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Unveiling Drought Tolerance Mechanisms in Soybean Seed Germination: New Insights from Physiological and Molecular Perspectives

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Abstract Drought tolerance in soybeans is crucial for ensuring sustainable crop production under increasing climate variability. This study aims to provide a comprehensive overview of the physiological and molecular mechanisms underlying drought tolerance during soybean seed germination, as well as integrative approaches and practical applications in breeding strategies. Physiological mechanisms include osmotic adjustment through proline and soluble sugar accumulation, water uptake and retention mediated by seed coat properties and aquaporins, and antioxidant defense systems involving both enzymatic and non-enzymatic antioxidants. On a molecular level, the study explores gene expression regulation by drought-responsive transcription factors, stress-inducible promoters and genes, signal transduction pathways including ABA-dependent and independent pathways, and genomic and proteomic approaches to identify drought-responsive genes and proteins. Integrative approaches such as systems biology and gene editing tools like CRISPR/Cas9 are discussed for their potential in enhancing drought tolerance. Practical applications focus on breeding strategies, highlighting marker-assisted selection and comparing conventional breeding with biotechnological methods. The study also addresses challenges and opportunities in developing drought-resilient soybean varieties, considering environmental variability, field conditions, and socioeconomic factors. The findings underscore the importance of a multi-faceted approach to improve drought tolerance in soybeans, with implications for global food security.

Keywords Drought tolerance; Soybean seed germination; Physiological mechanisms; Molecular mechanisms; Breeding strategies

1 Introduction

Soybean (*Glycine max* L.) is a globally significant crop, providing essential proteins and oils for human consumption and animal feed. However, its productivity is severely threatened by environmental stresses, particularly drought, which is exacerbated by climate change (Buezo et al., 2018; Zhao et al., 2020; Begum et al., 2022). Drought stress during the seed germination stage is especially detrimental, as it can inhibit seedling establishment and reduce overall crop yield (Aleem et al., 2020; Wang et al., 2022). Understanding the mechanisms underlying drought tolerance in soybeans is crucial for developing resilient cultivars that can withstand water-deficit conditions and ensure food security (Aleem et al., 2020; Sun et al., 2022; Xuan et al., 2022).

This study aims to provide a comprehensive overview of the physiological and molecular mechanisms that confer drought tolerance during soybean seed germination. By synthesizing findings from recent studies, we seek to highlight key genetic, biochemical, and physiological traits that contribute to drought resilience. The scope of this study includes the identification of drought-tolerance genes, the role of antioxidant metabolism, and the physiological adjustments that enable soybean seedlings to cope with water stress. Through this synthesis, we hope to offer new insights that can inform breeding programs and genetic engineering efforts aimed at enhancing drought tolerance in soybeans.

2 Physiological Mechanisms of Drought Tolerance in Soybean Seed Germination

2.1 Osmotic adjustment

Proline accumulation is a critical osmotic adjustment mechanism in soybean seed germination under drought conditions. Proline acts as an osmoprotectant, stabilizing proteins and membranes, and scavenging free radicals. Studies have shown that drought stress leads to a significant increase in proline content in soybean leaves, which helps in maintaining cell turgor and minimizing damage caused by dehydration (Fatema et al., 2023; Begum et al., 2022; Wang et al., 2022). Additionally, proline accumulation has been observed to correlate with drought tolerance in various soybean cultivars, indicating its essential role in osmotic adjustment (Figure1) (Du et al., 2020).

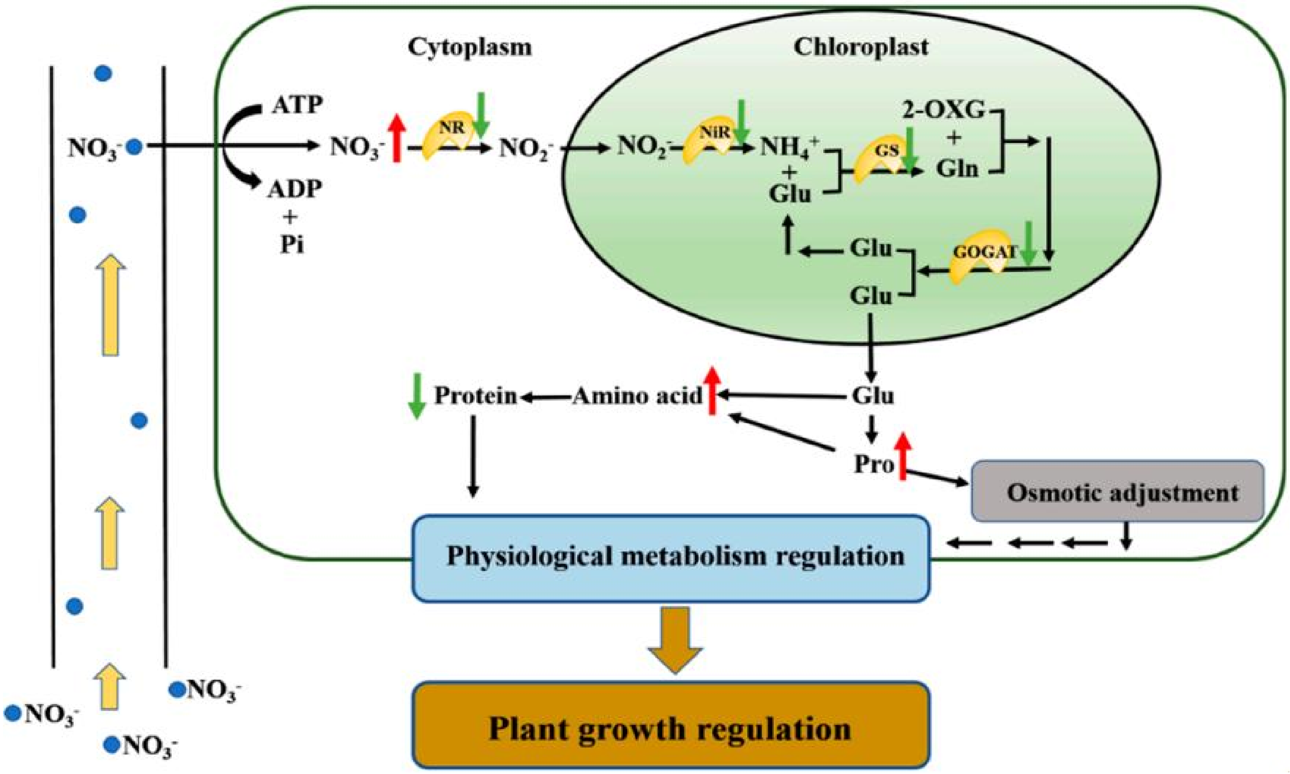

Figure 1 A model of the nitrogen regulation in soybean leaf in response to drought stress (Adopted from Du et al., 2020)
Image caption: Here, drought stress triggers a nitrogen-mediated tandem reaction in response to drought stress. Drought stress might change the expression of key regulatory metabolic genes and the activities of nitrogen metabolism enzymes, which regulate nitrogen distribution and utilization to adapt to environmental stress. NR, nitrate reductase; NiR, nitrite reductase; GS, glutamine synthetase; GOGAT, glutamate synthetase; Glu, glutamate; Gln, glutamine; 2-OXG, 2-oxoglutarate. Increased items under drought stress are marked with upward red arrows and decreased items are marked with downward green arrows (Adopted from Du et al., 2020)

Soluble sugars, such as sucrose and glucose, play a vital role in osmotic adjustment by maintaining osmotic balance and protecting cellular structures during drought stress. Study indicates that drought conditions lead to an increase in soluble sugar content in soybean plants, which contributes to osmotic adjustment and stress tolerance (Gurrieri et al., 2020; Moloi and Merwe, 2021; Wang et al., 2022). The accumulation of soluble sugars has been shown to be more significant than proline in some cases, highlighting their predominant role in counteracting osmotic stress (Gurrieri et al., 2020; Ozturk et al., 2020).

2.2 Water uptake and retention

The seed coat properties of soybean seeds influence water uptake and retention, which are crucial for germination under drought conditions. A robust seed coat can reduce water loss and enhance water retention, thereby supporting seed germination and seedling establishment during water-deficit conditions. Studies have demonstrated that drought-tolerant soybean varieties possess seed coats with better water retention capabilities, contributing to their overall drought resilience (Song et al., 2022; Fatema et al., 2023).

Aquaporins are water channel proteins that facilitate water transport across cell membranes, playing a significant role in water uptake and retention during drought stress. The expression of aquaporins is often upregulated in response to drought, enhancing the plant’s ability to maintain water balance and support seed germination. study has shown that aquaporins are crucial for maintaining cellular water homeostasis and improving drought tolerance in soybean (Sheteiwy et al., 2021; Fatema et al., 2023; Wang et al., 2024).

2.3 Antioxidant defense system

Enzymatic antioxidants, such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), play a pivotal role in mitigating oxidative stress caused by drought. These enzymes help in scavenging reactive oxygen species (ROS), thereby protecting cellular components from oxidative damage. Studies have reported increased activities of SOD, CAT, and POD in drought-tolerant soybean varieties, which contribute to their enhanced drought tolerance by maintaining cellular redox homeostasis (Moloi and Merwe, 2021; Begum et al., 2022; Wang et al., 2022).

Non-enzymatic antioxidants, such as ascorbate and glutathione, also play a crucial role in the antioxidant defense system during drought stress. These antioxidants work synergistically with enzymatic antioxidants to neutralize ROS and protect cells from oxidative damage. study indicates that drought stress leads to an increase in the levels of ascorbate and glutathione in soybean plants, which helps in maintaining cellular integrity and enhancing drought tolerance (Moloi and Merwe, 2021; Sheteiwy et al., 2021; Wang et al., 2022).

In summary, the physiological mechanisms of drought tolerance in soybean seed germination involve a complex interplay of osmotic adjustment, water uptake and retention, and antioxidant defense systems. The accumulation of proline and soluble sugars, the properties of the seed coat, the role of aquaporins, and the activities of enzymatic and non-enzymatic antioxidants are all critical factors that contribute to the drought resilience of soybean seeds. These insights provide a comprehensive understanding of the physiological responses of soybean to drought stress, which can inform breeding programs aimed at developing drought-tolerant soybean varieties.

3 Molecular Mechanisms of Drought Tolerance in Soybean Seed Germination

3.1 Gene expression regulation

Transcription factors (TFs) play a crucial role in regulating gene expression in response to drought stress. For instance, the WRKY transcription factor GmWRKY54 has been shown to enhance drought tolerance in soybean by activating genes involved in ABA and Ca^{2+} signaling pathways. GmWRKY54 directly binds to the promoter regions of genes such as *PYL8*, *SRK2A*, *CIPK11*, and *CPK3*, thereby promoting stomatal closure to reduce water loss (Wei et al., 2019). Another important TF, GmNFYB17, has been identified to regulate drought resistance by enhancing root growth and improving physiological traits such as relative water content (RWC) and superoxide dismutase (SOD) activity (Sun et al., 2022). Additionally, the GmHdz4 transcription factor, when edited via CRISPR/Cas9, has shown improved drought tolerance by promoting root system architecture and maintaining turgor pressure through osmolyte accumulation (Zhong et al., 2022).

Stress-inducible promoters and genes are critical for the activation of drought-responsive pathways. The *GmWRKY54* gene, driven by a drought-induced promoter (RD29a), has been shown to confer drought tolerance by activating a large number of stress-related genes (Wei et al., 2019). Similarly, the TGA transcription factor GmTGA17, which is strongly induced by drought stress, enhances tolerance by increasing chlorophyll and proline contents and reducing malondialdehyde (MDA) content in transgenic plants (Li et al., 2019). The GmNAC019 transcription factor also mediates drought tolerance in an ABA-dependent manner, demonstrating higher survival rates and stronger antioxidant defense under water-stressed conditions (Hoang et al., 2019).

3.2 Signal transduction pathways

Abscisic acid (ABA) plays a pivotal role in drought response by regulating various physiological and molecular processes. The GmWRKY54 transcription factor activates ABA receptors and SnRK2 kinases, which are upstream components of the ABA signaling pathway, thereby enhancing drought tolerance (Wei et al., 2019). The GmNAC019 transcription factor also functions as a positive regulator of ABA-mediated responses, leading to lower water loss rates and improved drought tolerance (Hoang et al., 2019). Additionally, the overexpression of the Arabidopsis bZIP transcription factor AREB1 in soybean has been shown to activate cross-signaling responses under drought stress, further highlighting the importance of ABA-dependent pathways (Fuhrmann-Aoyagi et al., 2020).

ABA-independent pathways also contribute to drought tolerance in soybean. For example, the comparative transcriptome analysis of drought-tolerant and sensitive soybean genotypes revealed that numerous differentially expressed genes (DEGs) are involved in signal transduction pathways such as calcium signaling and MAPK signaling, which are independent of ABA (Xuan et al., 2022). The GmHdz4 transcription factor, edited via CRISPR/Cas9, enhances drought tolerance through mechanisms that include antioxidant enzyme activity and osmolyte accumulation, which are not directly linked to ABA signaling (Zhong et al., 2022).

3.3 Genomic and proteomic approaches

Genomic approaches such as genome-wide association studies (GWAS) have identified several loci associated with drought tolerance in soybean. For instance, a study identified 26 SNPs related to drought tolerance during the germination stage, leading to the identification of 41 candidate genes (Figure 2) (Zhao et al., 2020). Another study identified 11 SNPs and 22 QTLs significantly associated with drought tolerance, with the *GmNFYB17* gene being a key candidate for further analysis (Sun et al., 2022). Additionally, RNA-seq analysis of wild soybean genotypes under drought stress identified thousands of DEGs involved in various stress responses, providing a comprehensive understanding of the genetic mechanisms underlying drought tolerance (Aleem et al., 2020).

Proteomic approaches have also been employed to identify proteins associated with drought tolerance. For example, a study identified 163 metabolites and 3 000 genes that are strongly regulated during water stress, including transcription factors and signaling components that are potential targets for improving drought responses (Tripathi et al., 2016). Proteomic profiling of soybean seedlings under drought stress revealed key pathways and proteins involved in stress responses, such as those related to cell wall remodeling and stress-related proteins (Xuan et al., 2022.)

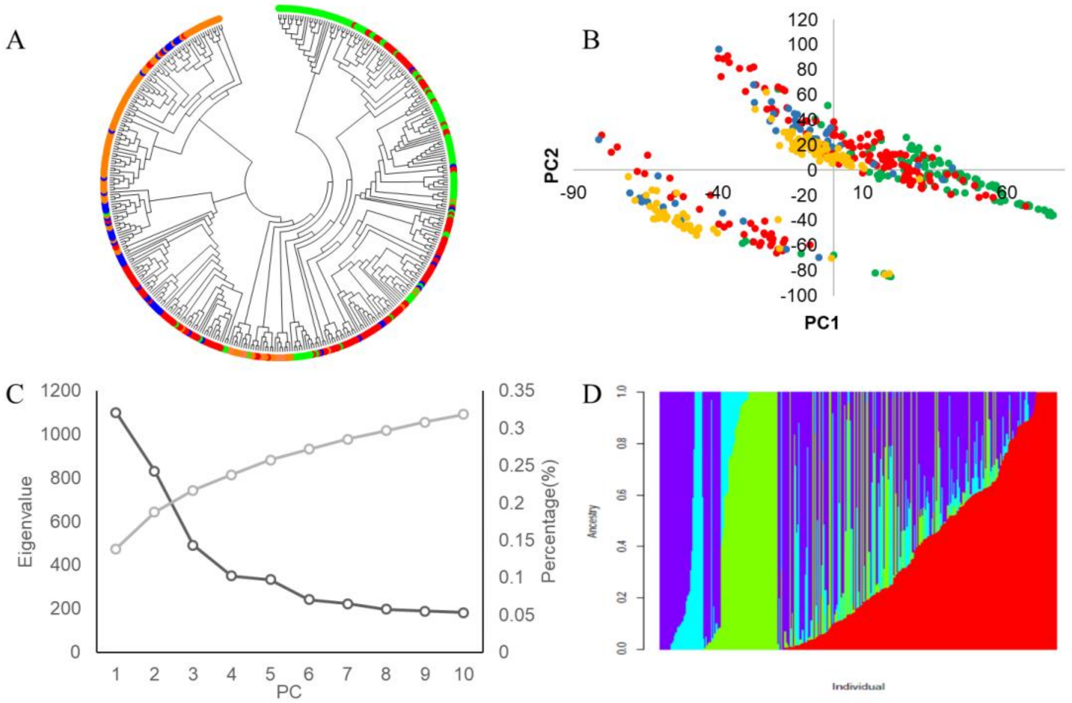

Figure 2 Genetic structure and relatedness of the 410 soybean accessions (Adopted from Zhao et al., 2020)

Image caption: (A) Neighbor--joining tree constructed using SNP data, foreign soybean germplasm expressed as green; soybean accessions from north are shown in red; those from the Huanghuaihai valley region are shown in blue; and those from the south valley region are shown in orange. (B) Principal component analysis for the entire panel of soybean accessions; (C) PCA eigenvalue performed by GAPIT using the pruned set of 200K SNP. As presented, the total variance explained by each principal component (PC) decreased from PC1 to PC4 and, after PC4, the variance explained by each further PC remained low and stable; (D) Clustering for PCA = 4 for the entire panel of soybean accessions. Each individual is represented by a vertical bar, as well as partitioned into colored segments, with the length of each segment representing the proportion of the individual's genome from groups when PCA = 4 (Adopted from Zhao et al., 2020)

4 Case Study: Gene Editing for Enhancing Drought Tolerance

4.1 Overview of CRISPR/Cas9 technology in plant breeding

CRISPR/Cas9 technology has revolutionized plant breeding by enabling precise and efficient genome editing. This system utilizes a guide RNA (gRNA) to direct the Cas9 nuclease to specific DNA sequences, where it introduces double-strand breaks. These breaks are then repaired by the plant's natural repair mechanisms, leading to targeted mutations. The simplicity, adaptability, and wide applicability of CRISPR/Cas9 make it a powerful tool for developing crop varieties with enhanced traits, including drought tolerance (Joshi et al., 2020; Kar et al., 2022; Kumar et al., 2023).

4.2 Application of CRISPR/Cas9 in modifying drought-responsive genes in soybeans

One notable example of CRISPR/Cas9 application in soybeans is the editing of the *GmHdz4* gene, a homeodomain-leucine zipper transcription factor. This gene plays a crucial role in drought stress response. By using CRISPR/Cas9 to create gmhdz4 mutants, researchers observed enhanced drought tolerance in soybean plants. The edited plants exhibited better growth, improved root system architecture, and higher activity of antioxidant enzymes, which helped in maintaining turgor pressure and reducing oxidative stress under drought conditions (Zhong et al., 2022). Another study targeted the *GmFAD2* genes, which are involved in fatty acid desaturation. By editing these genes, researchers were able to produce soybean plants with a high oleic acid content, which is associated with improved drought tolerance. The dual gRNA CRISPR/Cas9 system used in this study demonstrated high efficiency in creating targeted mutations, resulting in significant changes in the fatty acid profile of the soybean seeds (Do et al., 2019).

4.3 Potential and challenges of CRISPR/Cas9 for developing drought-resilient varieties

While CRISPR/Cas9 offers tremendous potential for developing drought-resilient soybean varieties, there are challenges that need to be addressed. One major concern is the off-target effects, where unintended genomic regions may be edited, leading to undesirable traits. Efforts to mitigate these effects include the use of shorter gRNAs and dual Cas9 nickases, which increase the specificity of the editing process (Erdoğan et al., 2023). Regulatory considerations also play a significant role in the adoption of CRISPR/Cas9-edited crops. The regulatory landscape varies across countries, with some viewing CRISPR-edited plants as genetically modified organisms (GMOs) and others not. This inconsistency can affect the commercialization and acceptance of CRISPR-edited crops. Therefore, it is crucial to develop clear and consistent regulatory frameworks to facilitate the use of CRISPR/Cas9 technology in agriculture (Joshi et al., 2020; Erdoğan et al., 2023). In conclusion, CRISPR/Cas9 technology holds great promise for enhancing drought tolerance in soybeans by enabling precise modifications of drought-responsive genes. However, addressing off-target effects and navigating regulatory challenges are essential for the successful implementation of this technology in developing drought-resilient crop varieties.

5 Integrative Approaches in Studying Drought Tolerance

5.1 Systems biology approaches and integrative analysis

The integration of multiple omics approaches, such as transcriptomics, proteomics, metabolomics, and phenomics, provides a comprehensive understanding of the complex mechanisms underlying drought tolerance in soybean. For instance, transcriptome profiling has identified numerous differentially expressed genes (DEGs) associated with drought tolerance, including those involved in water and auxin transport, cell wall/membrane integrity, antioxidant activity, and secondary metabolism (Aleem et al., 2020; Shahriari et al., 2022). Proteomic and metabolomic analyses further elucidate the biochemical pathways and metabolic networks that are activated in response to drought stress, such as photosynthesis and cytokinin dehydrogenase activity. Phenomics, which involves high-throughput phenotyping, allows for the assessment of drought tolerance traits at the whole-plant level, providing valuable data for breeding programs (Dubey et al., 2019; Fatema et al., 2023).

Network analysis and predictive modeling are essential tools for understanding the regulatory networks that govern drought responses in soybean. Gene co-expression analysis and protein-protein interaction (PPI) networks have identified key hub genes and transcription factors that play central roles in drought tolerance (Shahriari e al., 2022; Xuan et al., 2022). For example, core modules and significant hub genes such as *GLYMA_04G209700* and *GLYMA_02G204700* have been identified as potential candidates for improving drought tolerance. Predictive modeling can further enhance our understanding by simulating the interactions between different molecular components and predicting the outcomes of genetic modifications (Jogaiah et al., 2013).

5.2 Advanced tools and technologies for functional validation

High-throughput screening and phenotyping platforms are crucial for identifying and validating drought-tolerant soybean genotypes. These platforms enable the rapid assessment of large numbers of genotypes under controlled drought conditions, facilitating the identification of key traits and genetic markers associated with drought tolerance (Dubey et al., 2019; Zhao et al., 2020). For instance, genome-wide association studies (GWAS) have identified several single nucleotide polymorphisms (SNPs) and candidate genes related to drought tolerance during the germination stage. These high-throughput approaches provide valuable data for breeding programs aimed at developing drought-resilient soybean varieties.

CRISPR libraries and synthetic biology approaches offer powerful tools for functional validation and genetic engineering of drought tolerance traits in soybean. CRISPR/Cas9 technology allows for precise editing of target genes, enabling the validation of candidate genes identified through omics studies. For example, the overexpression and knockout of specific miRNAs, such as gma-miR398c, have been shown to significantly impact drought tolerance by regulating the expression of peroxisome-related genes. Synthetic biology approaches can further enhance drought tolerance by designing and constructing novel genetic circuits and pathways that improve stress resilience (Dubey et al., 2019; Zhou et al., 2020). By integrating these advanced approaches, researchers can gain a deeper understanding of the molecular and physiological mechanisms underlying drought tolerance in soybean, ultimately leading to the development of more resilient crop varieties.

6 Practical Applications and Future Directions

6.1 Breeding strategies for drought-tolerant soybeans

Marker-assisted selection (MAS) has emerged as a powerful tool in breeding drought-tolerant soybean varieties. By identifying and utilizing specific quantitative trait loci (QTLs) associated with drought tolerance, breeders can significantly enhance the efficiency and precision of selecting desirable traits. For instance, studies have identified multiple QTLs related to drought tolerance traits such as leaf wilting coefficient, excised leaf water loss, and seed yield under drought conditions (Du et al., 2009; Ren et al., 2020). The integration of MAS with traditional breeding methods allows for the rapid development of soybean cultivars with improved drought resistance, reducing the time and labor required for phenotypic selection (Pathan et al., 2007; Du et al., 2009).

Conventional breeding has played a crucial role in improving soybean drought tolerance over the past decades. However, it faces limitations due to the complex nature of drought tolerance traits, which are often quantitatively inherited and influenced by environmental factors (Pathan et al., 2007; Manavalan et al., 2009). In contrast, biotechnological approaches, including genetic engineering and functional genomics, offer new opportunities to enhance drought tolerance. For example, the use of candidate genes and genetic transformation techniques has shown promise in developing drought-resistant soybean lines (Manavalan et al., 2009). Combining conventional breeding with biotechnological tools can lead to more robust and resilient soybean varieties (Pathan et al., 2007; Manavalan et al., 2009; Dubey et al., 2019).

6.2 Challenges and opportunities in developing drought-resilient varieties

One of the primary challenges in breeding drought-tolerant soybeans is the variability in environmental conditions and field heterogeneity. Drought tolerance is a complex trait influenced by multiple factors, including soil type, water availability, and climatic conditions (Manavalan et al., 2009; Valliyodan et al., 2016). This variability makes it difficult to achieve consistent results across different locations and growing seasons. To address this challenge, researchers are employing high-throughput phenotyping and next-generation mapping approaches to better understand the genotype-environment interactions and identify stable QTLs for drought tolerance (Ren et al., 2020; Valliyodan et al., 2016).

The development and adoption of drought-tolerant soybean varieties also face socioeconomic and regulatory challenges. The acceptance of genetically modified (GM) crops varies across regions, and regulatory frameworks can impact the commercialization of biotechnologically enhanced varieties (Pathan et al., 2007; Manavalan et al., 2009). Additionally, the cost of developing and deploying new drought-tolerant varieties can be a barrier for resource-limited farmers. Addressing these challenges requires a collaborative effort between researchers, policymakers, and stakeholders to create supportive policies and ensure equitable access to improved soybean varieties (Pathan et al., 2007; Manavalan et al., 2009; Dubey et al., 2019).

In conclusion, the integration of marker-assisted selection, conventional breeding, and biotechnological approaches holds great promise for developing drought-tolerant soybean varieties. However, overcoming environmental variability and addressing socioeconomic and regulatory challenges are essential to fully realize the potential of these advancements. Future research should focus on refining breeding strategies, enhancing our understanding of drought tolerance mechanisms, and fostering an enabling environment for the adoption of resilient soybean cultivars.

7 Concluding Remarks

The study on drought tolerance mechanisms in soybean seed germination has yielded significant insights from both physiological and molecular perspectives. Genome-wide association studies (GWAS) identified several single nucleotide polymorphisms (SNPs) and candidate genes associated with drought tolerance during the germination stage. For instance, 26 SNPs were identified across 10 chromosomes, leading to the discovery of 41 candidate genes related to drought tolerance. Another study identified 15 SNPs associated with drought tolerance indices, with some SNPs located near previously mapped QTLs. Different soybean cultivars exhibit varied physiological responses to drought stress. For example, cultivar PI31 showed superior drought and salinity stress tolerance mechanisms, including enhanced photosynthesis, osmolyte accumulation, and antioxidative enzyme activity. Transcriptome analyses revealed numerous differentially expressed genes (DEGs) involved in drought response, including those related to water and auxin transport, antioxidant activity, and secondary metabolism. Additionally, metabolomics studies highlighted the role of osmotic compound accumulation and enhanced energy and secondary antioxidant metabolism in drought-tolerant wild soybean. The role of microRNAs (miRNAs) in regulating drought tolerance was elucidated, with gma-miR398c identified as a negative regulator of drought tolerance through its impact on peroxisome-related genes. These findings underscore the complex interplay of genetic, physiological, and molecular factors in conferring drought tolerance in soybean seed germination.

Future research should focus on several key areas to further enhance our understanding and application of drought tolerance mechanisms in soybean. One crucial area is the functional validation of candidate genes. Conducting functional studies to validate the roles of identified candidate genes and SNPs in drought tolerance will be essential. This could involve the use of gene editing techniques such as CRISPR to create drought-tolerant soybean varieties. Another important focus should be the integration of multi-omics approaches. Utilizing integrative multi-omics approaches, which combine genomics, transcriptomics, proteomics, and metabolomics, will provide a comprehensive understanding of the molecular networks involved in drought tolerance. This holistic view is crucial for identifying key regulatory pathways and potential targets for genetic improvement. Breeding programs must be developed and implemented to incorporate identified genetic markers and candidate genes into the production of drought-tolerant soybean cultivars. Marker-assisted selection and genomic-assisted selection should be key components of these programs, ensuring that the most promising genetic traits are effectively utilized. Additionally, it is important to investigate the interactions between drought tolerance mechanisms and other environmental stress factors, such as salinity and temperature. Understanding these interactions will enable the development of soybean varieties that can withstand multiple stress conditions, ultimately enhancing the resilience and productivity of soybean crops in diverse and challenging environments.

The insights gained from this study have significant implications for soybean production and global food security. By understanding and leveraging the genetic and molecular mechanisms of drought tolerance, it is possible to develop soybean varieties that are more resilient to drought conditions, thereby ensuring stable yields even in water-limited environments. Improved drought tolerance in soybean can contribute to more sustainable agricultural practices by reducing the need for irrigation and conserving water resources. This is particularly important in regions facing water scarcity. As soybean is a major source of protein and oil, enhancing its drought tolerance directly contributes to global food security. Drought-tolerant soybean varieties can help meet the growing demand for food in the face of climate change and increasing population. By continuing to explore and apply these findings, we can make significant strides in improving soybean production and ensuring food security in a changing global climate.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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