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## EPIGENOME EDITING & STRESS MEMORY: A NEW FRONTIER IN CLIMATE-RESILIENT AGRICULTURE

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**A**griculture in the 21st century faces unprecedented challenges due to climate change, soil degradation, and increasing biotic and abiotic stresses. Traditional breeding and transgenic technologies have significantly improved crop performance; however, they often require long development cycles and may involve permanent changes to DNA sequences. A promising alternative emerging from molecular biology is epigenome editing, a technology that modifies gene expression without altering the underlying DNA sequence. Closely linked to this is the phenomenon of stress memory, where plants “remember” previous exposure to stress and respond more efficiently upon re-exposure.

Epigenetics refers to heritable changes in gene function that do not involve changes in the DNA sequence. In plants, epigenetic regulation occurs primarily through DNA methylation, histone modifications, and chromatin remodeling. These modifications influence whether a gene is active or silent. Importantly, certain stress-induced epigenetic changes can be stable and sometimes transmitted to subsequent generations, contributing to what is termed transgenerational stress memory.

Epigenome editing combines precision genome-targeting tools with epigenetic modifiers, enabling researchers to activate or repress specific genes associated with stress tolerance. Unlike traditional genome editing, which changes nucleotide sequences, epigenome editing alters regulatory marks such as methyl groups on DNA or acetyl groups on histones. This makes it an attractive strategy for developing climate-resilient crops without introducing foreign DNA.

### Procedure

The process of epigenome editing and investigation of stress memory in plants involves several systematic steps:

## 1 Identification of Target Genes

The first step is identifying stress-responsive genes associated with traits such as drought tolerance, heat resistance, or pathogen defense. Techniques like RNA sequencing (RNA-seq), quantitative PCR, and transcriptome profiling help identify genes that are differentially expressed under stress conditions.

Simultaneously, epigenetic profiling methods such as:

- Bisulfite sequencing (for DNA methylation analysis)
- Chromatin immunoprecipitation sequencing (ChIP-seq)
- ATAC-seq (Assay for Transposase-Accessible Chromatin)

are used to determine epigenetic changes associated with stress responses.

## 2 Design of Epigenome Editing Tools

The most widely used system for epigenome editing is the CRISPR/dCas9 platform. In this system, a catalytically inactive Cas9 (dCas9) protein is fused with epigenetic modifier enzymes such as:

- DNA methyltransferases (for gene silencing)
- DNA demethylases (for gene activation)
- Histone acetyltransferases
- Histone deacetylases

A guide RNA (gRNA) directs the dCas9-effector complex to a specific genomic locus. Unlike conventional CRISPR-Cas9, this system does not cut DNA but instead modifies epigenetic marks at targeted sites.

## 3 Plant Transformation

The designed construct is introduced into plant cells through methods such as:

- Agrobacterium-mediated transformation
- Particle bombardment
- Protoplast transfection

Transformed cells are regenerated into whole plants using tissue culture techniques.

## 4 Validation of Epigenetic Changes

After regeneration, validation is essential to confirm successful editing. This includes:

- Methylation analysis through bisulfite sequencing
- ChIP assays to detect histone modifications
- Gene expression analysis through RT-qPCR
- Phenotypic evaluation under controlled stress conditions follows to assess improvements in tolerance.

## 5 Assessment of Stress Memory

To evaluate stress memory, plants are exposed to a mild stress treatment (priming), allowed to recover, and then re-exposed to stress. Improved performance upon re-exposure indicates memory formation. Researchers also examine whether epigenetic marks persist across developmental stages or generations.

### Principle

The principle of epigenome editing is based on the regulation of gene expression through reversible chemical modifications rather than changes in DNA sequence.

### 1 DNA Methylation

DNA methylation involves the addition of a methyl group to cytosine bases, typically in CG, CHG, or CHH contexts (where H = A, T, or C). Increased methylation in promoter regions usually suppresses gene expression, while demethylation activates genes.

Under stress conditions, plants often undergo dynamic methylation changes. These modifications can remain stable after stress removal, forming the molecular basis of stress memory.

### 2 Histone Modifications

DNA is wrapped around histone proteins to form nucleosomes. Chemical modifications to histone tails—such as acetylation, methylation, phosphorylation, and ubiquitination—alter chromatin structure and gene accessibility.

For example:

1. Histone acetylation generally activates gene expression.
2. Histone deacetylation represses transcription.

Stress exposure may increase activating histone marks at stress-responsive genes, enabling faster transcription during subsequent stress events.

### **3 Chromatin Remodeling**

Chromatin remodeling complexes reposition nucleosomes, making DNA more or less accessible to transcription machinery. This structural change is critical in regulating stress-induced gene expression.

### **4 Stress Memory Mechanism**

Stress memory can be categorized into:

- Somatic memory – persists within the same plant generation.
- Transgenerational memory – inherited by offspring.

When plants experience stress, epigenetic marks are established at specific genomic loci. If these marks are maintained after stress removal, they facilitate rapid gene reactivation during future stress exposure. This enhances survival and productivity under recurring stress conditions.

The principle underlying epigenome editing is to artificially induce or stabilize these beneficial epigenetic marks at targeted genes, thereby mimicking natural stress memory mechanisms.

### **Conclusion**

Epigenome editing and stress memory represent transformative advances in agricultural biotechnology. By modifying gene expression without altering DNA sequences, epigenome editing provides a precise, potentially reversible, and socially acceptable alternative to conventional genetic modification. It allows breeders and molecular biologists to enhance crop resilience against drought, heat, salinity, and pathogens.

Understanding stress memory mechanisms opens new avenues for sustainable crop improvement. Instead of relying solely on structural gene changes, scientists can harness

plants' inherent adaptive capacity. This is particularly relevant in the era of climate change, where repeated stress exposure is becoming common.

However, challenges remain. The stability of induced epigenetic modifications, potential off-target effects, and regulatory considerations need careful evaluation. Long-term field studies are essential to confirm the durability and heritability of engineered epigenetic traits.

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