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In-Depth Analysis of Physiological, Biochemical, and Molecular Bases of Drought Tolerance in Soybeans

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Abstract Drought tolerance in soybeans is critical for maintaining productivity under water-limited conditions. This study aims to elucidate the physiological, biochemical, and molecular mechanisms underlying drought tolerance in soybeans, integrating advanced breeding strategies to enhance crop resilience. Physiological aspects such as root morphology, aquaporins, osmotic adjustment, and stomatal regulation are examined to understand water uptake and retention. Biochemical defenses, including antioxidant systems, metabolic pathways, and membrane stability, are analyzed for their roles in stress mitigation. Molecular studies focus on gene expression, signal transduction, and omics approaches to identify key drought-responsive elements. Integrative systems biology, gene editing, and biotechnology are discussed for developing drought-tolerant varieties. Practical applications in breeding and field trials are highlighted, addressing environmental variability and stress management. The study concludes with future research directions, emphasizing novel genes, epigenetic regulation, and the challenges in translating research into practice. This comprehensive approach aims to improve soybean drought tolerance, contributing to food security and sustainable agriculture.

Keywords Drought tolerance; Soybeans; Physiological mechanisms; Biochemical defense; Molecular regulation

1 Introduction

Soybean (*Glycine max* L.) is a crucial crop globally, serving as a significant source of protein and oil for human and animal consumption. However, drought stress is a major environmental factor that severely limits soybean growth and productivity. The increasing frequency and intensity of drought events due to climate change have exacerbated this issue, making the development of drought-tolerant soybean varieties a critical area of research (Aleem et al., 2020; Xiong et al., 2020; Li et al., 2022). Drought stress affects various physiological, biochemical, and molecular processes in soybeans, leading to reduced yield and quality. Understanding the mechanisms underlying drought tolerance is essential for breeding and engineering soybean varieties that can withstand water-deficit conditions (Silvente et al., 2012; Zhao et al., 2022; Fatema et al., 2023).

Recent studies have employed advanced techniques such as RNA sequencing (RNA-seq), metabolomics, and transcriptome analysis to identify key genes, metabolic pathways, and physiological traits associated with drought tolerance in soybeans. For instance, differentially expressed genes (DEGs) involved in water and auxin transport, antioxidant activity, and secondary metabolism have been identified as crucial players in the drought response (Aleem et al., 2020; Li et al., 2022). Additionally, the use of novel nanomaterials like graphene oxide has shown promise in enhancing drought tolerance by improving water retention and activating stress-responsive genes (Zhao et al., 2022). These findings provide valuable insights into the complex network of responses that enable soybeans to cope with drought stress.

The study is to conduct an in-depth analysis of the physiological, biochemical, and molecular bases of drought tolerance in soybeans. By integrating data from various research approaches, including transcriptome profiling, metabolite analysis, and physiological assessments, this study aims to identify key genes and metabolic pathways involved in the drought response of soybean. Additionally, it seeks to elucidate the physiological and biochemical changes that occur in soybean under drought stress. Furthermore, this study investigates the potential of novel materials and genetic engineering approaches to enhance drought tolerance in soybean. This comprehensive analysis will contribute to a better understanding of the mechanisms underlying drought tolerance in soybeans. Ultimately, it will provide a foundation for developing more resilient soybean varieties through breeding and biotechnological interventions.

2 Physiological Bases of Drought Tolerance

2.1 Water uptake and retention mechanisms

Root morphology plays a critical role in water uptake and retention in soybeans under drought conditions. Enhanced root system architecture, including increased root length, surface area, and number of root tips, has been shown to improve water uptake efficiency. For instance, gene-editing of the GmHdz4 transcription factor in soybeans resulted in a significant increase in root system architecture parameters, which contributed to better drought tolerance (Figure 1) (Zhong et al., 2022). Additionally, the identification of genes such as *GmACX1*, *GmMS*, and *GmPEPCK*, which regulate root traits, underscores the importance of root morphology in drought resilience (Xiong et al., 2020).

Aquaporins are integral membrane proteins that facilitate water transport across cell membranes, playing a vital role in maintaining cellular water homeostasis under drought conditions. The soybean plasma membrane intrinsic protein GmPIP2;9, for example, has been shown to enhance water uptake and transport, leading to improved drought tolerance. Overexpression of GmPIP2;9 in transgenic soybean plants resulted in increased net CO_2 assimilation, stomatal conductance, and transpiration rates, indicating less stress and better water management (Lu et al., 2018). Furthermore, aquaporins modulate hydraulic conductance and root water transport properties, which are crucial for drought adaptation (Sousa et al., 2020; Patel and Mishra, 2021; Han, 2024).

2.2 Osmotic adjustment

Osmotic adjustment through the accumulation of osmolytes such as proline and soluble sugars is a key mechanism for maintaining cell turgor under drought stress. Studies have shown that drought-stressed soybean plants accumulate higher levels of soluble sugars, proteins, and proline, which help in osmotic balance and stress mitigation. The role of melatonin in enhancing osmolyte content and antioxidant enzyme activities further supports the importance of osmotic adjustment in drought tolerance (Cao et al., 2019).

Maintaining cell turgor is essential for plant survival under drought conditions. The accumulation of osmolytes helps in retaining water within cells, thereby maintaining turgor pressure. Gene-editing of GmHdz4 in soybeans has demonstrated better maintenance of turgor pressure through osmolyte accumulation, which contributes to higher drought tolerance (Zhong et al., 2022). Additionally, the role of aquaporins in regulating cellular water homeostasis further aids in maintaining cell turgor during water deficit (Sousa et al., 2020; Patel and Mishra, 2021).

2.3 Stomatal regulation and transpiration control

Stomatal regulation is crucial for controlling water loss through transpiration. Reducing stomatal density and adjusting stomatal aperture can significantly improve water-use efficiency. For instance, drought-stressed soybean leaves showed reduced mRNA levels of stomatal development genes, leading to a decrease in stomatal density and index, which helps in conserving water (Tripathi et al., 2016). High-yielding soybean varieties also exhibit physiological adjustments such as enhanced photoprotective defenses and higher intrinsic water-use efficiency under drought conditions (Buezo et al., 2018).

Abscisic acid (ABA) plays a pivotal role in stomatal regulation under drought stress. The transcription factor GmWRKY54, for example, enhances stomatal closure by activating genes in the ABA signaling pathway, thereby reducing water loss and conferring drought tolerance (Wei et al., 2019). The involvement of nitric oxide (NO) in altering stomatal characteristics and hydraulic conductivity further highlights the complex hormonal regulation mechanisms that contribute to drought resilience in soybeans (Sousa et al., 2020).

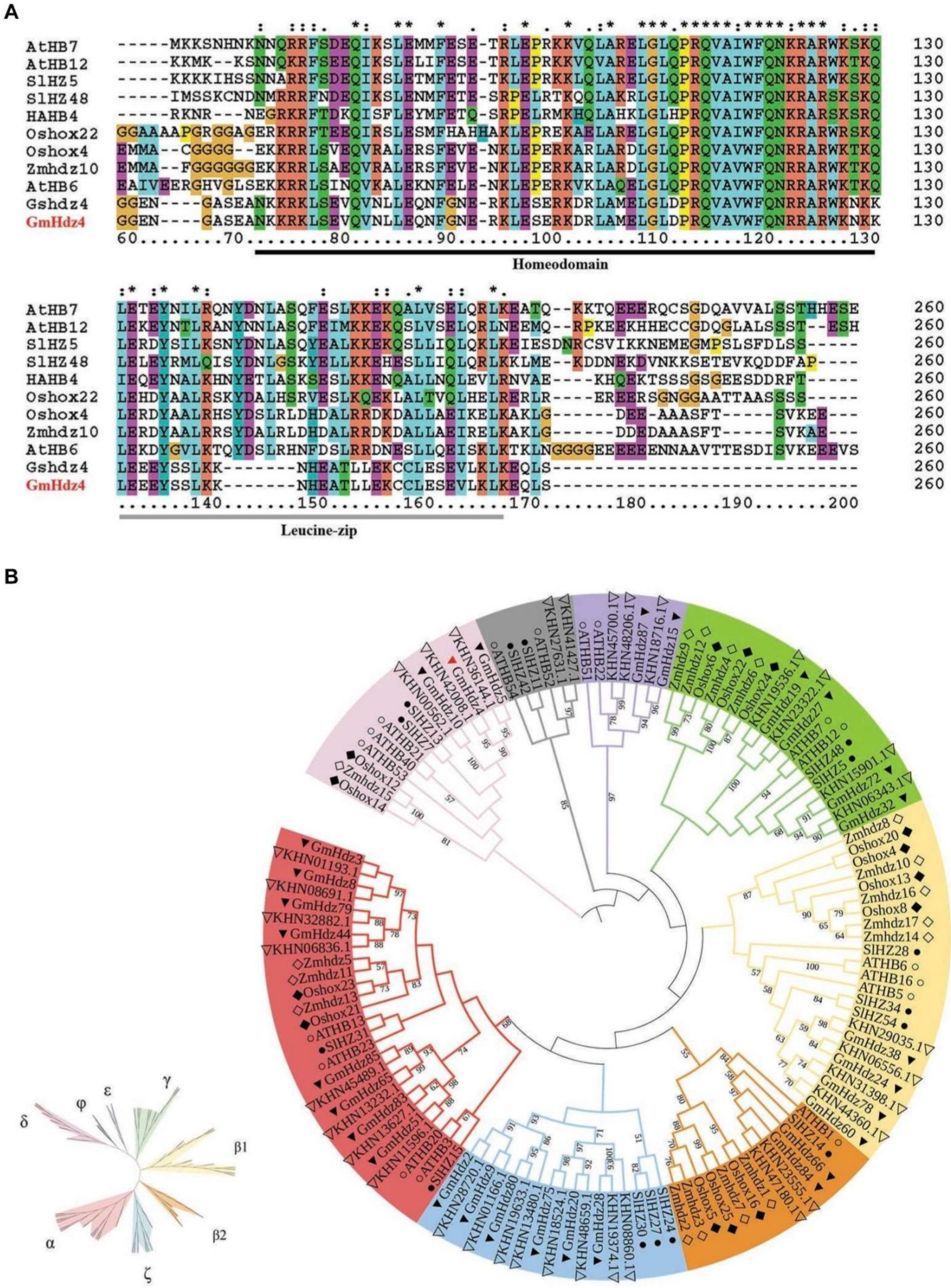

Figure 1 Sequence alignment and phylogenetic analysis (Adopted from Zhong et al., 2022)

Image caption: (A) Multiple sequence alignment for GmHdz4 and other drought stress-related HD-Zip I proteins. HD and LZ domains are underlined by black and gray, respectively. The corresponding IDs of Oshox22, Oshox4, AtHB7, AtHB12, AtHB6, Gshdz4, GmHdz4, HAHB4, Zmhdz10, SlHZ48, and SlHZ5, are Q7XUJ5.2, Q6K498.1, P46897.2, Q9M276.1, P46668.1, AOG74801, Glyma11g06940, XP_022022563, AFT92045, XP_004245456, and XP_004230017, respectively. (B) Phylogenetic analysis of HD-Zip I transcription factors from Arabidopsis, rice, maize, tomato, soybean, and wild soybean. The phylogenetic tree was built using NJ method in MEGA. Bootstrap support is indicated on the branches. Genes from each of the species are marked with different symbols: Arabidopsis (○), rice (■), maize (□), soybean (▼), wild soybean (▽), and tomato (●) (Adopted from Zhong et al., 2022)

3 Biochemical Bases of Drought Tolerance

3.1 Antioxidant defense systems

Enzymatic antioxidants play a crucial role in mitigating oxidative stress induced by drought conditions in soybeans. Superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) are key enzymes that help in scavenging reactive oxygen species (ROS). Studies have shown that the activities of these enzymes increase under drought stress, which helps in maintaining cellular homeostasis and protecting plant tissues from oxidative damage (Wang et al., 2022; Fatema et al., 2023). For instance, the drought-tolerant soybean variety ‘Heinong 44’ exhibited higher activities of CAT and POD compared to the drought-sensitive variety ‘Heinong 65’, indicating a robust enzymatic antioxidant defense system.

Non-enzymatic antioxidants such as ascorbate and glutathione also contribute significantly to the antioxidant defense system in soybeans under drought stress. These molecules act as ROS scavengers and help in maintaining the redox balance within the cells. The total antioxidant capacity (T-AOC) has been observed to increase significantly in drought-tolerant soybean varieties, suggesting an enhanced non-enzymatic antioxidant defense mechanism (Wang et al., 2022). This increase in T-AOC is crucial for mitigating the adverse effects of drought-induced oxidative stress.

3.2 Metabolic pathways and stress metabolites

Primary metabolites such as carbohydrates and amino acids play essential roles in the plant's response to drought stress. Carbohydrates serve as energy sources and osmoprotectants, while amino acids like proline accumulate in response to drought, aiding in osmotic adjustment and protecting cellular structures (Wang et al., 2022; Fatema et al., 2023). The accumulation of soluble sugars and proline has been reported to increase under drought conditions, contributing to the osmotic balance and stress tolerance in soybeans.

Secondary metabolites, including flavonoids and phenolics, are crucial for enhancing drought tolerance in soybeans. These compounds have antioxidant properties and help in protecting the plant cells from oxidative damage. The production of secondary metabolites is often upregulated in response to drought stress, contributing to the overall stress tolerance mechanism (Dubey et al., 2019; Aleem et al., 2020). For example, transcriptome analysis has revealed the upregulation of genes involved in secondary metabolism, indicating their role in drought response.

3.3 Membrane stability and lipid peroxidation

The lipid composition and fluidity of cellular membranes are critical factors in maintaining membrane stability under drought stress. Changes in lipid composition can affect membrane fluidity, which in turn influences the plant's ability to withstand drought conditions. Drought-tolerant soybean varieties have been shown to maintain better membrane stability, which is essential for preserving cellular integrity and function during water deficit (Castro et al., 2019; Fatema et al., 2023).

Lipid peroxidation is a common consequence of oxidative stress induced by drought, leading to membrane damage. However, drought-tolerant soybean varieties exhibit lower levels of lipid peroxidation, indicating effective protective mechanisms. The accumulation of malondialdehyde (MDA), a marker of lipid peroxidation, is significantly lower in drought-tolerant varieties, suggesting better protection against oxidative damage (Castro et al., 2019; Fatema et al., 2023). The overexpression of specific genes, such as GmNFYB17, has been shown to enhance drought resistance by reducing MDA content and improving membrane stability (Figure 2) (Sun et al., 2022).

In summary, the biochemical bases of drought tolerance in soybeans involve a complex interplay of antioxidant defense systems, metabolic pathways, and mechanisms to maintain membrane stability. These biochemical responses are crucial for enhancing the plant’s ability to cope with drought stress and ensure better growth and productivity under water-limited conditions (Buezo et al., 2018).

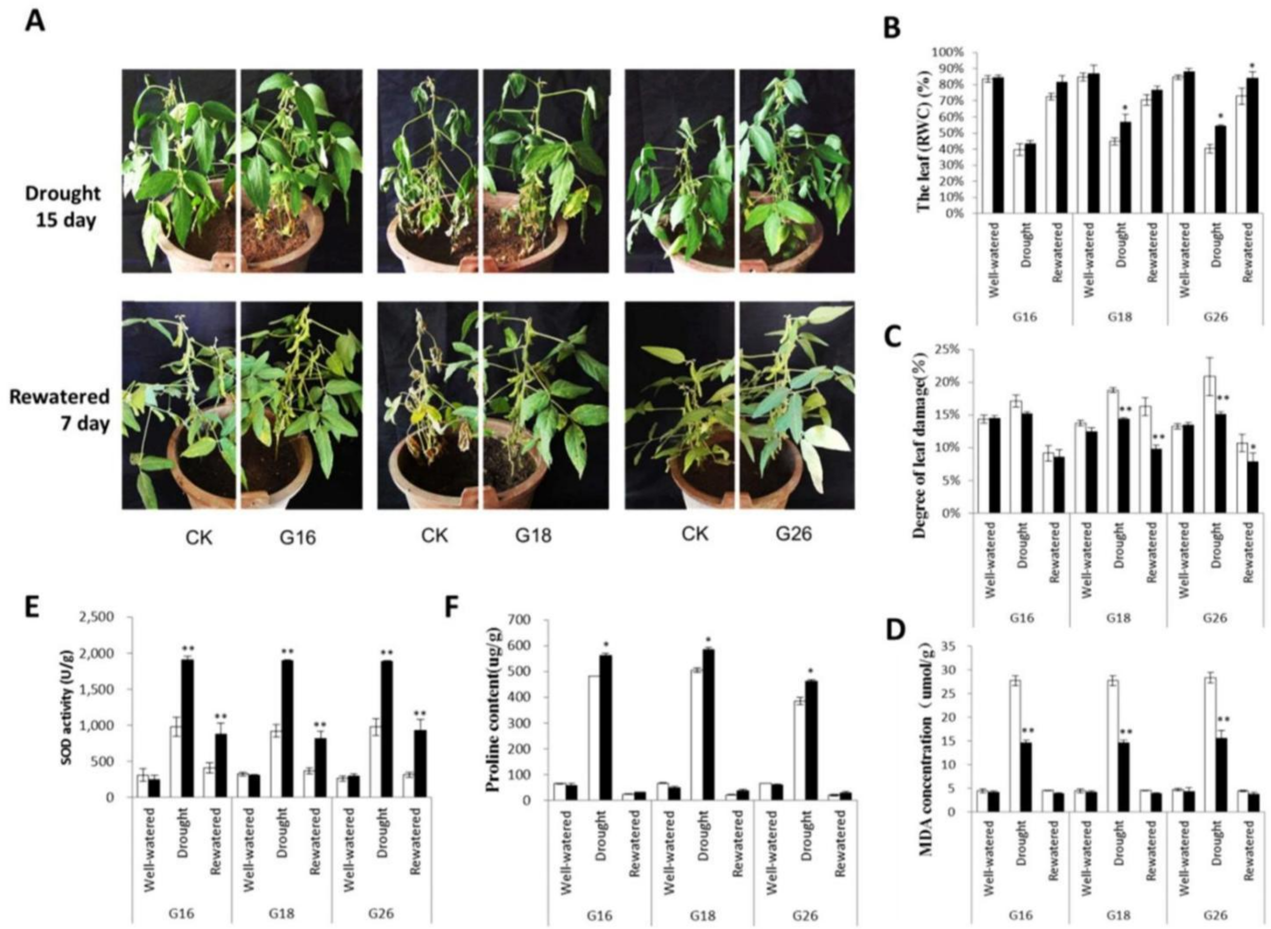

Figure 2 The GmNFYB17 transgenic soybean lines under drought treatment (Adopted from Sun et al., 2022)

Image caption: (A) Morphology of transgenic and non-transgenic plants under drought conditions. Water was withheld for 15 d, and then plants were re-watered for 7 d. G16, G18 and G26 are transgenic lines; CK is soybean DN50; (B, C) The leaf relative water content (RWC) and leaf damage of transgenic lines. G16, G18, G26 and non-transgenic control (CK) during the well-watered, drought and re-watered stage; (D-F) Comparison of physiological and biochemical indicators (MDA, SOD, Proline) between transgenic and non-transgenic plants. *: p-value ≤ 0.05; **: p-value ≤ 0.01 (Adopted from Sun et al., 2022)

4 Molecular Bases of Drought Tolerance

4.1 Gene expression and regulation

Transcription factors (TFs) play a crucial role in regulating gene expression in response to drought stress. Several TF families, including WRKY, MYB, DREB, and TGA, have been identified as key players in drought tolerance in soybeans. For instance, GmWRKY54 has been shown to enhance drought tolerance by activating genes in the ABA and Ca_2^+ signaling pathways, leading to reduced water loss through stomatal closure (Wei et al., 2019). Similarly, GmDREB2, a DREB-type TF, confers drought and high-salt tolerance by binding to dehydration-responsive elements (DREs) and activating downstream stress-responsive genes (Chen et al., 2007). Additionally, GmMYB84, an R2R3-MYB TF, contributes to drought resistance by modulating reactive oxygen species (ROS) levels and enhancing antioxidant enzyme activities (Wang et al., 2017).

Drought-inducible genes are crucial for enhancing drought tolerance in soybeans. The expression of these genes is often regulated by specific promoters that respond to drought stress. For example, the *GmWRKY54* gene, driven by a drought-induced promoter (RD29a), has been shown to confer drought tolerance in transgenic soybeans (Wei et al., 2019). Another study identified several drought-inducible genes, including those involved in ABA biosynthesis and signaling, such as PYL8, SRK2A, CIPK11, and CPK3, which are directly activated by GmWRKY54. Additionally, the identification of novel DNA elements, such as ABRE and CRT/DRE, in the promoters of drought-responsive genes provides potential targets for developing synthetic promoters to drive the expression of transgenes under drought conditions (Tripathi et al., 2016).

4.2 Signal transduction pathways

Abscisic acid (ABA) is a key hormone involved in the regulation of drought stress responses. ABA-dependent pathways play a significant role in mediating drought tolerance in soybeans. The AREB/ABF transcription factors, including AREB1, AREB2, and ABF3, are master regulators of ABA signaling and are required for the full activation of ABA-responsive genes under drought conditions (Yoshida et al., 2010). These TFs interact with SnRK2 protein kinases, such as SRK2D/SnRK2.2, to regulate the expression of downstream genes involved in drought tolerance. Additionally, the circadian clock components GmLHYs have been shown to negatively control drought tolerance by repressing ABA responses, highlighting the complex interplay between circadian regulation and ABA signaling (Wang et al., 2020).

In addition to ABA-dependent pathways, ABA-independent pathways also contribute to drought tolerance in soybeans. The DREB transcription factors, such as GmDREB1 and GmDREB2, play a crucial role in ABA-independent drought responses by binding to DREs in the promoters of stress-responsive genes (Figure 3) (Chen et al., 2007; Kidokoro et al., 2015). These TFs activate the expression of numerous genes involved in various abiotic stress responses, including cold, drought, and high salt, independently of ABA signaling (Kidokoro et al., 2015). Furthermore, the TGA transcription factors, such as GmTGA17, have been shown to enhance drought tolerance through ABA-independent mechanisms, including the regulation of chlorophyll and proline contents and the modulation of ABA-responsive marker genes (Li et al., 2019).

4.3 Genomic and proteomic studies

Genomic studies have identified a plethora of drought-responsive genes in soybeans. RNA-seq analysis of drought-tolerant and sensitive soybean genotypes revealed thousands of differentially expressed genes (DEGs) associated with drought stress (Aleem et al., 2020). These DEGs are involved in various biological processes, including water and auxin transport, cell wall/membrane integrity, antioxidant activity, and secondary metabolism (Aleem et al., 2020). Notably, several DEGs were located in QTL-rich regions associated with drought stress, and some exhibited non-synonymous SNP polymorphisms, making them potential candidates for further functional validation.

Proteomic studies have provided insights into the proteins involved in drought tolerance in soybeans. For instance, the identification of stress-responsive proteins, such as those involved in ABA and Ca_2^+ signaling, has highlighted their role in enhancing drought tolerance (Wei et al., 2019). Additionally, the proteomic profiling of drought-stressed soybean roots and leaves identified significant changes in the levels of metabolites and proteins, including a 160-fold increase in the coumestan coumestrol, which may serve as a biomarker for drought and a target for improving drought responses (Tripathi et al., 2016). These studies underscore the importance of integrating genomic and proteomic approaches to unravel the complex molecular mechanisms underlying drought tolerance in soybeans.

In summary, the molecular bases of drought tolerance in soybeans involve a complex network of gene expression and regulation, signal transduction pathways, and genomic and proteomic changes. Understanding these mechanisms provides valuable insights for developing strategies to enhance drought tolerance in soybeans and other crops.

5 Integrative Approaches to Drought Tolerance

5.1 Systems biology approaches

Systems biology approaches have been instrumental in understanding the complex mechanisms underlying drought tolerance in soybeans. By integrating transcriptomics, proteomics, and metabolomics, researchers have identified key genes, proteins, and metabolites involved in drought response. For instance, comprehensive RNA-seq analysis has revealed differentially expressed genes (DEGs) associated with water and auxin transport, cell wall/membrane integrity, antioxidant activity, and secondary metabolism, which are crucial for drought tolerance (Aleem et al., 2020). Similarly, integrative system biology analysis has identified significant up/downregulated DEGs and core modules that play vital roles in drought tolerance, such as photosynthesis and cytokinin dehydrogenase activity (Shahriari et al., 2022). Additionally, the identification of metabolites like coumestrol, which significantly increases under drought stress, provides potential biomarkers and targets for improving drought responses (Tripathi et al., 2016).

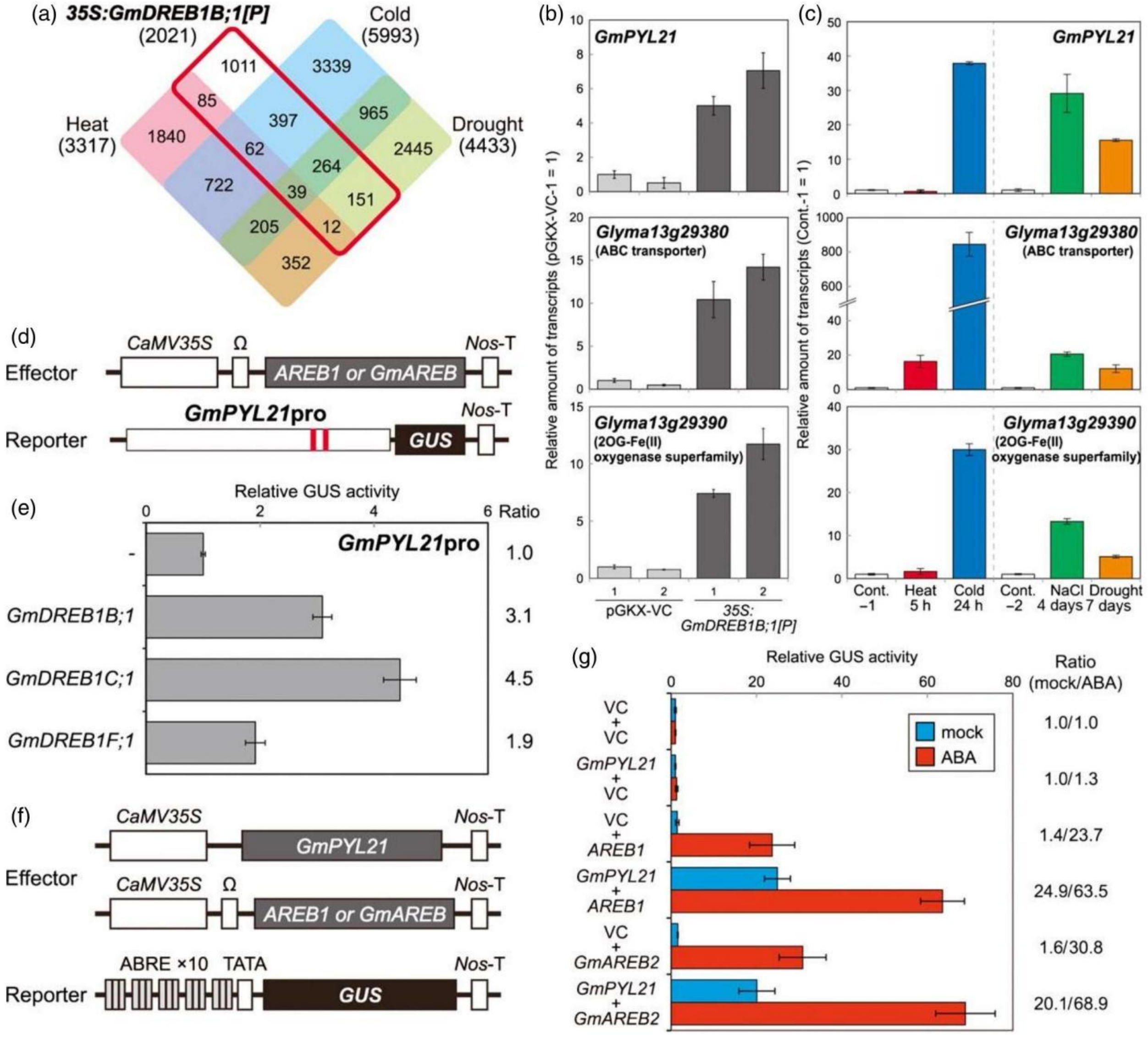

Figure 3 Transcriptome analysis of soybean protoplasts transiently expressing GmDREB1B;1 (Adopted from Kidokoro et al., 2015)
Image caption: (a) Venn diagrams comparing the up-regulated genes between the 35S:GmDREB1B;1[P] (red) and abiotic stress conditions, including cold (aqua), heat (orange), and drought (green). Genes that were significantly (FDR $P < 0.01$) up-regulated by more than two-fold in these transgenic plants are shown. The total numbers of the up-regulated genes are shown in parentheses. (b, c), Transcript levels of selected genes up-regulated by GmDREB1B;1 in soybean protoplasts (b) and soybean leaves subjected to abiotic stress conditions (c). The transcript amounts of each gene were measured by qRT-PCR. The amount in the protoplasts transfected with an empty vector (b) or at the start of the heat and cold stress treatments (c) was set to 1. The values represent the averages of three replicates, and the error bars indicate the SD. The conditions of the growth and stress treatments are same as in Figure 1(b). (d, f) Schematic diagram of the reporter construct used in the transactivation analysis. (e, g) Transactivation activities in soybean mesophyll protoplasts. The relative activity indicates the multiples of expression compared with the value obtained for the vector control. The error bars indicate the standard deviation of three technical replicates (Adopted from Kidokoro et al., 2015)

Network analysis and modeling are essential for constructing genome-scale networks that elucidate the interactions between various biological processes during drought stress. For example, gene co-expression analysis has predicted the biological roles of DEGs in drought tolerance, revealing significant hub genes and transcription factors that regulate drought-responsive genes. Furthermore, network maps integrating multiple functional genomics data have identified commonly regulated signaling pathways and genes following exposure to drought, which are crucial for developing stable drought-resistant crops (Jogaiah et al., 2013). Protein-protein interaction (PPI) network analysis has also highlighted significant hub genes and main transcription factors, providing insights into the regulatory mechanisms of drought tolerance (Shahriari et al., 2022).

5.2 Gene editing and biotechnology

Gene editing tools like CRISPR/Cas9 have revolutionized the field of plant biotechnology by enabling precise modifications of genes associated with drought tolerance. For instance, CRISPR/Cas9-mediated gene editing of the GmHdz4 transcription factor in soybean has shown enhanced drought tolerance by promoting better root system architecture, maintaining turgor pressure, and increasing antioxidant enzyme activity (Zhong et al., 2022). This approach provides new insights into the mechanisms by which gene editing can improve drought stress responses and offers potential targets for molecular breeding in soybeans.

Genetic engineering has been pivotal in developing drought-tolerant soybean varieties. Advances in genomic technologies, coupled with breeding approaches, have helped scientists unravel the genes responsible for drought tolerance in crops (Dubey et al., 2019). For example, the integration of functional genomics tools has identified several root-related and stress-specific candidates that contribute to drought resistance mechanisms in soybean. Additionally, the identification of transcription factors, such as ERF, MYB, NAC, bHLH, and WRKY, and their associated promoters, provides new targets for biotechnological improvement of drought responses (Tripathi et al., 2016). These genetic engineering strategies are crucial for enhancing drought tolerance and ensuring food security under changing climatic conditions.

6 Practical Applications and Breeding Strategies

6.1 Traditional and modern breeding approaches

Marker-assisted selection (MAS) has become a pivotal tool in breeding programs aimed at improving drought tolerance in soybeans. This approach leverages molecular markers linked to desirable traits, allowing for the selection of superior genotypes with greater precision and efficiency. For instance, the identification of quantitative trait loci (QTLs) associated with drought tolerance traits such as root system architecture and canopy wilting has facilitated the development of stress-tolerant soybean varieties (Valliyodan et al., 2016; Dhungana et al., 2021). The integration of MAS in breeding programs has been shown to expedite the introgression of drought-resilient genes, thereby enhancing the overall drought tolerance of soybean cultivars (Manavalan et al., 2009; Satpute et al., 2020).

Genomic selection (GS) represents a significant advancement over traditional breeding methods by utilizing genome-wide markers to predict the performance of breeding lines. This method has been particularly effective in crops like maize, where GS models such as Bayes B have demonstrated high prediction accuracies for drought tolerance traits (Shikha et al., 2017). In soybeans, the application of GS, combined with high-throughput phenotyping and next-generation sequencing, has enabled the identification and validation of key SNPs and candidate genes associated with drought tolerance (Valliyodan et al., 2016; Satpute et al., 2020). Advanced techniques such as RNA-Seq and transcriptome profiling have further elucidated the molecular pathways and genes involved in drought response, providing valuable insights for breeding programs (Aleem et al., 2020).

6.2 Field trials and environmental considerations

Field trials are essential for assessing the drought tolerance of soybean genotypes under real-world conditions. These trials involve the evaluation of various physiological, biochemical, and yield-related parameters under controlled drought stress environments. For example, studies have shown that genotypes like AGS383 exhibit superior drought tolerance by maintaining higher photosynthetic rates, leaf water potential, and root growth under drought conditions (Fatema et al., 2023). Additionally, the use of recombinant inbred line (RIL) populations has facilitated the mapping of QTLs for drought tolerance, providing a robust framework for evaluating and selecting drought-tolerant genotypes in the field (Ren et al., 2020; Dhungana et al., 2021).

Environmental variability poses a significant challenge in breeding for drought tolerance, as genotype-environment interactions can influence the expression of drought-related traits. To address this, breeding programs must consider the diverse environmental conditions under which soybeans are grown. Strategies such as multi-environment trials and the use of stress management practices (e.g., optimized irrigation and soil management) are crucial for accurately assessing the drought tolerance of soybean genotypes (Tuberosa and Salv, 2006; Toum et al., 2021). Moreover, understanding the molecular and physiological responses of soybeans to drought stress can inform the development of more resilient varieties capable of withstanding variable environmental conditions (Manavalan et al., 2009; Aleem et al., 2020). By integrating traditional and modern breeding approaches, conducting rigorous field trials, and considering environmental variability, breeding programs can develop soybean varieties with enhanced drought tolerance, ensuring stable yields and food security in the face of climate change.

7 Future Directions and Challenges

7.1 Emerging research areas

Recent studies have identified several novel genes and pathways that play crucial roles in drought tolerance in soybeans. For instance, the *GmWRKY54* gene has been shown to enhance drought tolerance by activating genes in the ABA and Ca_2^+ signaling pathways, which are critical for stomatal closure and water retention (Wei et al., 2019). Additionally, the identification of differentially expressed genes (DEGs) in drought-tolerant and sensitive soybean genotypes has revealed key pathways such as auxin transport, cell wall/membrane integrity, and antioxidant activity that are vital for drought response (Aleem et al., 2020; Xuan et al., 2022). Future research should focus on the functional validation of these candidate genes and pathways to develop drought-resistant soybean varieties.

Epigenetic regulation and stress memory are emerging as significant areas of research in plant stress tolerance. Epigenetic modifications, such as DNA methylation and histone modifications, can influence gene expression in response to environmental stresses. Studies have shown that epigenetic changes can lead to stress memory, where plants “remember” previous stress exposures and respond more effectively to subsequent stresses. Investigating the role of epigenetic regulation in soybean drought tolerance could provide new insights into developing crops with enhanced stress resilience (Dubey et al., 2019; Wang et al., 2022).

7.2 Challenges in research and application

One of the primary challenges in drought tolerance research is translating laboratory findings into practical applications in the field. While numerous genes and pathways have been identified as crucial for drought tolerance, their practical implementation in breeding programs remains a challenge. For example, the overexpression of GmNFYB17 has been shown to enhance drought resistance and yield in transgenic soybeans, but translating these findings into commercial varieties requires extensive field trials and regulatory approvals (Sun et al., 2022). Additionally, the use of CRISPR/Cas9 technology to edit genes like GmHdz4 has shown promise in enhancing drought tolerance, but the regulatory landscape for gene-edited crops is still evolving (Zhong et al., 2022).

The development and adoption of drought-tolerant soybean varieties are also influenced by socioeconomic and regulatory factors. Farmers need access to affordable and high-yielding drought-tolerant seeds, and there must be adequate support for the adoption of new technologies. Regulatory frameworks for genetically modified and gene-edited crops vary across regions, which can impact the commercialization of drought-tolerant soybean varieties. Addressing these challenges requires a collaborative approach involving researchers, policymakers, and stakeholders to ensure that scientific advancements translate into tangible benefits for farmers and contribute to global food security (Li et al., 2013; Dubey et al., 2019).

In conclusion, while significant progress has been made in understanding the physiological, biochemical, and molecular bases of drought tolerance in soybeans, several future directions and challenges remain. Focusing on novel genes and pathways, epigenetic regulation, and addressing translational and regulatory challenges will be crucial for developing drought-tolerant soybean varieties that can thrive under changing climatic conditions.

8 Concluding Remarks

The research on drought tolerance in soybeans has revealed significant insights into the physiological, biochemical, and molecular mechanisms that enable certain soybean varieties to withstand water-deficit conditions. Key findings include morpho-physiological and biochemical responses: drought stress adversely affects photosynthetic attributes, leaf production, pigment and water content, plant growth, and dry matter production in soybeans. However, drought-tolerant genotypes like AGS383 maintain healthier root and shoot growth, greater leaf area, and higher photosynthesis rates under drought conditions. Advances in genomic technologies and breeding approaches have identified specific genes and quantitative trait loci (QTLs) associated with drought tolerance. For instance, the *GmNFYB17* gene has been shown to enhance drought resistance and yield accumulation in transgenic soybean plants. Additionally, RNA-seq analysis has identified numerous differentially expressed genes (DEGs) that play roles in water and nutrient uptake, antioxidant activity, and stress signaling. The application of graphene oxide (GO) as a soil water retention agent has been found to significantly enhance drought stress tolerance in soybeans by improving root parameters, increasing the activities of defense enzymes, and upregulating drought-related genes.

The findings from these studies have profound implications for soybean production and global food security. The identification of drought-tolerant genotypes and the underlying genetic mechanisms provide valuable resources for breeding programs aimed at developing high-yielding, drought-resistant soybean varieties. This can lead to more resilient soybean crops that can thrive in water-limited environments, thereby ensuring stable food production. The use of innovative approaches such as the application of nanomaterials like graphene oxide can enhance the drought tolerance of soybean plants, reducing the need for excessive irrigation and promoting sustainable agricultural practices. As climate change continues to exacerbate drought conditions, the development of drought-tolerant soybean varieties will be crucial in maintaining crop yields and food security. Integrating physiological, biochemical, and molecular insights into breeding strategies can help mitigate the adverse effects of climate change on soybean production.

The research on drought tolerance in soybeans has made significant strides, but there is still much to be explored. Future research should focus on the functional validation of candidate genes. Further studies are needed to validate the function of identified candidate genes and their role in drought tolerance. This will involve detailed genetic and physiological analyses to confirm their effectiveness in different environmental conditions. Combining genomics, transcriptomics, proteomics, and metabolomics can provide a comprehensive understanding of the complex networks involved in drought tolerance. This integrative approach will facilitate the identification of key regulatory pathways and potential targets for genetic improvement. Conducting extensive field trials to evaluate the performance of drought-tolerant genotypes under real-world conditions is essential. These trials will help in selecting the most promising varieties for large-scale cultivation and incorporation into breeding programs. In conclusion, the advancements in understanding the physiological, biochemical, and molecular bases of drought tolerance in soybeans hold great promise for enhancing crop resilience and ensuring food security in the face of increasing environmental challenges. Continued research and innovation in this field will be pivotal in developing sustainable agricultural practices and securing the future of global food production.

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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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