

Physiological Responses and Variety Screening for Drought Tolerance in Soybeans During Flowering and Podding

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Abstract Drought tolerance is crucial for soybean cultivation due to its significant impact on crop yield and sustainability. This study aims to synthesize current research on the physiological, biochemical, and molecular responses of soybean to drought stress, with a focus on identifying traits and mechanisms that confer drought tolerance. The study examines the physiological responses to drought during the flowering and podding stages, including water relations, osmotic adjustment, stomatal conductance, transpiration, photosynthetic activity, and reproductive development. It also explores biochemical and molecular responses, highlighting antioxidant defense mechanisms, hormonal regulation, and gene expression related to drought tolerance. Furthermore, various screening methods for drought-tolerant varieties are discussed, encompassing field and controlled environment techniques, as well as the use of physiological and biochemical markers. Case studies of successful breeding programs and notable drought-tolerant soybean varieties are presented, alongside traditional and modern breeding strategies. This study provides a comprehensive understanding of the strategies employed by soybean plants to cope with drought stress, offering valuable insights for future research and breeding efforts aimed at enhancing drought tolerance in soybean. The findings are expected to inform breeding programs and contribute to the development of drought-resilient cultivars, thereby improving soybean production and food security.

Keywords Drought tolerance; Soybean; Physiological responses; Biochemical markers; Breeding strategies

1 Introduction

Soybean (*Glycine max* L.) is a critical crop globally, providing essential protein and oil for human consumption and animal feed. However, its production is significantly hampered by drought stress, which is one of the most severe abiotic factors affecting crop yield and quality. Drought stress during the flowering and podding stages is particularly detrimental, leading to substantial reductions in seed yield and quality (Moloi and Merwe, 2021; Wang et al., 2022; Fatema et al., 2023). The ability of soybean plants to withstand drought conditions is therefore of paramount importance for ensuring food security and agricultural sustainability, especially in the face of climate change and increasing water scarcity (Dubey et al., 2019; Wang et al., 2022a).

Drought tolerance in soybeans involves a complex interplay of morphological, physiological, and biochemical mechanisms. These include maintaining water uptake and retention, enhancing root growth, adjusting osmotic balance, and activating antioxidant defenses to mitigate oxidative damage (Buezo et al., 2018; Wang et al., 2022b; Fatema et al., 2023). Identifying and developing drought-tolerant soybean varieties is crucial for stabilizing yields under water-limited conditions and for expanding soybean cultivation into arid and semi-arid regions (Arya et al., 2021; Wang et al., 2022).

The study is to evaluate the physiological responses and screen various soybean varieties for drought tolerance during the critical flowering and podding stages. This study aims to synthesize current research findings on the morpho-physiological and biochemical adaptations of soybean to drought stress, highlighting the key traits and mechanisms that confer drought tolerance. Specifically, this study will examine the impact of drought stress on soybean growth, photosynthesis, and yield components. It will identify physiological and biochemical markers associated with drought tolerance in soybean and evaluate the performance of different soybean genotypes under drought conditions to identify potential drought-tolerant varieties. Furthermore, this study will discuss the

implications of these findings for soybean breeding programs aimed at developing drought-resilient cultivars. By integrating insights from various studies, this study seeks to provide a comprehensive understanding of the strategies employed by soybean plants to cope with drought stress and to inform future research and breeding efforts aimed at enhancing drought tolerance in this vital crop.

2 Physiological Responses to Drought During Flowering and Podding

2.1 Water relations and osmotic adjustment

Drought stress significantly impacts the water potential in soybean plants, leading to a decrease in leaf water potential. This reduction in water potential is a critical response mechanism that helps the plant to maintain cellular turgor and continue metabolic activities under water-limited conditions. For instance, in a study involving soybean genotypes, it was observed that water deficit conditions led to a marked decrease in leaf water potential, which was more pronounced in drought-sensitive varieties compared to drought-tolerant ones (Wang et al., 2022a; Silva et al., 2022).

Osmolytes such as proline and soluble sugars play a crucial role in osmotic adjustment under drought conditions. These compounds help in stabilizing proteins and membranes, scavenging free radicals, and maintaining cell turgor. Research has shown that the content of proline and soluble sugars increases significantly in soybean leaves under drought stress, contributing to enhanced drought tolerance. For example, drought-tolerant varieties exhibited higher levels of proline and soluble sugars compared to drought-sensitive varieties, which helped them to better withstand water deficit conditions (Wang et al., 2022a; Silva et al., 2022).

2.2 Stomatal conductance and transpiration

Stomatal conductance is a critical factor in regulating water loss and gas exchange in plants. Under drought conditions, soybean plants typically exhibit reduced stomatal conductance to minimize water loss through transpiration. This response is essential for conserving water and maintaining cellular hydration. Studies have indicated that drought-tolerant soybean varieties tend to have better control over stomatal closure, which helps them to reduce water loss more effectively than drought-sensitive varieties (Buezo et al., 2018; Silva et al., 2022).

Drought stress leads to a reduction in transpiration rates as a result of decreased stomatal conductance. This reduction is a protective mechanism to conserve water. However, it also impacts the plant's ability to assimilate carbon dioxide, affecting photosynthesis. High-yielding soybean varieties have been observed to enhance their water use efficiency under drought conditions by optimizing their transpiration rates and maintaining higher productivity levels despite reduced water availability (Figure 1) (Buezo et al., 2018; Molinari et al., 2021).

2.3 Photosynthetic activity and chlorophyll fluorescence

Drought stress adversely affects photosynthetic activity in soybean plants by reducing the rate of carbon assimilation. This reduction is primarily due to stomatal closure, which limits the availability of carbon dioxide for photosynthesis. Additionally, drought stress can lead to damage in the photosynthetic apparatus, further reducing photosynthetic efficiency. Studies have shown that drought-tolerant soybean varieties are able to maintain higher photosynthetic rates under water deficit conditions compared to sensitive varieties, which is crucial for sustaining growth and productivity (Buezo et al., 2018; Iqbal et al., 2019; Silva et al., 2022).

Chlorophyll fluorescence is a valuable tool for assessing the impact of drought stress on the photosynthetic efficiency of plants. It provides insights into the health of the photosynthetic apparatus and the extent of stress experienced by the plant (Pereira et al., 2019). Research has demonstrated that drought-tolerant soybean varieties exhibit less reduction in chlorophyll fluorescence parameters under drought conditions, indicating better maintenance of photosynthetic activity and lower stress levels compared to sensitive varieties (Buezo et al., 2018; Du et al., 2020; Delavar et al., 2023).

2.4 Reproductive development and yield components

Drought stress during the flowering stage can significantly impact the reproductive development of soybean plants. It can lead to delayed flowering, reduced pod formation, and ultimately lower yield. High-yielding soybean

varieties have been observed to adopt strategies such as faster transition into the reproductive stage to avoid the most severe drought periods, thereby mitigating the adverse effects on flowering and pod formation (Buezo et al., 2018).

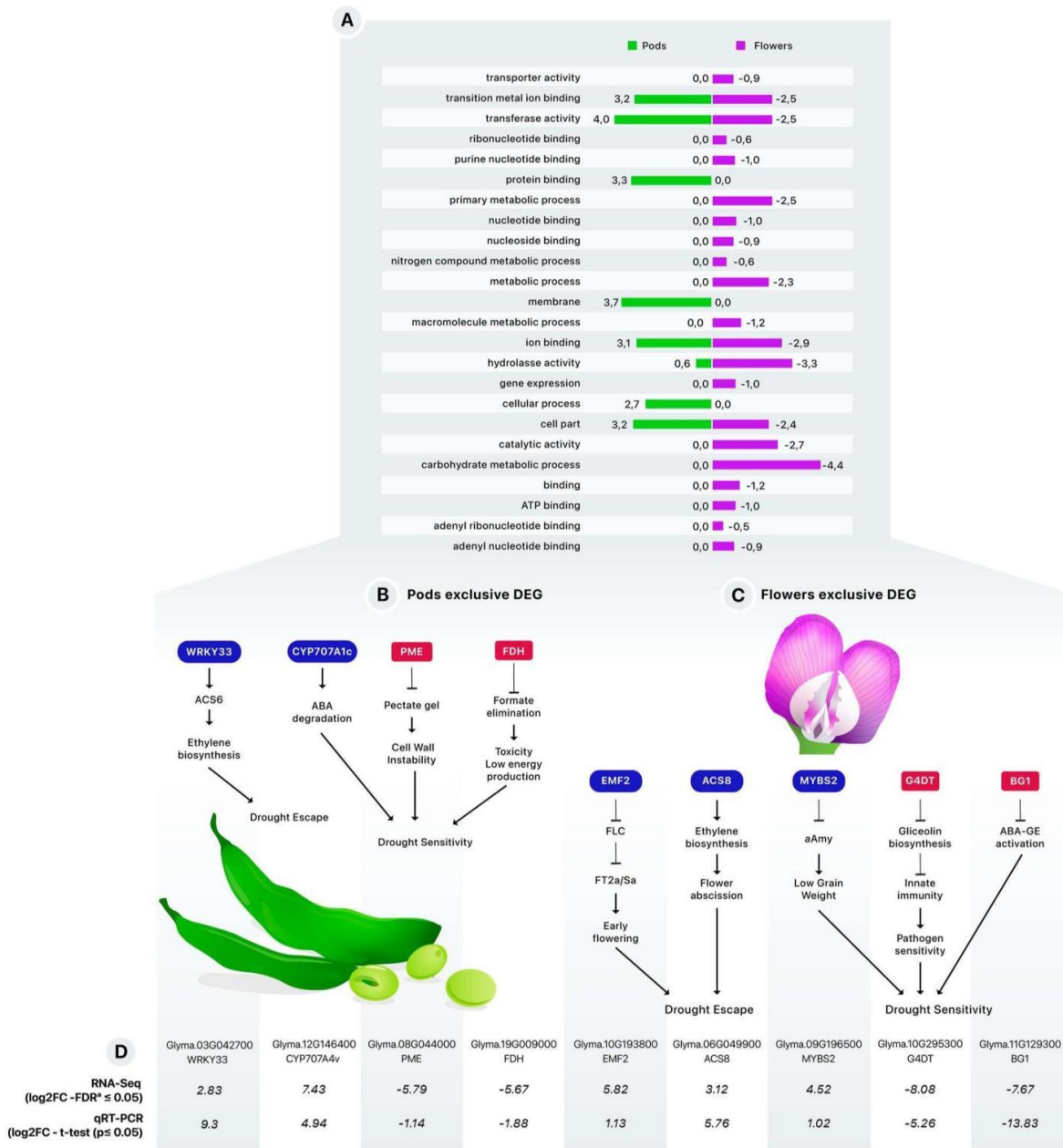


Figure 1 Comparative analysis of gene expression and pathways in drought-sensitive soybean cultivar BR 16 under water-deficit conditions (Adapted from Molinari et al., 2021)

Image caption: (A) Genetic ontology analysis of DEG identified in flower (R2 developmental stage) and pods (R4 developmental stage) of drought-sensitive soybean cultivar BR 16 subjected to water-deficit conditions. Data include both expression profiles, up and down-regulated genes. Green bars represent genes associated with GO terms identified in pods and purple ones in flowers. Positive and negative numbers associated with the bars, stand for up and down-regulated expression profiles of genes associated with the respective GO term category. (B). Drought-responsive pathways affected by WD condition in soybean cv. BR16. Legend: Blue circles stand for up-regulation and red ones for down-regulation. (C). Comparison of expression analysis performed using RNA-Seq and qRT-PCR techniques for genes differentially expressed in flower and pod of drought-sensitive soybean cultivar BR 16 subjected to WD treatment compared to C condition. JOURNAL OF PLANT INTERACTIONS 193 (Adopted from Molinari et al., 2021)

Drought stress during the podding stage can severely affect seed development, pod set, and grain filling, leading to reduced yield and quality. Drought-tolerant soybean varieties tend to exhibit better resilience in these aspects by maintaining higher rates of pod set and grain filling under water-limited conditions. This resilience is attributed to their ability to sustain physiological and metabolic functions during drought stress, ensuring better reproductive success and yield stability (Buezo et al., 2018; Wang et al., 2022b; Silva et al., 2022).

By understanding these physiological responses and screening for drought-tolerant varieties, it is possible to develop soybean cultivars that can better withstand drought conditions, ensuring stable productivity and food security (Figure 2) (Tripathi et al., 2016).

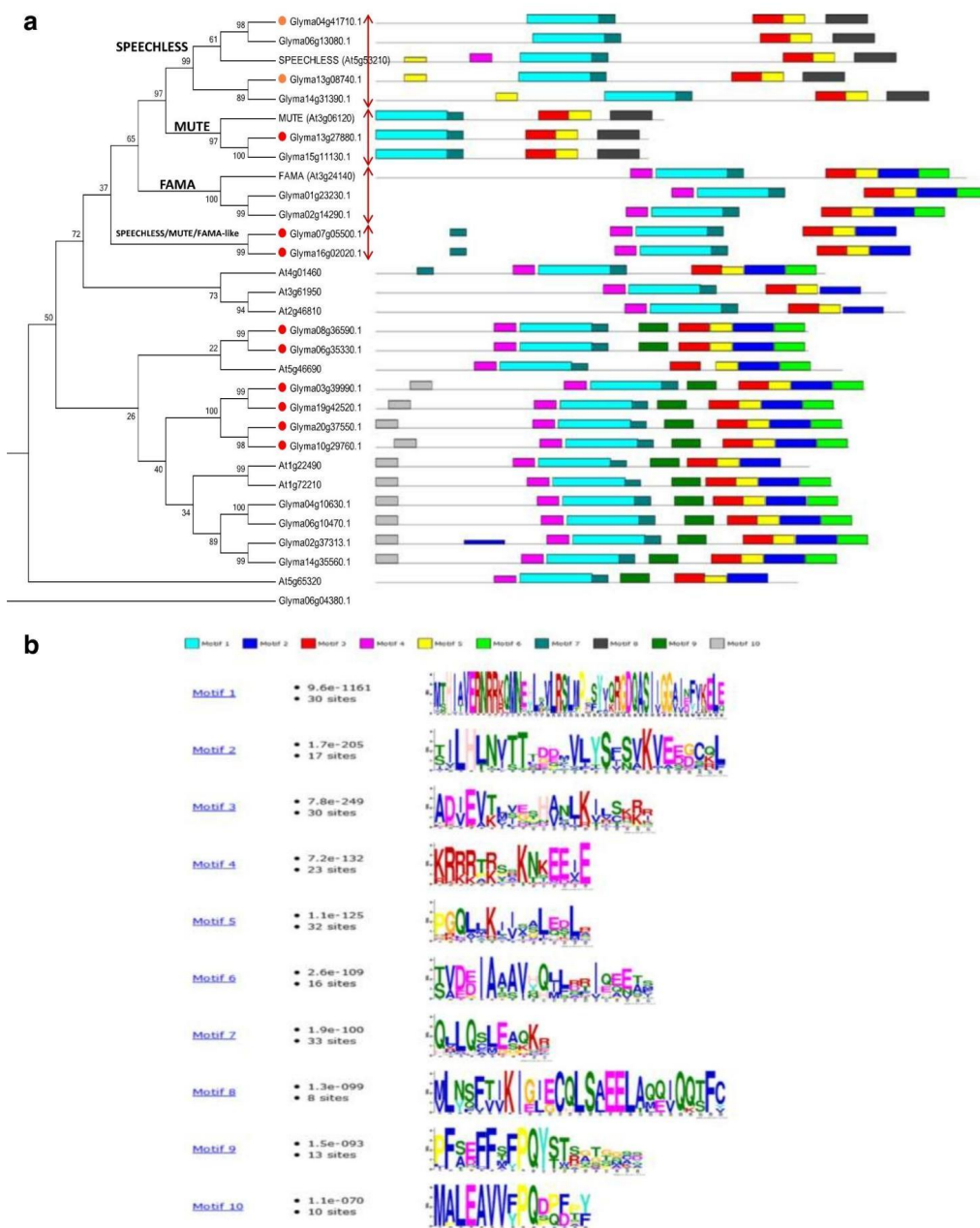


Figure 2 Phylogenetic analyses of the Group 10 (Ia) bHLH subfamily from soybean and Arabidopsis (Adopted from Tripathi et al., 2016)

Image caption: a Neighbor Joining phylogenetic tree derived from a MUSCLE alignment of the full length proteins. SPEECHLESS, MUTE and FAMA-like genes are indicated. Numbers indicate bootstrap values from 1 000 replicates. Red dots denote 8-fold down-regulation and orange dots 5-fold. To the right of each protein is a cartoon of the protein architecture derived by MEME analysis. b The conserved protein motifs produced by MEME analysis (Adopted from Tripathi et al., 2016)

3 Biochemical and Molecular Responses to Drought Stress

3.1 Antioxidant defense mechanisms

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) and protecting cellular components from oxidative damage. Studies have shown that the activities of these enzymes increase significantly under drought stress. For instance, the activities of SOD, CAT, and POD were observed to increase in soybean leaves under water deficit conditions, which helps in reducing the accumulation of malondialdehyde (MDA), a marker of lipid peroxidation (Wang et al., 2022a; Zhao et al., 2022). Additionally, the application of graphene oxide (GO) was found to enhance the activities of these enzymes, further improving drought tolerance in soybeans (Rezayian et al., 2020; Zhao et al., 2022; Fatema et al., 2023).

Non-enzymatic antioxidants such as ascorbate and glutathione also play a significant role in the defense against oxidative stress. These molecules act as ROS scavengers and help in maintaining the redox balance within the cells. Under drought conditions, the levels of ascorbate and glutathione are often elevated, contributing to the overall antioxidant capacity of the plant. For example, the activities of ascorbate peroxidase (APX) and glutathione reductase (GR) were found to increase in vegetable-type soybean cultivars under drought stress, indicating their role in enhancing drought tolerance (Rao and Chaitanya, 2019; Darmanti et al., 2020; Moloi and Merwe, 2021).

3.2 Hormonal regulation

Abscisic acid (ABA) is a key hormone involved in the regulation of plant responses to drought stress. ABA levels typically increase under drought conditions, leading to various physiological and molecular changes that help the plant cope with water deficit. ABA plays a crucial role in stomatal closure, reducing water loss through transpiration, and activating stress-responsive genes. In soybeans, the application of GO was found to increase ABA content significantly, which in turn enhanced drought tolerance by improving water retention and activating defense mechanisms (Buezo et al., 2018; Zhao et al., 2022).

Apart from ABA, other hormones such as ethylene, cytokinins, and auxins also play important roles in drought response. Ethylene is involved in the regulation of stress responses and senescence, while cytokinins and auxins are crucial for growth and development. Under drought conditions, the balance between these hormones is altered to optimize the plant's response to stress. For instance, drought stress was found to trigger changes in hormone biosynthesis in soybean flowers and pods, leading to early flowering and reduced grain weight as part of the drought escape mechanism (Molinari et al., 2021).

3.3 Gene expression and signal transduction pathways

Drought-responsive genes and transcription factors play a pivotal role in the regulation of plant responses to water deficit. These genes are involved in various processes such as osmotic adjustment, antioxidant defense, and hormone signaling. RNA-Seq analysis has identified numerous differentially expressed genes (DEGs) in soybean under drought conditions, including those related to water and auxin transport, cell wall/membrane stability, and antioxidant activity (Aleem et al., 2020). Key transcription factors such as DREB (dehydration-responsive element-binding) proteins are also upregulated, enhancing the plant's ability to withstand drought stress (Aleem et al., 2020; Zhao et al., 2022).

Signal transduction pathways play a critical role in the perception and response to drought stress. These pathways involve the activation of various receptors and signaling molecules that transmit stress signals to the nucleus, leading to the activation of stress-responsive genes. In soybeans, the application of GO was found to enhance the expression of drought-related genes such as GmP5CS, GmGOLS, GmDREB1, and GmNCED1, which are involved in osmotic adjustment, sugar metabolism, and ABA biosynthesis (Zhao et al., 2022; Wang et al., 2022a). Additionally, the transcriptome profiling of wild soybean has revealed the involvement of multiple signaling pathways, including those related to secondary metabolism and transcription factor activities, in drought tolerance (Aleem et al., 2020).

By understanding these biochemical and molecular responses, researchers can develop strategies to enhance drought tolerance in soybean varieties, ensuring better yield and productivity under water-limited conditions.

4 Screening Methods for Drought Tolerant Varieties During Flowering and Podding

4.1 Field screening techniques for identifying drought tolerant varieties

Field trials are essential for evaluating the drought tolerance of soybean varieties, particularly during the critical flowering and podding stages. These trials should be designed to simulate drought conditions accurately and assess the physiological and agronomic responses of different soybean genotypes. A randomized complete block design (RCBD) with multiple replications is recommended to ensure the reliability of the results (Wang et al., 2022a). To target the flowering and podding stages, drought stress can be imposed by controlling soil moisture content. For instance, maintaining soil moisture at 40% of field capacity can effectively simulate drought conditions. Additionally, the use of pot experiments to control soil moisture and evaluate survival percentages after drought stress can provide valuable insights into the drought tolerance of different soybean varieties (Fatema et al., 2023).

Evaluating agronomic traits under drought conditions is crucial for identifying drought-tolerant soybean varieties. Key traits to assess include yield, flower retention, and pod number. Drought stress during the flowering stage can significantly impact these traits, leading to reduced yield and flower retention (Buezo et al., 2018). For instance, studies have shown that drought stress can adversely affect pod and seed production, grain size, and seed yield, with genotypic variations being evident (Wang et al., 2022a). High-yielding varieties under drought conditions often exhibit enhanced photoprotective defenses and higher intrinsic water use efficiency, which contribute to their productivity (Wang et al., 2022b). Moreover, physiological traits such as leaf area, plant height, and membrane stability index (MSI) should also be evaluated, as they are indicative of a plant's ability to withstand drought stress. Varieties that maintain higher values of these traits under drought conditions are considered more drought-tolerant (Moloi and Merwe, 2021; Wang et al., 2024).

In summary, field trials targeting the flowering and podding stages, combined with the evaluation of key agronomic and physiological traits, are essential for screening and identifying drought-tolerant soybean varieties. These methods provide a comprehensive understanding of the genotypic responses to drought stress and aid in the development of resilient soybean cultivars.

4.2 Controlled environment screening

Controlled environment screening is essential for understanding the drought tolerance of soybean varieties, particularly during critical reproductive stages such as flowering and podding. Growth chamber and greenhouse experiments allow for precise control of environmental variables, facilitating the study of physiological and biochemical responses to drought stress. For instance, a study conducted in a greenhouse setting revealed significant reductions in leaf number, plant height, stem girth, leaf area, and flower number under drought conditions, highlighting the sensitivity of these traits to water deficit during the flowering stage (Puobi et al., 2023). Another study emphasized the importance of maintaining high water use efficiency and biomass accumulation to sustain pod number and seed yield under drought stress, which was observed in certain drought-tolerant soybean cultivars (Gebre et al., 2022).

To simulate field conditions and induce drought stress, various techniques such as polyethylene glycol (PEG) treatment and controlled water deficit are employed. These methods help in creating a consistent and reproducible drought environment for screening purposes. For example, a study used controlled soil moisture content to simulate different degrees of drought, revealing that drought-tolerant varieties exhibited less reduction in physiological traits compared to drought-sensitive ones (Wang et al., 2022a). Similarly, another experiment utilized pot trials with controlled watering regimes to assess the drought tolerance of soybean genotypes, identifying key traits such as leaf area index (LAI) and root-to-shoot ratio (RSR) as reliable indicators of drought resistance (Yan et al., 2020).

4.3 Physiological and biochemical markers for efficient screening

Physiological traits are critical markers for screening drought tolerance in soybeans. Traits such as leaf water potential, chlorophyll content, and stomatal conductance provide insights into the plant's ability to maintain water status and photosynthetic efficiency under drought conditions. For instance, drought-tolerant soybean varieties were found to maintain higher leaf water potential and chlorophyll content, which are indicative of better water retention and photosynthetic capacity (Fatema et al., 2023). Additionally, stomatal conductance measurements revealed that drought-tolerant genotypes could regulate water loss more effectively, contributing to their overall drought resilience (Buezo et al., 2018). Biochemical markers such as antioxidant enzyme activities and osmolyte accumulation are also valuable for screening drought tolerance. Increased activities of enzymes like peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) have been associated with enhanced drought tolerance, as they help mitigate oxidative stress caused by drought. Moreover, the accumulation of osmolytes such as proline and soluble sugars plays a crucial role in osmotic adjustment and cellular protection under water deficit conditions. Studies have shown that drought-tolerant soybean varieties exhibit higher levels of these osmolytes, which aid in maintaining cell turgor and stabilizing proteins and membranes during drought stress (Moloi and Merwe, 2021; Wang et al., 2022b).

In summary, controlled environment screening using growth chambers and greenhouses, along with the application of drought simulating techniques, provides a robust framework for evaluating drought tolerance in soybeans. The selection of physiological and biochemical markers further enhances the efficiency of screening, enabling the identification of drought-tolerant varieties that can be utilized in breeding programs to develop resilient soybean cultivars.

5 Case Studies and Successful Breeding Programs

5.1 Case studies of drought tolerant varieties

Several soybean varieties have been identified for their notable drought tolerance characteristics. For instance, the variety N-3001 (HY1) has demonstrated a high intrinsic water use efficiency and a strategy of faster transition into the reproductive stage to avoid drought periods, enhancing its photoprotective defenses and investing in growth and productivity under water-limited conditions (Buezo et al., 2018). Another variety, AGS383, has shown minimal adverse effects under drought conditions, maintaining healthier root and shoot growth, greater leaf area, and higher photosynthesis, which contribute to its heavier grains and higher yield under both drought and normal conditions (Fatema et al., 2023). Additionally, the variety Heinong 44 (HN44) has been identified for its significant increase in antioxidant enzyme activities and osmotic regulatory substances under drought stress, which helps in maintaining cell membrane stability and overall plant health (Wang et al., 2022a; Wang et al., 2022b).

Field performance and yield stability of drought-tolerant soybean varieties have been extensively studied. For example, the variety AGS383 has been noted for its yield stability, producing heavier grains and maintaining higher seed yield under drought conditions compared to other genotypes (Fatema et al., 2023). Similarly, the variety Heinong 44 (HN44) has shown better performance under drought conditions by maintaining higher levels of antioxidant activities and osmotic regulatory substances, which contribute to its drought tolerance and yield stability (Wang et al., 2020; Wang et al., 2022b). Furthermore, a study on various soybean genotypes identified LD24, JD36, and TF31 as highly drought-tolerant during the vegetative growth phase, and TD37 and LD26 during the reproductive growth phase, indicating their potential for stable yield under drought conditions (Yan et al., 2020).

5.2 Breeding strategies for enhancing drought tolerance

Traditional breeding approaches have focused on selecting traits that enhance reproductive resilience under drought conditions. For example, the selection of traits such as leaf area index (LAI) and root-to-shoot ratio (RSR) has been effective in identifying drought-resistant cultivars during the vegetative growth phase. Additionally, traits like the number of seeds per pod (SPP) and yield per plant (YPP) have been used to screen for drought

resistance during the reproductive phase (Yan et al., 2020). These traditional methods rely on phenotypic selection and have been instrumental in developing varieties like Heinong 44 and Tiefeng 31, which exhibit strong drought tolerance (Wang et al., 2020; Wang et al., 2022a).

Modern breeding techniques such as marker-assisted selection (MAS) and genomic selection (GS) have revolutionized the development of drought-tolerant soybean varieties. These techniques allow for the identification and selection of specific genes associated with drought tolerance. For instance, RNASeq and agro-physiological characterization have been used to identify genes involved in hormone biosynthesis and cell wall stability in response to water deficit, providing insights into tissue-specific mechanisms of drought tolerance (Molinari et al., 2021). Additionally, the use of MAS has facilitated the screening of soybean varieties at the molecular level, enabling the identification of drought-resistant genes and their incorporation into breeding programs (Wang et al., 2022a). These advanced techniques have the potential to accelerate the development of high-yielding, drought-tolerant soybean varieties, ensuring stable production under water-limited conditions.

By integrating both traditional and modern breeding approaches, significant progress has been made in developing soybean varieties that can withstand drought stress, particularly during the critical flowering and podding stages. These efforts are crucial for ensuring food security and sustainable agricultural practices in the face of climate change.

6 Challenges and Future Directions

6.1 Challenges in drought tolerance research

One of the primary challenges in drought tolerance research is the significant environmental variability and the complexity of field conditions. Drought stress can vary greatly in intensity, duration, and timing, making it difficult to standardize experiments and compare results across different studies. For instance, the study by Buezo et al. (2018) highlights the variability in physiological and photochemical responses among different soybean varieties under mild drought conditions. Similarly, emphasizes the challenge of simulating different degrees of drought in controlled environments to study the physiological changes in soybean plants. This variability complicates the identification and validation of drought-tolerant traits and genotypes (Wang et al., 2022b).

Another significant challenge is the interaction between genotype and environment (GxE), which can obscure the genetic basis of drought tolerance. The study by demonstrates that different soybean genotypes exhibit varied responses to drought stress at different developmental stages (Molinari et al., 2021; Puobi et al., 2023), indicating a strong GxE interaction. Additionally, discusses the difficulty in identifying drought-tolerant genotypes due to the complex interplay between multiple phenotypic and yield-related characteristics under varying environmental conditions (Yan et al., 2020; Fatema et al., 2023). This interaction necessitates extensive multi-environment trials to accurately assess the drought tolerance of different genotypes.

6.2 Future research directions

Future research should focus on integrating physiological, biochemical, and molecular approaches to gain a comprehensive understanding of drought tolerance mechanisms. The study by suggests that a network of biochemical mechanisms, including antioxidative enzyme activities and osmotic regulatory substances, plays a crucial role in drought tolerance during critical growth stages (Moloi and Merwe, 2021). Moreover, highlights the importance of transcriptome profiling to identify differentially expressed genes and molecular pathways associated with drought tolerance (Aleem et al., 2020). By combining these approaches, researchers can uncover the complex interactions between various physiological and molecular processes that contribute to drought tolerance.

The development of new screening technologies and tools is essential for advancing drought tolerance research. High-throughput phenotyping platforms and advanced imaging techniques can facilitate the rapid and accurate assessment of drought-related traits in large populations. For example, discusses the use of pot experiments to screen soybean varieties for drought tolerance at the seedling stage (Wang et al., 2022a), providing valuable insights into the early-stage responses of different genotypes. Additionally, emphasizes the need for innovative

screening methods to evaluate the drought tolerance of commercial soybean cultivars under realistic field conditions (Arya et al., 2021; Gebre et al., 2022; Xu et al., 2023). These advancements will enable more efficient and precise identification of drought-tolerant genotypes, accelerating the breeding and development of resilient soybean varieties (Dhungana et al., 2021).

By addressing these challenges and pursuing these future research directions, the scientific community can make significant strides in understanding and improving drought tolerance in soybeans, ultimately enhancing crop productivity and resilience in the face of climate change.

7 Concluding Remarks

This study has provided a comprehensive analysis of the physiological responses and variety screening for drought tolerance in soybeans during the critical flowering and podding stages. Key findings include biochemical mechanisms, where drought stress significantly increased the activities of antioxidative enzymes such as ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and glutathione reductase (GR) in certain soybean cultivars, particularly at the flowering stage. These enzymes play a crucial role in minimizing hydrogen peroxide (H₂O₂) production and lipid peroxidation, thereby enhancing drought tolerance. Proline and total soluble sugars (TSS) were found to be important osmotic regulators under drought conditions. Their levels increased significantly in response to drought, aiding in osmotic adjustment and stress mitigation. Drought stress adversely affected various morpho-physiological traits such as leaf production, plant height, stem girth, and leaf area. However, certain genotypes like AGS383 showed minimal adverse effects, maintaining healthier growth and higher yield under drought conditions. Significant genotypic variations were observed in drought tolerance. Some genotypes, such as AGS429 and AGS383, exhibited superior drought tolerance by maintaining higher photosynthetic efficiency, water use efficiency, and biochemical stability.

The findings of this study have several important implications for soybean production and food security: Breeding programs can benefit from the identification of drought-tolerant genotypes such as AGS429 and AGS383. These genotypes provide valuable genetic resources for breeding programs aimed at developing high-yielding, drought-tolerant soybean varieties. They can be used as parent material to enhance drought tolerance in future cultivars. Understanding the physiological and biochemical responses of soybeans to drought stress can inform agronomic practices. For instance, the application of osmoprotectants or antioxidants could be explored to mitigate drought stress and improve yield stability. As climate change intensifies the frequency and severity of droughts, the development and cultivation of drought-tolerant soybean varieties will be crucial for ensuring food security. These varieties can help stabilize soybean production in drought-prone regions, thereby contributing to global food security.

In conclusion, this study underscores the importance of screening and identifying drought-tolerant soybean varieties to enhance resilience against water-deficit conditions. Future research should focus on further investigation into the molecular mechanisms underlying drought tolerance, including gene expression and regulatory pathways, which will provide deeper insights into the genetic basis of drought resilience. Conducting extensive field trials across different environmental conditions will validate the laboratory findings and ensure the practical applicability of drought-tolerant genotypes. Combining physiological, biochemical, and molecular approaches will offer a holistic understanding of drought tolerance and facilitate the development of robust, high-yielding soybean varieties capable of withstanding drought stress. By addressing these areas, we can make significant strides in improving soybean production and ensuring food security in the face of climate change.

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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.

References

- Aleem M., Raza M., Haider M., Atif R., Ali Z., Bhat J., and Zhao T., 2020, Comprehensive RNA-seq analysis revealed molecular pathways and genes associated with drought tolerance in Wild Soybean (*Glycine soja* Sieb. & Zucc.), *Physiologia Plantarum*, 172(2): 707-732.
<https://doi.org/10.1111/ppl.13219>
- Arya H., Singh M., and Bhalla P., 2021, Towards developing drought-smart soybeans, *Frontiers in Plant Science*, 12: 750664.
<https://doi.org/10.3389/fpls.2021.750664>
- Buezo J., Sanz-Saez A., Moran J., Soba D., Aranjuelo Í., and Esteban R., 2018, Drought tolerance response of high-yielding soybean varieties to mild drought: physiological and photochemical adjustments, *Physiologia Plantarum*, 166(1): 88-104.
<https://doi.org/10.1111/ppl.12864>
- Darmanti S., Hastuti E., and Suedy S., 2020, Exogenous hydrogen peroxide induces an antioxidative defense system against drought stress in soybean [*Glycine max* (L.) Merr.] crops, *The Journal of Animal and Plant Sciences*, 31(1): 213-220.
<https://doi.org/10.36899/japs.2021.1.0208>
- Delavar E., Faramarzi A., Ajalli J., Nazari N., and Abdi M., 2023, Piriformospora indica symbiosis and iron oxide nanoparticles alleviates drought stress in soybean plants through improved on photosynthetic gas exchange and sucrose phosphate synthase and acid phosphatase, *Romanian Agricultural Research*, (40): 81-94.
<https://doi.org/10.59665/rar4008>
- Dhungana S., Park J., Oh J., Kang B., Seo J., Sung J., Kim H., Shin S., Baek I., and Jung C., 2021, Quantitative trait locus mapping for drought tolerance in soybean recombinant inbred line population, *Plants*, 10(9): 1816.
<https://doi.org/10.3390/plants10091816>
- Du Y., Zhao Q., Chen L., Yao X., and Xie F., 2020, Effect of drought stress at reproductive stages on growth and nitrogen metabolism in soybean, *Agronomy*, 10(2): 302.
<https://doi.org/10.3390/agronomy10020302>
- Dubey A., Kumar A., AbdAllah E., Hashem A., and Khan M., 2019, Growing more with less: Breeding and developing drought resilient soybean to improve food security, *Ecological Indicators*, 105: 425-437.
<https://doi.org/10.1016/J.ECOLIND.2018.03.003>
- Fatema M., Mamun M., Sarker U., Hossain M., Mia M., Roychowdhury R., Ercişli S., Marc R., Babalola O., and Karim M., 2023, Assessing morpho-physiological and biochemical markers of soybean for drought tolerance potential, *Sustainability*, 15(2): 1427.
<https://doi.org/10.3390/su15021427>
- Gebre M., Rajcan I., and Earl H., 2022, Genetic variation for effects of drought stress on yield formation traits among commercial soybean [*Glycine max* (L.) Merr.] cultivars adapted to Ontario, Canada, *Frontiers in Plant Science*, 13: 1020944.
<https://doi.org/10.3389/fpls.2022.1020944>
- Iqbal N., Hussain S., Raza M., Yang C., Safdar M., Brestič M., Aziz A., Hayyat M., Asghar M., Wang X., Zhang J., Yang W., and Liu J., 2019, Drought tolerance of soybean (*Glycine max* L. Merr.) by improved photosynthetic characteristics and an efficient antioxidant enzyme activities under a split-root system, *Frontiers in Physiology*, 10: 786.
<https://doi.org/10.3389/fphys.2019.00786>
- Molinari M., Fuganti-Paglierini R., Marcolino-Gomes J., Barbosa D., Marin S., Mertz-Henning L., Nepomuceno A., and Filho E., 2021, Flower and pod genes involved in soybean sensitivity to drought, *Journal of Plant Interactions*, 16: 187-200.
<https://doi.org/10.1080/17429145.2021.1921293>
- Moloi M., and Merwe R., 2021, Drought tolerance responses in vegetable-type soybean involve a network of biochemical mechanisms at flowering and pod-filling stages, *Plants*, 10(8): 1502.
<https://doi.org/10.3390/plants10081502>
- Pereira Y., Rodrigues W., Lima E., Santos L., Silva M., and Lobato A., 2019, Brassinosteroids increase electron transport and photosynthesis in soybean plants under water deficit, *Photosynthetica*, 57(1): 181-191.
<https://doi.org/10.32615/PS.2019.029>
- Puobi R., Asare A., Otwe E., and Galyuon I., 2023, Drought tolerance in soybean (*Glycine max* L. Merr.) genotypes during the flowering stage of development, *Journal of Advances in Biology and Biotechnology*, 26(11): 1-14.
<https://doi.org/10.9734/jabb/2023/v26i11663>
- Rao D., and Chaitanya K., 2019, Changes in the antioxidant intensities of seven different soybean (*Glycine max* (L.) Merr.) cultivars during drought, *Journal of Food Biochemistry*, 44(2): e13118.
<https://doi.org/10.1111/jfbc.13118>
- Rezayian M., Ebrahimzadeh H., and Niknam V., 2020, Nitric oxide stimulates antioxidant system and osmotic adjustment in soybean under drought stress, *Journal of Soil Science and Plant Nutrition*, 20: 1122-1132.
<https://doi.org/10.1007/s42729-020-00198-x>

- Silva A., Silva C., Rosa V., Santos M., Kuki K., Dal-Bianco M., Bueno R., Oliveira J., Brito D., Costa A., and Ribeiro C., 2022, Metabolic adjustment and regulation of gene expression are essential for increased resistance to severe water deficit and resilience post-stress in soybean, *PeerJ*, 10: e13118.
<https://doi.org/10.7717/peerj.13118>
- Tripathi P., Rabara R., Reese R., Miller M., Rohila J., Subramanian S., Shen Q., Morandi D., Bücking H., Shulaev V., and Rushton P., 2016, A toolbox of genes, proteins, metabolites and promoters for improving drought tolerance in soybean includes the metabolite coumestrol and stomatal development genes, *BMC Genomics*, 17: 102.
<https://doi.org/10.1186/s12864-016-2420-0>
- Wang G., Zhou Q., He M., Zhong X., and Tang G., 2020, Wilting index and root morphological characteristics used as drought-tolerance variety selection at the seedling stage in soybean (*Glycine max* L.), *Plant Growth Regulation*, 92: 29-42.
<https://doi.org/10.1007/s10725-020-00617-0>
- Wang X., Li X., and Dong S., 2022b, Screening and identification of drought tolerance of spring soybean at seedling stage under climate change, *Front. Sustain. Food Syst.*, 6: 988319.
<https://doi.org/10.3389/fsufs.2022.988319>
- Wang X., Wu Z., Zhou Q., Wang X., Song S., and Dong S., 2022a, Physiological response of soybean plants to water deficit, *Frontiers in Plant Science*, 12: 809692.
<https://doi.org/10.3389/fpls.2021.809692>
- Wang H.Y., Yao X.D., Guo Y., Wang L., and Yang M.D., 2024, In-depth analysis of physiological, biochemical, and molecular bases of drought tolerance in soybeans, *Legume Genomics and Genetics*, 15(5): 257-269.
<https://doi.org/10.5376/lgg.2024.15.0025>
- Xu Y., Song D., Qi X., Asad M., Wang S., Tong X., Jiang Y., and Wang S., 2023, Physiological responses and transcriptome analysis of soybean under gradual water deficit, *Frontiers in Plant Science*, 14: 1269884.
<https://doi.org/10.3389/fpls.2023.1269884>
- Yan C., Song S., Wang W., Wang C., Li H., Wang F., Li S., and Sun X., 2020, Screening diverse soybean genotypes for drought tolerance by membership function value based on multiple traits and drought-tolerant coefficient of yield, *BMC Plant Biology*, 20: 321.
<https://doi.org/10.1186/s12870-020-02519-9>
- Zhao L., Wang W., Fu X., Liu A., Cao J., and Liu J., 2022, Graphene oxide, a novel nanomaterial as soil water retention agent, dramatically enhances drought stress tolerance in soybean plants, *Frontiers in Plant Science*, 13: 810905.
<https://doi.org/10.3389/fpls.2022.810905>

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