

Molecular Mechanisms and Application Prospects for Breeding for Drought Tolerance in Maize

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Abstract

As a crop of critical global significance, maize requires substantial water throughout its growth cycle. However, with climate change intensifying and water resources becoming increasingly scarce, drought has emerged as a primary limitation on maize productivity. By delving into the molecular underpinnings of drought resistance in maize, researchers have pinpointed an array of drought-resilience genes, transcriptional regulators, and signal transduction pathways, offering a valuable genetic reservoir for enhancing maize's drought resistance. Advanced molecular tools, including genome editing and marker-assisted selection, have streamlined the targeted optimization and transference of drought-resistance genes, thereby significantly strengthening drought resilience in maize varieties. This paper reviews the intricate molecular bases of drought tolerance and the regulatory frameworks surrounding essential genes in maize, while discussing the future potential of molecular breeding strategies tailored to enhance drought tolerance. Ultimately, this study aims to furnish both a theoretical foundation and technical guidance for advancing drought-resistant maize breeding initiatives.

Keywords

Maize, Drought tolerance, Molecular mechanism, Gene regulation, Breeding technology, Molecular marker-assisted selection, Gene editing.

1. Introduction

Maize (*Zea mays* L.), recognized as a critical global food source, sustains populations across many nations. With the increasing impact of global climate change, drought has emerged as a key constraint on maize yield and quality[1]. Drought not only diminishes soil moisture levels but also impedes photosynthesis, root growth, and nutrient uptake in maize, resulting in significant reductions in crop growth and yield[2]. Therefore, investigating maize drought tolerance and deciphering its molecular mechanisms hold considerable agricultural and economic importance, as these insights provide foundational support for breeding maize varieties with enhanced drought tolerance and yield.

Progress in breeding drought-tolerant maize varieties has been achieved, with traditional methods like cross-breeding and selective breeding yielding several varieties that exhibit improved drought resilience[3]. However, the complexity and multifaceted nature of drought stress pose challenges for conventional breeding, including extended selection cycles and limited efficiency. With advancements in molecular biology and genomics, research into the molecular basis of drought tolerance in maize has advanced significantly[4]. Techniques such as genome-wide association studies, transcriptomics, and gene function analysis have identified numerous drought tolerance-related genes and regulatory factors, offering innovative strategies and technical approaches for molecular breeding.

Elucidating the molecular mechanisms underlying drought tolerance in maize not only enhances understanding of the physiological and molecular responses of maize to drought but also lays the groundwork for precise gene localization and functional analysis of drought tolerance traits[5]. Techniques such as molecular marker-assisted selection and gene editing can expedite the development of drought-tolerant maize varieties, boosting crop resilience and yield[6]. As genomics and epigenomics research progresses, future studies will further advance the molecular design and precise control of drought tolerance breeding in maize, offering potential solutions to the challenges posed by climate change.

2. Overview of Molecular Mechanisms of Drought Tolerance in Maize

The molecular mechanisms underlying drought tolerance in maize encompass regulation across various physiological, molecular, and genetic layers [7]. When subjected to drought stress, maize responds through a cascade of physiological adjustments [8]. Water loss triggers cellular dehydration, which maize counters by enhancing water uptake through its root system, reducing water loss via stomatal closure, and producing osmoregulatory compounds (e.g., proline, glycerol, etc.). Additionally, activation of the antioxidant system enables maize to manage drought-induced oxidative stress, with these physiological adaptations serving as essential protective mechanisms that support maize survival in drought-prone conditions, showed in Figure 1 :

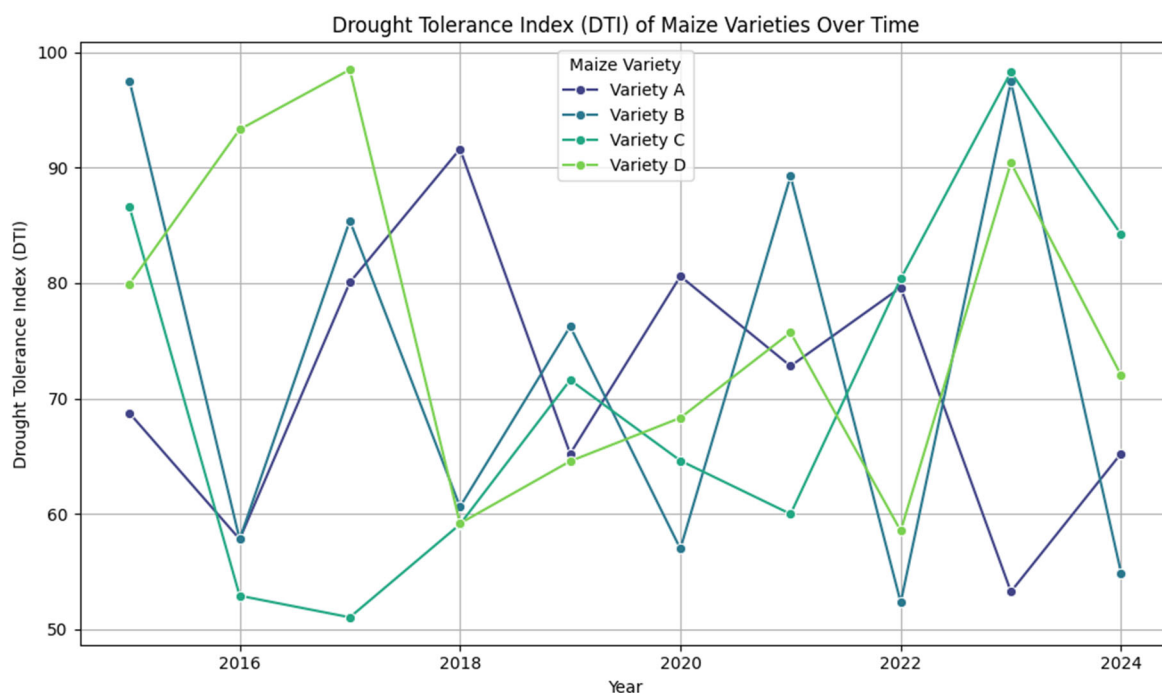


Figure 1. Drought Tolerance Index (DTI) of Maize Varieties Over Time

On a molecular level, certain genes associated with water metabolism, osmoregulation, and antioxidant responses in maize show altered expression patterns under drought conditions[9]. Genes within the abscisic acid (ABA) signaling pathway, for instance, modulate stomatal closure, root development, and the synthesis of drought-resistance proteins. By adjusting gene expression, maize can promptly respond to drought stress and enhance its drought resilience, with these gene regulatory mechanisms forming the molecular foundation for drought tolerance in maize[10].

Transcription factors also play an essential role in maize's drought tolerance. Families of transcription factors like NAC, bZIP, and WRKY can modulate the expression of drought-tolerance genes by binding to promoter regions. Studies have shown that NAC transcription factors can control root growth in maize under drought conditions, optimize water use, and enhance drought resilience by activating drought-responsive genes. These transcription factors thus hold a central position in regulating drought tolerance. Drought Tolerance Index (DTI):

$$DTI = \frac{Y_d}{Y_w} \times 100\% \quad (1)$$

Signaling pathways play a crucial role in regulating drought tolerance in maize. The ABA signaling pathway serves as the central regulatory network under drought conditions, improving drought resilience by regulating stomatal closure and increasing the expression of drought-resistance genes. Other signaling pathways, such as CIPK (CDPK-interacting protein kinase) and MAPK (mitogen-activated protein kinase), also participate in modulating drought tolerance in maize. These pathways enable maize to detect drought stimuli and activate a range of molecular responses to manage physiological adaptations, thus strengthening the plant's drought resilience. Comprehensive studies of these molecular mechanisms can provide more precise theoretical insights and technical foundations for drought-tolerant breeding initiatives.

3. Regulatory Mechanisms of Drought Tolerance Genes in Maize

The regulatory mechanisms of drought tolerance genes in maize are complex and multilayered, involving multiple aspects of gene expression, transcription factor regulation and signaling pathways. These mechanisms work together to ensure the adaptation and growth of maize in arid environments. The regulation of drought tolerance gene expression is achieved through the regulation of hormone signaling pathways such as abscisic acid (ABA) and transcription factors, which can precisely control water metabolism, antioxidant response and stomatal regulation. By binding to the promoters of specific genes, they activate or inhibit the expression of the target genes, thus enhancing drought tolerance in maize. Signaling pathways, such as the ABA-dependent signaling pathway and the CIPK/MAPK pathway, play a key role in the perception and regulation of drought response. Through these regulatory mechanisms, maize can efficiently respond to drought stress, thus enhancing its drought tolerance.

3.1. Identification and function of drought resistance related genes

With advancements in genomics technology, methods for identifying drought tolerance genes have become increasingly varied. Initially, traditional genetic approaches like hybridization analysis and quantitative trait locus (QTL) mapping allowed researchers to locate regions associated with drought tolerance. Today, genomic techniques such as genome-wide association studies (GWAS) and whole genome resequencing enable more efficient identification of drought-tolerance genes across diverse maize varieties. Additionally, transcriptomic and epigenomic studies have introduced new avenues for uncovering drought-responsive genes, highlighting the critical role of gene regulatory networks in maize's drought tolerance.

Several drought tolerance genes in maize have been identified and studied for their functions. These genes contribute to mechanisms like water uptake, stomatal regulation, and antioxidant defenses. For instance, members of the ZmDREB (Dehydration Responsive Element-Binding) gene family regulate water metabolism and cellular osmotic balance during drought stress, while ZmAREB (ABA-Responsive Element-Binding) genes are pivotal in the ABA signaling pathway, modulating stomatal closure to limit water loss. Additionally, genes like ZmSOD (Superoxide Dismutase) play protective roles by scavenging reactive oxygen species, thereby

reducing oxidative damage induced by drought and enhancing overall drought resilience in maize.

Beyond the functions of these individual genes, the regulatory mechanisms of drought tolerance genes have been thoroughly explored. Many drought-resistant genes are regulated by transcription factors; NAC, bZIP, and WRKY families, for example, bind to gene promoters under drought conditions to modulate gene expression, thus bolstering maize's drought tolerance. The activation of the ABA signaling pathway further reinforces gene regulation, as ABA, a key drought hormone, enhances maize drought tolerance by controlling stomatal closure and promoting the synthesis of drought-protective proteins, showed in Figure 2 :

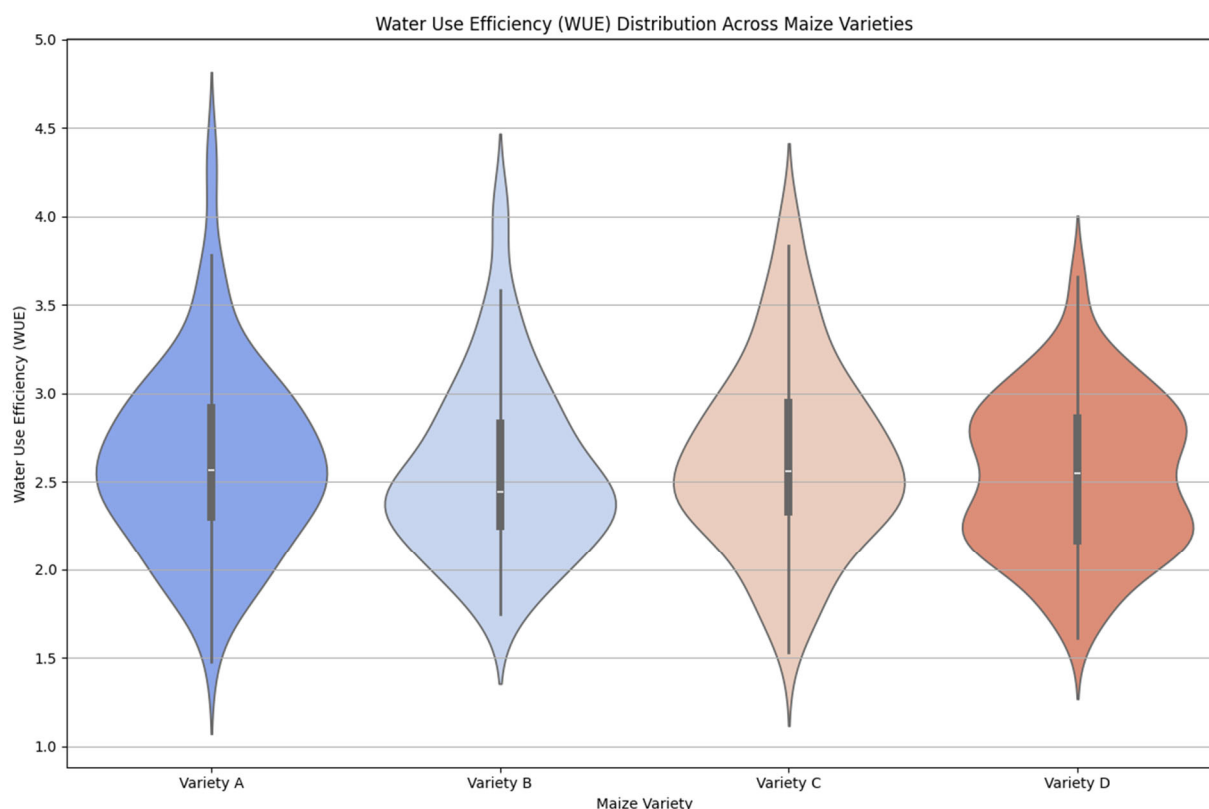


Figure 2. Water Use Efficiency (WUE) Distribution Across Maize Varieties

With the continuous discovery and functional elucidation of drought tolerance-related genes, molecular marker-assisted selection (MAS) and gene editing techniques have become important tools for breeding drought tolerance in maize. By introducing drought tolerance genes, the drought tolerance performance of maize varieties can be significantly improved. Gene editing technologies, such as CRISPR/Cas9, have already achieved initial results in drought tolerance improvement, making it possible to precisely regulate specific drought tolerance genes. With the discovery of more drought-resistant genes and a deeper understanding of their regulatory networks, maize drought tolerance breeding will become more precise and efficient, contributing to food security and sustainable agricultural development.

3.2. Transcription factors and their regulatory networks

Transcription factors are key proteins that regulate gene expression and are able to regulate the transcriptional activity of genes by binding to their promoter or enhancer regions. In the regulation of drought tolerance in maize, transcription factors play a crucial role. It has been found that a variety of transcription factors exhibit significant up- or down-regulation under

drought stress, including members of the NAC, bZIP, and WRKY families. These transcription factors are able to sense drought signals and enhance maize adaptation to drought environment by regulating the expression of relevant drought tolerance genes. The transcription factors not only affect stomatal regulation and root growth, but also regulate the activity of antioxidant enzymes to help maize cope with drought-induced oxidative damage. Water Use Efficiency (WUE):

$$WUE = \frac{\text{Biomass}}{\text{Transpired Water}} \quad (2)$$

The NAC family of transcription factors plays a crucial role in regulating drought tolerance in plants, including maize. Members of the NAC family can enhance water use efficiency and antioxidant responses in maize under drought stress by binding to specific gene promoter regions. For instance, ZmNAC1 promotes the growth of the maize root system, improving its capacity to absorb water, while ZmNAC2 regulates drought-related gene expression, enhancing the plant's ability to resist drought stress. These functional studies highlight the critical role of NAC transcription factors in drought tolerance, offering potential molecular targets for improving drought resistance in maize breeding.

bZIP transcription factors, another widely distributed class of plant transcription factors, are known to be crucial for plant responses to environmental stress. Particularly in drought stress, bZIP transcription factors interact with the abscisic acid (ABA) signaling pathway, a key regulator of drought response. ABA, the primary hormone responsible for drought response, activates bZIP transcription factors, which regulate stomatal closure, the expression of drought-resistant genes, and other adaptive mechanisms. By combining with ABA, bZIP transcription factors initiate the expression of genes that produce drought-related proteins such as antioxidant enzymes and dehydration-responsive proteins, significantly enhancing maize drought tolerance.

WRKY transcription factors are another critical class involved in the plant response to drought stress. These factors regulate the expression of drought-responsive genes by binding to their promoters, and they not only participate in the ABA signaling pathway but also interact with other stress-related signaling pathways, such as those for salinity and low temperature. For example, ZmWRKY2 has been shown to regulate the expression of drought-resistant genes, thereby improving maize's drought tolerance. The study of WRKY transcription factors offers valuable insights into the molecular response mechanisms of maize under drought stress, providing new approaches for improving drought tolerance through gene manipulation.

3.3. Signaling pathways and hormone regulation

Signaling pathways play a crucial role in how plants, including maize, respond to drought stress by enabling adaptive and rapid physiological changes. The abscisic acid (ABA) signaling pathway is one of the most important core pathways in drought response. As a key phytohormone, ABA regulates stomatal closure, which reduces water loss through transpiration, and activates the expression of genes associated with drought resistance. The CIPK (CDPK-interacting protein kinase) and MAPK (mitogen-activated protein kinase) pathways also contribute significantly to maize's response to drought stress. These pathways work by activating transcription factors and other related genes, coordinating the cellular adaptation processes necessary for drought tolerance.

Under drought conditions, ABA accumulates and triggers a series of molecular events that enhance plant drought tolerance. ABA binds to the PYR/PYL proteins via its receptor, ABAR (ABA receptor), which inhibits the activity of protein phosphatase 2C (PP2C), leading to the activation of SnRK2 (Sucrose Non-fermenting Related Kinase 2). The ABA signaling pathway

regulates various processes such as stomatal closure, antioxidant enzyme synthesis, and the accumulation of osmoregulatory substances. These responses collectively minimize water loss, thus enhancing drought tolerance in maize. Additionally, ABA interacts with other hormones, such as cytokinin and gibberellin, to coordinate the plant's overall response to drought stress.

In addition to the ABA pathway, the CIPK and MAPK signaling pathways also play significant roles in drought regulation. The CIPK pathway interacts with calcium (Ca^{2+}) signaling to regulate osmotic adjustment and water uptake at the cellular level, thereby improving the plant's ability to survive under drought conditions. The MAPK pathway, through the phosphorylation of protein kinases, activates transcription factors that regulate the expression of drought-resistant genes. The activation of these pathways in maize optimizes water management and enhances the plant's drought tolerance.

Plant hormones involved in drought stress not only function individually but also coordinate with each other to modulate drought tolerance. ABA typically promotes stomatal closure and reduces water loss, while cytokinin enhances water uptake by stimulating root growth. Gibberellins contribute to plant growth and development under drought stress, helping maize maintain growth despite limited water availability. Jasmonic acid, a hormone involved in plant defense, works synergistically with ABA to strengthen drought tolerance. The interactions between these hormones provide a sophisticated molecular regulatory framework that is essential for understanding and improving drought tolerance in maize breeding.

4. Strategies and Practices for Breeding for Drought Tolerance in Maize

Traditional breeding methods for drought tolerance in maize include crossbreeding and varietal improvement, which enhance the adaptability of maize to drought by selecting and breeding drought-tolerant parents and superior varieties. The drought tolerance of maize varieties can be progressively improved by selecting maize germplasm that exhibits high drought tolerance under natural conditions for crossing and screening. In addition, conventional field trials and methods for evaluating drought tolerance phenotypes have played a fundamental role in breeding for drought tolerance in maize. Despite the partial success of conventional breeding methods, they have long cycles and limited effects, and thus need to be combined with modern biotechnological tools to promote the further development of maize drought tolerance breeding.

Molecular marker-assisted breeding (MAS) provides a more precise tool for breeding for drought tolerance in maize. By utilizing molecular markers related to drought tolerance, breeders are able to screen for superior drought-tolerant genotypes at the genotype stage, speeding up the selection process. methods such as QTL (quantitative trait loci) targeting and GWAS (genomic linkage analysis) have helped to identify a number of drought-tolerant-related genes and marker loci. Using these molecular markers, breeders are able to better screen drought tolerance genes at the gene level, improving breeding efficiency and accuracy. The genetic basis of drought tolerance traits in maize has been further elucidated through MAS technology, which can help develop more efficient drought-tolerant maize varieties.

Gene editing technology, especially CRISPR/Cas9 technology, has become an important tool for drought tolerance breeding in maize. By precisely modifying specific genes in the maize genome, gene editing technology is able to realize the targeted regulation of drought-related genes, thus effectively improving the drought tolerance of maize. By editing genes that control water metabolism, stomatal regulation, antioxidant and other related genes, it can enhance the survival ability of maize in drought environment. In addition, gene editing technology can accelerate the breeding process and reduce the uncertainty and long period of time in traditional breeding methods, and its application in maize drought tolerance breeding is very promising.

In the practical application of maize drought tolerance breeding, an integrated breeding strategy combining traditional breeding, molecular marker-assisted breeding (MAS) and gene editing technology has become an important way to enhance maize drought tolerance. Through the synergistic effect of different breeding methods, maize varieties with both high yield and excellent drought tolerance can be selected quickly and accurately. Drought tolerance genes are identified through genomics technology, and drought tolerance traits are accurately evaluated in combination with field environmental and phenotypic data to select the best breeding materials. In addition, changes in environmental factors and climate change have put forward higher requirements for drought-tolerant maize breeding, and the breeding work needs to be flexible to cope with changing climatic conditions, so as to promote the rapid popularization and application of drought-tolerant maize varieties.

5. Conclusion

Breeding for drought tolerance in maize is one of the important ways to cope with global climate change and water shortage. Through in-depth research on the molecular mechanisms of drought tolerance in maize, especially the analysis of drought tolerance-related genes, transcription factors and signaling pathways, the physiological and molecular responses of maize under drought stress have been revealed, providing a theoretical basis for the mining and application of drought tolerance genes. The rapid development of modern breeding technologies, especially molecular marker-assisted breeding (MAS) and gene editing technologies, provides a more precise and efficient means for breeding drought tolerance in maize. Through the combined use of these technologies, the selection and breeding of drought-tolerant maize varieties can be accelerated and the drought tolerance of maize can be significantly improved.

The genetic complexity of drought tolerance traits, the impact of climate change on maize growth, and the diversity of drought tolerance mechanisms in different environments. Future breeding efforts not only need to integrate traditional breeding methods and modern biotechnology, but also need to strengthen the combination of genomics, phenomics and environmental data to cope with changing climatic conditions and to realize efficient and sustainable drought-tolerant breeding in maize, and drought-tolerant breeding in maize will surely make an important contribution to the global food security and sustainable development of agriculture.

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