

Molecular Mechanisms of Abiotic Stress Tolerance in Wheat: Insights from Genetic Engineering

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Wheat (*Triticum aestivum* L.) is one of the most vital staple crops worldwide, contributing significantly to global food security. However, its productivity is increasingly threatened by abiotic stresses such as drought, salinity, and heat, which are exacerbated by climate change. These stresses disrupt cellular homeostasis, impair photosynthesis, and reduce yield potential. Traditional breeding methods have made progress, but they are time-consuming and limited by the existing genetic variability. In this context, genetic engineering emerges as a powerful tool to enhance wheat's resilience to abiotic stresses by introducing specific genes responsible for stress tolerance. This article delves into the molecular mechanisms underlying abiotic stress tolerance in wheat, exploring

recent advancements in genetic engineering techniques such as CRISPR-Cas9, transgenics, and RNA interference (RNAi), aimed at ensuring sustainable wheat production.

1. Molecular Mechanisms of Abiotic Stress Tolerance in Wheat

A. Osmo protectant Synthesis and Ion Homeostasis

Abiotic stresses cause osmotic imbalance in wheat cells, leading to dehydration and ionic toxicity. Genetic engineering has enabled the introduction of genes responsible for the synthesis of Osmo protectants like proline, glycine betaine, and trehalose.

These compounds help maintain cell turgor and membrane stability under stress conditions.

For instance, overexpression of the P5CS gene, which is involved in proline biosynthesis, has been shown to enhance drought tolerance in wheat.

B. Antioxidant Defense Mechanisms



Abiotic stresses such as heat and salinity induce the accumulation of reactive oxygen species (ROS), which cause oxidative damage to cellular components. Genetic engineering has been utilized to enhance the expression of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX). For example, transgenic wheat lines expressing higher levels of SOD and CAT have shown improved tolerance to oxidative stress.

C. Heat Shock Proteins (HSPs) and Chaperones

Heat shock proteins (HSPs) act as molecular chaperones that prevent protein denaturation under heat stress. Genetic engineering has successfully introduced HSP70 and HSP90 genes into wheat, enhancing thermotolerance and ensuring yield stability under high temperatures.

2. CRISPR-Cas9: A Revolution in Wheat Genomics

The advent of CRISPR-Cas9 technology has transformed the landscape of wheat genetic engineering by enabling precise genome editing. Researchers have successfully employed CRISPR to knock out negative regulators of stress response pathways and enhance stress tolerance. For instance, the knockout of TaERF3 (an ethylene response factor) has been shown to improve salt and drought tolerance in wheat. Additionally, CRISPR has been instrumental in targeting ROS-scavenging pathways to minimize oxidative damage under stress conditions. The simplicity, efficiency, and precision of CRISPR make it a promising tool for developing climate-resilient wheat varieties.

3. Epigenetic Modifications and Stress Memory in Wheat

Recent studies have highlighted the significance of epigenetic modifications such as DNA methylation, histone acetylation, and non-coding RNAs in regulating stress-responsive genes in wheat. Epigenetic changes can induce stress memory, allowing wheat to respond more effectively to recurrent stress episodes. For instance, the methylation of promoter regions of drought-responsive genes has been associated with enhanced drought tolerance. Genetic engineering approaches targeting DNA methyltransferases (DNMTs) and histone acetyltransferases (HATs) offer new possibilities for stabilizing stress memory in wheat.

4. Enhancing Photosynthetic Efficiency Under Stress

Abiotic stresses often impair photosynthesis by disrupting chloroplast function and reducing CO₂ assimilation. Genetic engineering has been used to upregulate Rubisco activate genes and introduce C4 pathway enzymes into wheat to enhance photosynthetic efficiency under stress conditions. For example, the expression of PEPC (phosphoenolpyruvate carboxylase) and PPDK (pyruvate phosphate dikinase) has been shown to improve carbon fixation efficiency under heat and drought stress. Improving photosynthesis can significantly contribute to yield stability under adverse conditions.

5. Role of Phytohormones in Stress Tolerance

Phytohormones such as abscisic acid (ABA), salicylic acid (SA), jasmonic acid (JA), and ethylene play a crucial role in modulating wheat's response to abiotic stresses. Genetic engineering has been utilized to

enhance the biosynthesis and signalling pathways of these hormones to improve stress tolerance. For example, the overexpression of NCED (9-cis-epoxycarotenoid dioxygenase), a key enzyme in ABA biosynthesis, has been shown to enhance drought resistance by regulating stomatal closure and osmotic adjustment. Similarly, the manipulation of ethylene-responsive transcription factors (ERFs) has been reported to improve tolerance to salt and heat stress by modulating stress-responsive genes.

6. The Role of MicroRNAs (miRNAs) in Stress Tolerance

MicroRNAs (miRNAs) are small, non-coding RNAs that regulate post-transcriptional gene expression and play a pivotal role in mediating wheat's response to abiotic stresses. Genetic engineering approaches targeting miRNA-mediated pathways have been explored to enhance stress tolerance. For example, overexpression of miR398 has been shown to regulate Cu/Zn-SOD and improve oxidative stress tolerance. Similarly, targeting miR319 has been associated with improved tolerance to drought and salinity by modulating auxin signalling.

7. Enhancing Water and Nutrient Use Efficiency

Abiotic stresses such as drought and salinity significantly impact water and nutrient uptake in wheat. Genetic engineering has been used to introduce aquaporin genes (PIP2;1 and TIP1;1) to enhance water use efficiency (WUE) under drought stress. Similarly, the introduction of NHX (sodium/hydrogen antiporter) and HKT (high-affinity potassium transporter) genes has shown promise in improving salt tolerance by maintaining ion homeostasis. Enhancing WUE and

nutrient efficiency can help sustain wheat productivity under water-limited and saline conditions.

Challenges and Future Directions

Despite the significant progress, the genetic engineering of wheat faces challenges such as regulatory hurdles, off-target effects, public acceptance, and the need for extensive field trials. The development of cisgenic approaches, which involve the introduction of genes from the same or closely related species, could address some of these concerns. Moreover, the integration of machine learning and bioinformatics for predicting gene targets and optimizing transformation protocols can accelerate the development of stress-resilient wheat varieties. A multi-omics approach combining genomics, transcriptomics, proteomics, and metabolomics is essential to unravel the complex molecular networks involved in abiotic stress tolerance. Public-private partnerships (PPPs) can also play a pivotal role in funding research and accelerating the commercialization of stress-resilient wheat.

Conclusion

Genetic engineering has unlocked new possibilities for enhancing abiotic stress tolerance in wheat by targeting Osmo protection, antioxidant defence, heat shock response, and transcriptional regulation pathways. The use of advanced tools like CRISPR-Cas9, RNAi, and epigenetics has made it possible to develop climate-resilient wheat varieties with greater precision and efficiency. However, for these innovations to translate into sustainable agricultural practices, it is essential to address ethical, regulatory, and public acceptance challenges. A synergistic approach combining

biotechnology, traditional breeding, and sustainable agronomy holds the key to securing wheat production in a rapidly changing climate.

References

1. Zhang, Y., et al. (2023). *Epigenetic Modifications in Wheat Under Abiotic Stress*. Plant Molecular Biology Journal.