cold cause a number of genes in plants to express and produce different proteins, such as chaperones or late embryogenesis abundant (LEA) proteins, etc., in different sections of the plant, leading to developing distinct abiotic stress tolerance mechanisms in plants (Kazuko & Shinozaki, 2006). Abiotic stresses produce distinctive DNA methylation patterns, which either promote or decrease gene transcription. Regulons of some stress-sensitive genes are dehydration-responsive element-binding (DREB) or C-repeat binding factor (CBF), basic-leucine zipper (bZIP), and zinc-finger proteins (Hu et al., 2006). A unique cis-acting element called the C-repeat/dehydration response element (CRT/DRE) responds to drought, cold, and high-salt stress (Yamaguchi et al., 1994). By looking for DNA-binding proteins that bind to the CRT/DRE motif, CBF proteins have been successively identified since their discovery (Stockinger et al., 1997; Liu et al., 1998). Three cold-induced CBF genes (*CBF1/DREB1B*, *CBF2/DREB1C*, and *CBF3/DREB1A*), are present in Arabidopsis and are organized in a tandem

manner on chromosome 4. According to Liu et al. (1998) and Gilmour et al. (1998), *CBF1-3* are *APETALA2/ETHYLENE-RESPONSIVE (AP2/ERF1)* type transcription factors that directly bind to the conserved CRT/DRE motifs in the promoters of COR genes (known as CBF regulons) and activate their expression in cold stress circumstances. When its own CBF genes, specifically *LeCBF1*, are overexpressed, the cold-sensitive tomato (*Lycopersicon esculentum*), becomes freezing-tolerant (Zhang et al., 2004).

Genetic modification in plants for abiotic stress tolerance

Despite the advantages of commercially available genetically engineered plants and their success in combating abiotic stresses, this technology is still not universally adopted due to public misconceptions, which restricts its use in developing abiotic resistant varieties. The main issue with transgenic technology may be that the gene source used to create transgenic crops is frequently acquired from unrelated organisms, such as microorganisms, plants, and animals. Genome editing technology can better handle this problem. Making small genome changes through chemical and

physical mutagens in crop plants is called mutation breeding (a traditional way of crop improvement). The success rate or odds of attaining a desirable genotype are quite low since mutational breeding causes changes in the genome in a random manner. However, modifications are made in genome editing at specific locations using sequence-specific nucleases that cause double-strand breaks in the target genomic loci chosen for editing. As a result, more frequently, a desirable genotype can be obtained. TALENs and CRISPR/Cas9 are important genome editing tools (Voytas, 2013). There are many possibilities for developing crop plants with any desirable character by advanced genome editing methods using several available crop genome sequence information. Among genome editing technologies, CRISPR/Cas9 genome editing involves simple cloning and designing techniques, with the same Cas9 can be used with several guide RNAs targeting various genomic regions. Additionally, there are ways to improve the specificity and effectiveness of gene editing approaches because of the accessibility of Cas9 enzymes from various bacterial species. These methods will create non-genetically modified (Non-GMO) crops with the desired characteristic that will increase their yield potential under biotic and abiotic stress conditions (Wang et al., 2014).

CRISPR-Cas9

Utilizing the CRISPR/Cas9 system allows for forward genetics, which can be used to research the genetics of abiotic stress response and contribute to the development of crop types that are resistant to stress. Using CRISPR/Cas-based genome editing techniques, Shi et al. (2017) have created a maize variety with increased yield under drought stress. The research has focused on ARGOS8, which inhibits ethylene reactions. Increased drought tolerance was reported in plants with improved ARGOS8 expression (Shi et al., 2017). In another research, truncated gRNAs (tru-gRNAs) and Cas9 were driven by a tissue-specific AtEF1 promoter, which mutated the abiotic stress-responsive gene OST2/AHA1 and increased stomatal responses in Arabidopsis. To improve salinity tolerance, rice genes OsRR22 and OsNAC041 have also been targeted (Zhang et al., 2019; Bo et al., 2019). By utilizing the RNase/DNase capabilities of *Acidamino coccus* Cas12a (Cpf1) for multiplexed genome editing, a recent work effectively targeted 25 distinct genomic targets (Campa et al., 2019). Water, urea, H₂O₂, and silicon are some of the most important solutes for solute transport control, and aquaporins are among the best candidates for abiotic stress augmentation (Zargar et al., 2017).



Recent studies revealed that, some other transporter proteins also involved in improving abiotic stress tolerance by genome editing (Vishwakarma et al., 2019; Ahmad et al., 2019). Increased gene expression using synthetic transcriptional activators and repressors (Piatek et al., 2015). C-repeat binding factors (CBFs) are responsible for plants' adaptation to cold temperatures. Producing triple mutant *CBF1,2,3* lines through conventional genetic crossing is extremely difficult because the *CBF1-3* loci are all on the same chromosome.

Therefore, creating single, double, and triple mutants of the CBF genes was effectively accomplished by utilizing the genome editing technique CRISPR/Cas9. When exposed to cold, the cbfs triple mutants are the most vulnerable to the effects of freezing stress among any of the other mutants. According to RNA sequencing investigation of the triple mutants, 10–20% of COR gene expression is CBF dependent (Jia et al.,

2016; Zhao et al., 2016). These results support the idea that CBFs are important regulators that perform redundant functions in plants' cold adaptation. These experimental findings suggest that the CRISPR/Cas system can be effectively used for this innovative purpose and will eventually be the method of choice for targeting minor genes of complex quantitative features associated with abiotic stress (Mushtaq et al., 2018).

CRISPR for drought tolerance

Since drought tolerant trait is complicated and quantitative, several physiological and biochemical mechanisms are involved in developing drought tolerance (Bhat et al., 2020). To increase public acceptance of genome-edited crops, experiments are being planned to alter the genes implicated in pathways for drought tolerance by using genome editing tools (Li et al., 2022). Numerous studies have documented how CRISPR confers plant drought tolerance (Table 1).

Table 1. Gene modification for drought tolerance through genome editing

Crop	Gene	Modification	Reference
Rice	<i>MYB5, DERF1</i>	Down-regulation	Zhang et al., 2014
Rice	<i>SRL1, SRL2, and ERA1</i>	knockout	Liao et al., 2019; Ogata et al., 2020
Rapeseed	<i>A6RGA</i>	Insertion/deletion	Wu et al., 2020
Arabidopsis	<i>OST2</i>	Insertion/deletion	Osakabe et al., 2016
Arabidopsis	<i>STL1</i>	deletion	Zhang et al., 2016
Arabidopsis	<i>miR169a</i>	Knockout	Du et al., 2017
Arabidopsis	<i>AVP1</i>	Activation	Park et al., 2017
Arabidopsis	<i>AREB1</i>	Activation	Roca Paixao et al., 2019
Arabidopsis	<i>TRE1</i>	Silencing	Nunez-Munoz et al., 2021
Maize	<i>Abh2</i>	Suppression	Liu et al., 2020
Maize	<i>abh2</i>	Insertion/deletion	Liu et al., 2020
Tomato	<i>LBD40</i>	Insertion	Liu et al., 2020
Tomato	<i>GID1a</i>	Insertion	Illouz-Eliaz et al., 2020

CRISPR for salt tolerance

Plants can tolerate salinity by activating more molecular and physiological processes and pathways (Munns & Tester, 2008). To control osmotic adjustment during salt stress, genome editing technologies have been used to target genes involved in ion transport (Volkov, 2015). Several studies have been conducted to show the possibility of development of salt tolerance in plants through CRISPR (Table 2).

**Table 2. Gene modification for salt tolerance through genome editing**

Crop	Gene	Modification	Reference
Rice	<i>OsmiR535</i>	Knockout	Yue et al., 2020
Rice	<i>RR9, RR10</i>	Deletion	Wang et al., 2019
Rice	<i>SPL10</i>	Insertion and Deletion	Lan et al., 2019
Rice	<i>RR22</i>	Insertion and Deletion	Zhang et al., 2019; Han et al., 2022
Rice	<i>PQT3</i>	Insertion and Deletion	Alfatih et al., 2020
Tomato	<i>SlHyPRP1</i>	Knockdown	Tran et al., 2021
Tomato	<i>ABIG1</i>	deletion	Ding et al., 2022
Arabidopsis	<i>SOS1</i> (salt overly sensitive 1)	Overexpression	Yue et al., 2012
Arabidopsis	<i>C/VIF1</i>	Insertion and Deletion	Yang et al., 2020
Barley	<i>HvHKT2;1</i>	Overexpression	Mian et al., 2011
Cotton	<i>AITR</i> genes	Deletion	Wang et al., 2021
Maize	<i>STL1</i>	Insertion and Deletion	Wang et al., 2022

CRISPR/Cas9 for cold stress

A crop's growth and yield are negatively impacted by cold stress, which can be separated into freezing stress (0°C) and chilling stress (0-15°C) based on the temperature difference (Guo et al., 2018). Mechanical damage to cells and metabolic activity malfunction are the most prevalent symptoms of cold stress. (Yadav, 2010). Low temperatures can harm agricultural crop species, impairing their growth, production, and survival ability (Sanghera et al., 2011). Many researchers have researched several crops to generate cold-tolerant crops through CRISPR (Table 3)

Table 3. Gene modification for cold resistance through genome editing

Crop	Gene	Modification	Reference
Rice	<i>OsPRP1</i>	Knockout	Nawaz et al., 2019
Rice	<i>OsMYB30</i>	Knockout	Zeng et al., 2020
Rice	<i>OsAnn3</i>	Knockout	Shen et al., 2017
Rice	<i>OsAnn5</i>	Knockout	Que et al., 2020
Tomato	<i>CBF1</i>	Knockout	Wang et al., 2017
poplar	<i>PtPYRL1</i> and <i>PtPYRL5</i> genes	Overexpression	Yu et al., 2017

Conclusion

Our understanding of genetic manipulation and optimization of genome editing technology will grow with notable advancements toward developing crop varieties with abiotic stress resistance. In due course, genome editing tools could be incorporated with conventional and marker-assisted breeding efforts to get the desired improved varieties. Identifying multiple abiotic stress tolerant genotypes are most important for developing new varieties for a changing climate. Together, these initiatives will make significant progress in mitigating the consequences of climate change, especially drought, salt stress, and cold stress, and will improve agricultural productivity, ultimately improving food security.

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