Improving Abiotic Stress Tolerance in Crops through Genome editing Technologies

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Introduction

Abiotic stresses, such as drought, salinity, extreme temperatures (heat and cold), mineral deficiencies, UV-B radiation, and imbalanced light conditions, significantly impact plant growth and crop productivity. Drought leads to dehydration and reduced photosynthesis, while salinity causes ion toxicity and nutrient imbalances. Extreme temperatures can damage cellular structures and disrupt metabolic processes. Mineral deficiencies hinder essential nutrient uptake, and increased UV-B radiation can cause molecular damage. Light-related stresses, including excess light causing photo oxidative damage and insufficient light limiting photosynthesis, also affect plant health. These combined stresses result in substantial yield losses, challenging agricultural productivity and sustainability (He *et al.* 2018). Genome editing refers to a suite of biotechnological techniques that enable precise modifications to the DNA by addition, removal, or alteration of genetic material at specific locations within the genome. Genome editing technology leverages the natural DNA repair mechanisms of cells to introduce specific genetic modifications.

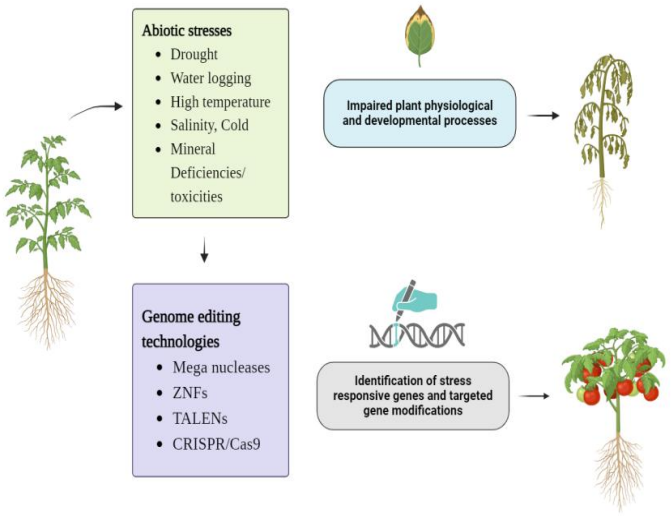

Fig. 1 Overview of the impact of Abiotic stresses and Genome editing on the plant

The specific sequence of DNA is recognized by the sequence specific nucleases that are inserted into the genome and cause double stranded breaks. The targeted generation of double-strand breaks is repaired primarily through two host DNA repair mechanisms such as Non-Homologous End Joining and Homologous Directed Repair mechanisms.

Non-Homologous End Joining (NHEJ)

NHEJ is a rapid repair mechanism that rejoins the two broken ends of DNA without requiring a homologous template. The process is often error-prone leading to insertions or deletions (indels) causing frameshift mutations. NHEJ is classified into Classical NHEJ (cNHEJ) and Alternative NHEJ (aNHEJ). Classical NHEJ protects broken DNA ends from degradation and prevents the joining of unlinked broken ends. Alternative NHEJ takes over DSB repair in the absence of cNHEJ, leading to more inversions and translocations aNHEJ is necessary for creating patch insertions during DSB repair (Gehrke et al., 2022).

Homologous Recombination (HR)

HR is a high-fidelity repair mechanism that uses a homologous sequence as a template for repair. Since it uses a homologous template, HR is generally error-free and precise. Used for precise insertion, correction, or replacement of DNA sequences by providing a homologous DNA template with the desired changes. Ideal for correcting point mutations or introducing specific genetic changes

Site-directed nucleases (SDNs) are engineered enzymes designed to introduce precise cuts in the DNA at specific locations. SDNs rely on the cell's natural DNA repair mechanisms to get targeted modifications. Based upon the genetic modifications induced they are commonly categorized into SDN1, SDN2 and SDN3

SDN1: SDN1 induces double-strand breaks (DSBs). The DSBs are repaired by the NHEJ pathway, which directly ligates the broken DNA ends.

SDN2: SDN2 also induces DSBs, but repairing is guided by a homologous DNA template. HDR allows precise modifications at the break site, such as specific nucleotide changes, small insertions, or deletions, guided by the template.

SDN3: Similar to SDN2, SDN3 induces DSBs, and the repair is guided by a homologous DNA template. However, SDN3 involves the integration of larger DNA sequences, such as entire genes or regulatory elements. HDR facilitates the insertion of larger genetic constructs, allowing the addition of new genes or multiple traits at a single locus. The various types of genome editing technologies evolved includes Meganucleases (Homing Endonucleases), Zinc Finger Nucleases (ZFNs), TALENs (Transcription Activator-Like Effector Nucleases) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9.

1. Meganucleases (Homing Endonucleases)

Meganucleases are naturally occurring enzymes that recognize and cleave larger DNA sequences >12. The double-strand breaks caused by meganucleases are repaired by the host's homologous repair system. Among various meganucleases, I-SceI and I-CreI are the most widely used for genome editing. I-SceI is located in the 21S rRNA gene of *Saccharomyces cerevisiae* of mitochondrial DNA, recognizes the 5′-TAGGGATAACAGGGTAAT-3′. I-CreI, found in the 23S rRNA gene of the chloroplast in *Chlamydomonas reinhardtii*, targets the 22 bp sequence 5′-CAAAACGTCGTGAGACAGTTTG-3′ (Prieto et al., 2018). Due to their difficulty in reengineering to target new DNA sequences limits the application of meganucleases in genome editing.

2. Zinc Finger Nucleases (ZFNs)

ZFNs are artificially synthesized nucleases consisting of a DNA-binding domain composed of DNA binding domain (3-6 zinc finger repeats that can recognize 9-18 bp) and a nuclease domain consisting of the restriction enzyme FokI derived from (*Flavobacterium okeanokoites* I). The FokI domain functions as a dimer, requiring two ZFN monomers to bind adjacent DNA sequences. Once dimerized, FokI cleaves the target DNA sequence, inducing a double-strand break (DSB) that can be repaired either through non-homologous end joining (NHEJ) or homologous recombination (HR). ZFNs can be designed using various protein engineering techniques to target virtually any new DNA sequence. Enhanced specificity and activity of engineered ZFNs have facilitated successful gene knockouts to disable gene function and introduce gain-of-function mutations (Petolino 2015).

Table 3: Key characteristics of different genome editing technologies

Features	Meganucleases	ZFNs	TALENs	CRISPR/Cas9
Endonuclease	I-SecI	FokI	FokI	Cas
Target Sequence Length	12-40 bp	18-35 bp	25-40 bp	20 bp
Targeting Flexibility	high	Moderate	High	Very High
Repair mechanism	HDR	NHEJ	HDR	NHEJ
Cost	High	High	High	Low
Off-Target Effects	Low	Moderate	Low	high

3. TALENs (Transcription Activator-Like Effector Nucleases)

TALENs are engineered nucleases that are composed of a DNA-binding domain derived from transcription activator-like effectors (TALEs) and a FokI nuclease domain. Each TALE repeat can recognize a single base pair, allowing for highly specific targeting by assembling multiple repeats. TALEs are transcription factors naturally produced by plant pathogens of the Xanthomonas genus during infection (Doyle et al., 2013). Similar to ZFNs, TALENs require dimerization of the FokI nuclease domains for DNA cleavage. TALENs offer a highly customizable approach to genome editing, allowing precise modifications such as gene knockout, gene correction, and insertion of specific sequences. They have been used in a wide range of organisms for both research and biotechnological applications.

4. CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats)

CRISPR/Cas9 is a revolutionary genome editing tool espoused from a bacterial immune system of different archaeal and bacterial species. It consists of a guide RNA (gRNA) that directs the Cas9 enzyme to specific genomic sequences, where it induces DSBs. The gRNA is programmable and guides Cas9 to cleave DNA at precise locations. The DSBs trigger cellular DNA repair mechanisms, leading to gene knockout, gene insertion, or precise nucleotide changes through HDR or NHEJ pathways. At first, Cas9 isolated from

Streptococcus pyogenes was predominantly used for genome editing experiments. However, in recent years, several variants of Cas9 have been developed through enhancements of the wild-type enzyme such as Cas3, Cas12, and Cas13. Among which, Cas9 has emerged as the preferred choice followed by Cas12. Compared to other genome editing tools, this system is simpler, more versatile, and cost-effective, making it widely used for improving various traits in the plants. The various genes modified using CRISPR/Cas to enhance abiotic stress tolerance in different crop plants are summarized in Table 2.

Table 2. The enhancement of abiotic stress resistance in various plants using CRISPR/Cas

Crop	Gene edited	Target trait	Outcome	Reference
Soybean	GmSALT3	Salt tolerance	Enhanced salt tolerance by regulating ion homeostasis and reducing sodium accumulation in roots	(Do et al., 2021)
Barley	HvCBF4	Cold tolerance	Enhanced cold tolerance through increased expression of cold-responsive transcription factors	(Wang et al., 2021)
Tomato	SlMAPK3	Cold tolerance	Improved cold tolerance through enhanced expression of cold-responsive genes	(Wang et al., 2020)
Cotton	GhWRKY33	Heat and drought tolerance	Improved heat and drought tolerance by modulating the expression of stress-related genes	(He et al., 2020)
Rice	OsDREB1F	Drought tolerance	Enhanced drought tolerance by improving root architecture and water use efficiency	(Xu et al., 2019)
Potato	StDREB2	Drought tolerance	Enhanced drought tolerance through increased expression of stress-responsive genes	(Kim et al., 2019)
Wheat	TaHKT1;5-D	Salinity tolerance	Improved salinity tolerance by reducing sodium uptake and maintaining potassium homeostasis	(Wang et al., 2018)
Maize	ARGOS8	Drought and heat tolerance	Increased yield under drought and heat stress conditions by altering ethylene response pathways	(Shi et al., 2017)
Arabidopsis	AtERF74	Oxidative stress tolerance	Increased tolerance to oxidative stress through enhanced ROS scavenging activity	(Zhang et al., 2016)

Conclusion

Genome editing, particularly through the use of CRISPR/Cas9, offers a transformative approach to enhancing abiotic stress tolerance in crops. By enabling precise modifications of key genes associated with stress responses, it has led to significant improvements in traits such as drought, salinity, cold, and heat tolerance. The ability to target and modify specific genetic sequences allows for the development of crop varieties that are better equipped to withstand adverse environmental conditions, thereby improving yield and sustainability.

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