

Marker-Assisted Selection Strategies for Drought Tolerance in Soybean and Future Perspectives

Xiaofei Tang, Yongguo Xue, Dan Cao, Xiaoyan Luan, Qi Liu, Zifei Zhu, Xinlei Liu ✉

Soybean Research Institute, Heilongjiang Academy of Agricultural Science, Harbin, 150086, China

✉ Corresponding email: nkyddsxl@163.com

Legume Genomics and Genetics, 2024 Vol.15, No.6 doi: [10.5376/lgg.2024.15.0028](https://doi.org/10.5376/lgg.2024.15.0028)

Received: 07 Nov., 2024

Accepted: 08 Dec., 2024

Published: 20 Dec., 2024

Copyright © 2024 Tang et al., This is an open access article published under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Preferred citation for this article:

Tang X.F., Xue Y.G., Cao D., Luan X.Y., Liu Q., Zhu Z.F., and Liu X.L., 2024, Marker-assisted selection strategies for drought tolerance in soybean and future perspectives, Legume Genomics and Genetics, 15(6): 291-302 (doi: [10.5376/lgg.2024.15.0028](https://doi.org/10.5376/lgg.2024.15.0028))

Abstract Soybean is a crucial crop for global food security, yet its growth and yield are highly susceptible to drought stress. This study reviews the application of marker-assisted selection (MAS) in breeding drought-tolerant soybean varieties, summarizing the identification and utilization of quantitative trait loci (QTL) and key candidate genes associated with drought tolerance. MAS enables the precise selection of drought tolerance traits using molecular markers, significantly shortening breeding cycles and enhancing the efficiency of drought-resistant variety development. The study finds that drought tolerance, a complex trait controlled by multiple genes and significantly affected by environmental factors, requires the integration of genomic selection and high-throughput genotyping technologies to improve MAS accuracy and applicability. The paper discusses potential future directions, including the integration of climate-resilient agricultural practices and emerging technologies in MAS, offering comprehensive theoretical and practical guidance for advancing drought-tolerant soybean breeding.

Keywords Marker-assisted selection; Drought tolerance; Soybean breeding; Quantitative trait loci; Genomic selection

1 Introduction

Drought stress is one of the most significant abiotic factors adversely affecting agricultural productivity worldwide. Soybean (*Glycine max* L.), a crucial crop for food security, is particularly sensitive to drought conditions, which can severely impact its yield and production stability (Valliyodan et al., 2016; Dubey et al., 2019). The interaction of drought with other stress factors, such as high temperatures, exacerbates the negative effects on soybean growth and productivity. As climate change continues to intensify, the frequency and severity of drought events are expected to increase, posing a further threat to soybean production and global food security.

Given the critical role of soybean in global agriculture, developing drought-tolerant varieties is essential to ensure stable yields under water-limiting conditions. Enhancing drought tolerance in soybean can help mitigate the adverse effects of drought stress, thereby improving yield stability and contributing to food security (Dubey et al., 2019). The success of breeding programs aimed at improving drought tolerance relies heavily on the genetic diversity present in the germplasm base and the identification of traits associated with drought resistance (Valliyodan et al., 2016). By focusing on traits such as root system architecture, water-use efficiency, and nitrogen fixation, researchers can develop soybean varieties that are better equipped to withstand drought conditions (Fatema et al., 2023).

Marker-assisted selection (MAS) is a powerful tool in plant breeding that utilizes molecular markers to select for desirable traits, such as drought tolerance, more efficiently and accurately than traditional breeding methods. MAS enables the identification and incorporation of specific genes or quantitative trait loci (QTLs) associated with drought tolerance into elite soybean varieties (Cattivelli et al., 2008). Advances in genomic technologies, such as high-throughput sequencing and genotyping platforms, have facilitated the discovery of numerous markers linked to drought tolerance traits (Valliyodan et al., 2016). By integrating MAS with conventional breeding approaches, researchers can accelerate the development of drought-tolerant soybean varieties, ultimately enhancing crop resilience and productivity under adverse environmental conditions (Ren et al., 2020).

This study summarizes the latest research advancements and technological progress in genomics, focusing on how these developments assist in identifying and utilizing effective drought-resistant markers. It analyzes the genetic diversity and key traits related to drought tolerance in soybeans, while exploring future research directions and potential strategies to enhance drought resilience. The aim is to investigate the role and contribution of marker-assisted selection (MAS) technology in breeding drought-tolerant soybean varieties.

2 Soybean and Drought Stress

2.1 Overview of soybean cultivation and its economic importance

Soybean (*Glycine max* L.) is a globally significant crop, contributing approximately 57% of the total oilseed production worldwide (Shaheen et al., 2016). It is a major source of protein and oil, making it crucial for both human consumption and animal feed. The economic importance of soybean is underscored by its role in food security and its extensive use in various industries, including food, feed, and biofuel production (Dubey et al., 2019). The crop's adaptability to different climatic conditions and its ability to fix atmospheric nitrogen through symbiosis with rhizobia make it a valuable component of sustainable agricultural systems (Valliyodan et al., 2016).

2.2 Physiological and biochemical responses of soybean to drought stress

Drought stress significantly impacts soybean growth and productivity by altering its physiological and biochemical processes. Under drought conditions, soybean plants exhibit reduced photosynthetic activity, leaf production, and water content, leading to stunted growth and lower dry matter production (Figure 1) (Arya et al., 2021). Biochemically, drought stress induces the accumulation of osmoprotectants such as proline and malondialdehyde (MDA), which help mitigate oxidative damage (Wang et al., 2022; Fatema et al., 2023). Additionally, drought stress enhances the activity of antioxidant enzymes like peroxidase (POD), catalase (CAT), and Ascorbic Acid Peroxidase (APX), which play crucial roles in protecting the plant from oxidative stress (Wang et al., 2022). High-yielding and drought-tolerant soybean varieties have been shown to maintain better root and shoot growth, higher photosynthesis rates, and greater water use efficiency under drought conditions (Buezo et al., 2018).

2.3 Challenges in breeding for drought tolerance in soybean

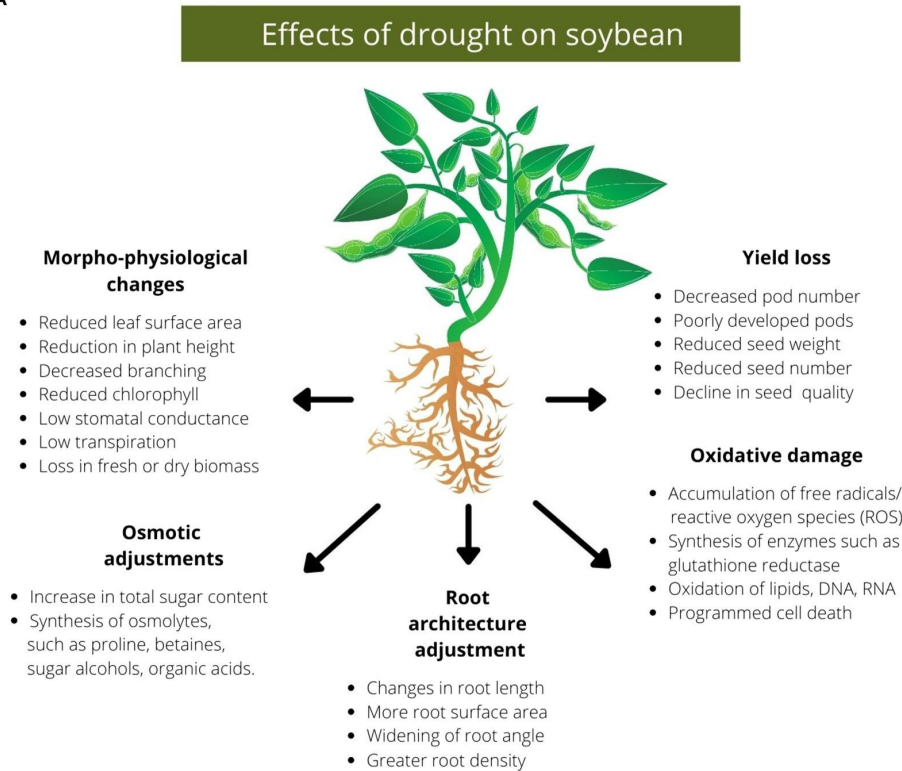
Breeding for drought tolerance in soybean presents several challenges due to the complex nature of drought resistance traits, which are often quantitative and influenced by multiple genetic and environmental factors (Manavalan et al., 2009). Traditional breeding methods, which rely on direct selection for yield stability across multiple locations, are time-consuming and labor-intensive. The low heritability of yield under drought conditions further complicates the breeding process. Advances in genomic technologies, such as the availability of the whole soybean genome sequence and high-throughput phenotyping, have facilitated the identification of quantitative trait loci (QTLs) and candidate genes associated with drought tolerance (Xiong et al., 2020). However, integrating these genomic tools with conventional breeding approaches requires a comprehensive understanding of the physiological and molecular mechanisms underlying drought tolerance (Arya et al., 2021). Additionally, the limited genetic variability in soybean germplasm poses a significant hurdle, necessitating the exploration of diverse genetic resources to enhance drought tolerance (Valliyodan et al., 2016). Despite these challenges, the development of drought-tolerant soybean cultivars through marker-assisted selection and genetic engineering holds promise for improving soybean resilience to water-limited conditions (Shaheen et al., 2016; Dubey et al., 2019).

3 Marker-Assisted Selection: Concept and Applications

3.1 Definition and principles of marker-assisted selection

Marker-assisted selection (MAS) is a process in plant breeding that uses molecular markers to select plants with desirable traits. The principle behind MAS is to identify specific DNA sequences (markers) that are linked to traits of interest, such as drought tolerance, and use these markers to guide the selection process. This allows for more precise and efficient breeding compared to traditional methods, as it enables the early identification of plants that carry the desired traits (Ribaut and Ragot, 2006; Torres et al., 2010).

A



B

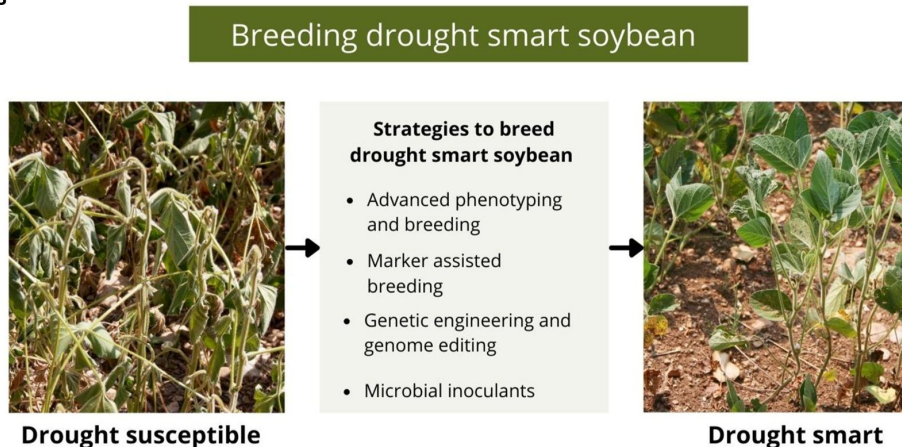


Figure 1 (A) A diagram depicting various effects of drought on soybean, (B) An outline of strategies to breed drought-smart soybeans (Adopted from Arya et al., 2021)

3.2 Types of molecular markers used in MAS

Several types of molecular markers are commonly used in MAS, including Simple Sequence Repeats (SSR): Also known as microsatellites, SSRs are short, repetitive DNA sequences that are highly polymorphic and widely distributed throughout the genome. They are useful for genetic mapping and diversity studies (Nugroho et al., 2020). Single Nucleotide Polymorphisms (SNP): SNPs are single base-pair variations in the DNA sequence. They are abundant and can be used for high-resolution mapping and genotyping (He et al., 2014; Patil et al., 2016; Ouyang et al., 2022). Random amplified polymorphic DNA (RAPD): RAPD markers are generated by amplifying random segments of genomic DNA with single primers of arbitrary nucleotide sequence. They are useful for identifying genetic diversity and mapping. Kompetitive allele-specific PCR (KASP): KASP markers are used for genotyping and are based on competitive allele-specific PCR. They are highly specific and useful for marker-trait association studies (Eltaher et al., 2023; Zhou and Guo, 2024).

3.3 Advantages of MAS over traditional breeding methods

MAS offers several advantages over traditional breeding methods. Efficiency: MAS allows for the selection of desirable traits at the seedling stage, reducing the time and resources needed for field trials (Ribaut and Ragot, 2006). Precision: By targeting specific genetic markers, MAS increases the accuracy of selecting plants with the desired traits, leading to more consistent and reliable outcomes (Ouyang et al., 2022; Eltaher et al., 2023). Speed: MAS accelerates the breeding process by enabling the early identification of desirable traits, thus shortening the breeding cycle (Patil et al., 2016). Cost-Effectiveness: Although the initial setup for MAS can be expensive, it ultimately reduces the costs associated with extensive field trials and phenotypic evaluations (He et al., 2014; Hassan et al., 2023).

3.4 Examples of MAS applications in other crops

MAS has been successfully applied in various crops to improve traits such as drought tolerance, disease resistance, and yield. Common bean (*Phaseolus vulgaris* L.): MAS has been used to identify RAPD markers associated with drought resistance, leading to improved performance under stress conditions. Maize (*Zea mays* L.): Marker-Assisted Backcross (MABC) selection has been employed to improve grain yield under drought conditions, resulting in hybrids that perform significantly better under water stress (Ribaut and Ragot, 2006). Faba bean (*Vicia faba* L.): MAS has been used to develop resistance to diseases such as ascochyta blight and rust, as well as to improve traits like growth habit and nutritional value (Torres et al., 2010). Rice (*Oryza sativa* L.): MAS has been utilized to introgress QTLs associated with drought tolerance into elite rice lines, enhancing their ability to withstand adverse conditions (Hassan et al., 2023).

4 Genetic Basis of Drought Tolerance in Soybean

4.1 Key genetic loci and quantitative trait loci (QTLs) associated with drought tolerance

Drought tolerance in soybean is a complex trait controlled by multiple genetic loci. Several studies have identified key QTLs associated with drought tolerance. For instance, a study using a Recombinant Inbred Line (RIL) population from a cross between drought-tolerant and drought-sensitive cultivars identified five QTLs on five chromosomes, with one QTL on chromosome 16 accounting for 17.177% of the phenotypic variation (Ouyang et al., 2022). Another study identified 10 QTLs on seven chromosomes, with significant loci on chromosomes 1, 2, 7, 10, 14, 19, and 20, explaining up to 12.9% of the phenotypic variance (Figure 2) (Dhungana et al., 2021). Additionally, a comprehensive study on a Chinese cultivated soybean population detected 75 and 64 QTLs for drought tolerance indicators, explaining 54.7% and 47.1% of phenotypic variance, respectively (Wang et al., 2020).

4.2 Major genes involved in drought tolerance mechanisms

Several genes have been implicated in the mechanisms of drought tolerance in soybean. For example, nine candidate genes were identified within a QTL on chromosome 16, including Glyma.16G036700, Glyma.16G036400, and Glyma.16G036600, which are annotated as NAC transport factor, GATA transport factor, and BTB/POZ-MATH proteins, respectively (Ouyang et al., 2022). Another study identified 177 candidate genes grouped into nine categories, with ABA and stress responders being major components (Wang et al., 2020). Furthermore, RNA-seq analysis of wild soybean identified differentially expressed genes (DEGs) associated with water and auxin transport, cell wall/membrane integrity, antioxidant activity, and transcription factor activities, highlighting the complexity of the genetic response to drought stress (Aleem et al., 2020).

4.3 Importance of identifying QTLs for MAS

Identifying QTLs is crucial for Marker-Assisted Selection (MAS) as it allows for the precise incorporation of drought tolerance traits into new soybean varieties. The identification of QTLs linked to drought tolerance traits enables breeders to select for these traits more efficiently, thereby accelerating the development of drought-tolerant cultivars. For instance, the QTLs identified in various studies can be used to develop molecular markers that facilitate the selection of drought-tolerant genotypes in breeding programs (Ren et al., 2020; Dhungana et al., 2021; Ouyang et al., 2022). The establishment of QTL-allele matrices, as demonstrated in the Chinese cultivated soybean population, provides a robust framework for predicting optimal crosses and enhancing drought tolerance through MAS (Wang et al., 2020).

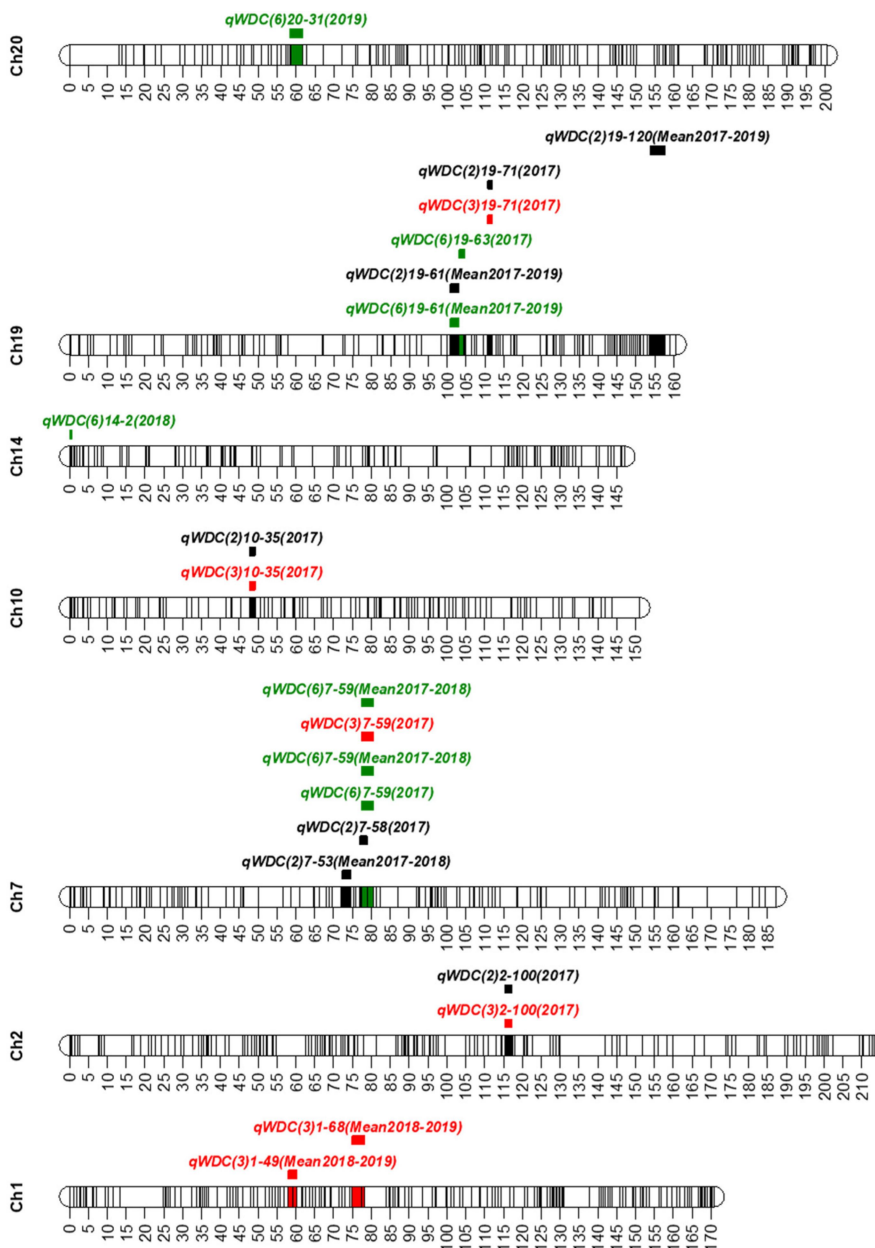


Figure 2 Positions of the QTLs for drought tolerance on seven chromosomes (Ch) (Adopted from Dhungana et al., 2021)

5 Marker Development for Drought Tolerance in Soybean

5.1 Strategies for developing molecular markers linked to drought tolerance traits

Developing molecular markers linked to drought tolerance traits in soybean involves several strategies. One primary approach is the identification and mapping of Quantitative Trait Loci (QTLs) associated with drought tolerance. This can be achieved through the use of Recombinant Inbred Line (RIL) populations derived from crosses between drought-tolerant and drought-sensitive cultivars. For instance, studies have identified multiple QTLs related to drought tolerance traits such as plant height and seed weight per plant using high-density genetic maps constructed with specific locus amplified fragment sequencing (SLAF-Seq) technology (Ren et al., 2020). Additionally, the use of Single Nucleotide Polymorphism (SNP) markers has been instrumental in constructing genetic maps and identifying QTLs associated with drought tolerance (Dhungana et al., 2021; Ouyang et al., 2022).

Another strategy involves the use of Genome-Wide Association Studies (GWAS) to identify loci associated with drought tolerance. This method leverages the genetic diversity present in large germplasm collections to uncover significant associations between genetic markers and drought tolerance traits (Wang et al., 2020). Furthermore,

transcriptome profiling using RNA-Seq can identify differentially expressed genes (DEGs) under drought conditions, providing insights into the genetic mechanisms underlying drought tolerance and identifying potential candidate genes for marker development (Aleem et al., 2020).

5.2 High-throughput sequencing technologies and genotyping

High-throughput sequencing technologies, such as Whole-Genome Resequencing (WGRS) and RNA-Seq, have revolutionized the field of genotyping and marker development. WGRS allows for the comprehensive analysis of genetic variation across the entire genome, facilitating the identification of SNPs and structural variants associated with drought tolerance (Patil et al., 2016). This technology has been used to identify high-quality SNP markers that can be employed in marker-assisted selection (MAS) programs to develop drought-tolerant soybean cultivars.

RNA-Seq, on the other hand, provides a detailed view of the transcriptome, enabling the identification of DEGs under drought stress. This approach has been used to compare root transcriptome profiles of drought-tolerant and drought-sensitive soybean genotypes, leading to the identification of key genes and pathways involved in drought response (Aleem et al., 2020). The integration of high-throughput sequencing data with phenotypic data allows for the precise mapping of QTLs and the development of robust molecular markers for drought tolerance.

5.3 Importance of genome-wide association studies (GWAS) and linkage mapping

Genome-Wide Association Studies (GWAS) and linkage mapping are critical tools in the identification of genetic loci associated with drought tolerance in soybean. GWAS leverages the natural genetic variation present in diverse germplasm collections to identify significant associations between genetic markers and drought tolerance traits. This approach has been used to identify numerous QTLs and candidate genes associated with drought tolerance in soybean. The use of multi-locus multi-allele GWAS has further enhanced the resolution and accuracy of QTL mapping, providing a comprehensive understanding of the genetic architecture of drought tolerance (Wang et al., 2020).

Linkage mapping, on the other hand, involves the use of bi-parental populations to map QTLs associated with drought tolerance. This method has been successfully employed to identify QTLs related to various drought tolerance traits, such as relative water content, root length, and grain yield, in soybean (Can, 2011). The integration of GWAS and linkage mapping results can provide a more complete picture of the genetic basis of drought tolerance, facilitating the development of effective MAS programs for breeding drought-tolerant soybean cultivars.

6 Marker-Assisted Breeding Programs in Soybean

6.1 Overview of MAS breeding programs targeting drought tolerance in soybean

Marker-Assisted Selection (MAS) has emerged as a powerful tool in the breeding of drought-tolerant soybean varieties. This approach leverages molecular markers to identify and select for desirable traits, thereby accelerating the breeding process and improving the precision of selection. The application of MAS in soybean breeding programs has focused on identifying quantitative trait loci (QTLs) associated with drought tolerance and introgressing these traits into elite cultivars.

Several studies have demonstrated the effectiveness of MAS in improving drought tolerance in various crops, which provides a strong foundation for its application in soybean. For instance, MAS has been successfully used to improve drought adaptation in maize by introgressing favorable alleles at target regions involved in yield components and flowering traits, resulting in significant yield improvements under water-limited conditions (Ribaut and Ragot, 2006). Similarly, in common bean, MAS has been employed to identify RAPD markers associated with drought resistance, leading to improved performance under stress conditions. These successes highlight the potential of MAS to enhance drought tolerance in soybean by targeting specific QTLs and genes associated with drought resistance.

In soybean, the identification of QTLs related to drought tolerance has been a key focus. For example, a study identified multiple QTLs associated with drought tolerance in a Recombinant Inbred Line (RIL) population, which could be utilized in MAS to develop drought-tolerant soybean varieties (Dhungana et al., 2021).

Additionally, genomic resources such as whole-genome sequences and high-throughput marker genotyping platforms have facilitated the discovery of markers and genes for drought tolerance, further supporting the implementation of MAS in soybean breeding programs (Valliyodan et al., 2016).

6.2 Key successes and challenges in these programs

The application of MAS in soybean breeding programs has yielded several notable successes. One significant achievement is the identification and validation of QTLs associated with drought tolerance, which has enabled the development of soybean varieties with improved drought resilience. For instance, the identification of QTLs on chromosomes 1, 2, 7, 10, 14, 19, and 20 has provided valuable targets for MAS, facilitating the breeding of drought-tolerant soybean cultivars (Dhungana et al., 2021). Moreover, the use of context-specific MAS (CSM) has demonstrated significant yield gains in elite soybean populations, with selected sublines showing up to 5.8% higher yields compared to their mother lines (Sebastian et al., 2010).

Despite these successes, several challenges remain in the implementation of MAS for drought tolerance in soybean. One major challenge is the complexity of drought tolerance as a trait, which involves multiple genes and environmental interactions. This complexity can make it difficult to achieve consistent improvements across different genetic backgrounds and environmental conditions. For example, the performance of MAS-derived populations can vary substantially across different genetic backgrounds, highlighting the need for careful characterization of donor alleles within elite backgrounds before implementing MAS-based breeding (Singh et al., 2022).

Another challenge is the cost and resource requirements associated with MAS. While advances in genotyping technologies have made MAS more feasible, the initial investment in developing and validating markers can be substantial. Additionally, the integration of MAS with conventional breeding methods requires careful planning and coordination to ensure the successful introgression of target traits.

7 Case Study: Marker-Assisted Selection for Drought Tolerance in Soybean

7.1 Detailed description of a specific MAS breeding program or case study

In this case study, we focus on a Marker-Assisted Selection (MAS) breeding program aimed at improving drought tolerance in soybean. The program utilized a high-yielding but drought-sensitive cultivar, 'Zhonghuang 35', and a drought-tolerant cultivar, 'Jindou 21', to develop F6:9 Recombinant Inbred Lines (RILs). The primary goal was to identify Quantitative Trait Loci (QTL) associated with drought tolerance traits, such as plant height and seed weight per plant, and to use these markers to accelerate the breeding of drought-tolerant soybean varieties (Ren et al., 2020).

7.2 Methodology: selection of markers, phenotyping, and genotyping approaches

The methodology involved several key steps. Selection of Markers: Specific locus amplified fragment sequencing (SLAF-Seq) technology was employed to construct a high-density genetic map containing 8 078 SLAF markers distributed across 20 soybean chromosomes. This map facilitated the identification of QTL associated with drought tolerance traits. Phenotyping: Field tests were conducted under two conditions: irrigation and drought. Plant height and seed weight per plant were used as indicators of drought tolerance. These phenotypic traits were measured to assess the performance of the RILs under both conditions (Ren et al., 2020). Genotyping: The genetic map was used to perform Additive-Inclusive Composite Interval Mapping (ICIM-ADD) to identify QTL. This approach allowed for the precise localization of QTL on the soybean genome, which were then linked to the phenotypic traits measured during the field tests (Sreenivasa et al., 2020).

7.3 Results: identification of key markers and their impact on drought tolerance

The study identified a total of 23 QTL related to drought tolerance. Key findings include seven QTL (qPH2, qPH6, qPH7, qPH17, qPH19-1, qPH19-2, and qPH19-3) were associated with plant height and were located on chromosomes 2, 6, 7, 17, and 19. Five QTL (qSWPP2, qSWPP6, qSWPP13, qSWPP17, and qSWPP19) were linked to seed weight per plant and were found on chromosomes 2, 6, 13, 17, and 19. Three common QTL (qPH6/qSWPP6, qPH17/qSWPP17, and qPH19-3/qSWPP19) were identified for both plant height and seed

weight per plant, indicating regions of the genome that have a significant impact on multiple drought tolerance traits (Ren et al., 2020).

7.4 Lessons learned and practical implications

Several important lessons and practical implications emerged from this MAS breeding program. Efficiency of SLAF-seq technology: The use of SLAF-Seq technology proved to be highly effective in constructing a detailed genetic map and identifying QTL associated with drought tolerance. This technology can be applied to other breeding programs aiming to improve stress tolerance in crops. Importance of multi-trait QTL: The identification of QTL that influence multiple traits, such as plant height and seed weight, highlights the potential for developing soybean varieties with comprehensive drought tolerance. These multi-trait QTL can be prioritized in breeding programs to achieve more robust drought-resistant cultivars. Genetic diversity utilization: The study underscores the importance of utilizing genetic diversity in breeding programs. By crossing drought-sensitive and drought-tolerant cultivars, the program was able to identify valuable genetic markers that can be used to enhance drought tolerance in soybean. Field phenotyping: Accurate phenotyping under field conditions is crucial for the success of MAS programs. The use of both irrigated and drought conditions allowed for a comprehensive assessment of the RILs' performance, ensuring that the identified markers are truly associated with drought tolerance (Ren et al., 2020).

8 Challenges and Limitations of MAS in Soybean Breeding

8.1 Limitations in marker development and identification

Marker development and identification are critical steps in Marker-Assisted Selection (MAS) for drought tolerance in soybean. One of the primary limitations is the complexity of drought tolerance as a polygenic trait, which involves multiple quantitative trait loci (QTLs) (Ren et al., 2020). The identification of these QTLs requires extensive genetic mapping and phenotyping, which can be resource-intensive and time-consuming. For instance, the study by identified 10 QTLs for drought tolerance in soybean (Dhungana et al., 2021), but the phenotypic variance explained by each QTL was relatively low, indicating the need for further refinement and validation of these markers. Additionally, the effectiveness of identified markers can vary across different genetic backgrounds and environmental conditions, as seen in the study on alfalfa where the performance of MAS-derived populations varied significantly across different genetic backgrounds (Singh et al., 2022).

8.2 Challenges in phenotyping for drought tolerance

Phenotyping for drought tolerance presents several challenges, primarily due to the variability in environmental conditions and the complex nature of drought responses. Accurate phenotyping requires controlled environments to simulate drought conditions and measure relevant traits such as leaf wilting, excised leaf water loss, and relative water content (Du et al., 2009). However, field conditions are often unpredictable, making it difficult to obtain consistent and reliable phenotypic data. The study by highlighted the importance of selecting cost-efficient and reliable markers for phenotyping, such as pubescence, stomatal density, and canopy temperature depression, which showed high consistency across different phenological stages. Despite these advancements, phenotyping remains a bottleneck in MAS due to the labor-intensive and time-consuming nature of the process.

8.3 Integration of MAS with other breeding strategies

Integrating MAS with other breeding strategies, such as Genomic Selection (GS), can enhance the efficiency and effectiveness of breeding programs. While MAS focuses on specific QTLs, GS uses genome-wide markers to predict the breeding value of individuals, allowing for the selection of superior genotypes based on their overall genetic potential (Ribaut and Ragot, 2006). The study on maize demonstrated the potential of combining MAS with backcrossing to improve grain yield under drought conditions, suggesting that similar approaches could be beneficial for soybean breeding. However, the integration of MAS with GS and other strategies requires careful consideration of the genetic architecture of drought tolerance and the development of robust statistical models to predict breeding values accurately. Additionally, the high cost of genotyping and the need for large training populations can be limiting factors in the widespread adoption of these integrated approaches (Hassan et al., 2023).

9 Future Perspectives of MAS in Soybean Drought Tolerance Breeding

9.1 Emerging technologies in MAS

The integration of emerging technologies such as CRISPR and Genomic Selection (GS) into Marker-Assisted Selection (MAS) holds significant promise for enhancing drought tolerance in soybean. CRISPR technology allows for precise gene editing, enabling the introduction of specific drought-tolerant traits into soybean genomes with high accuracy and efficiency (Rosero et al., 2020). Genomic selection, on the other hand, leverages whole-genome information to predict the performance of breeding lines, thus accelerating the breeding process by selecting the best candidates early in the breeding cycle (Valliyodan et al., 2016; Cerrudo et al., 2018). These technologies, when combined with traditional MAS, can significantly enhance the genetic gain for drought tolerance by targeting multiple genes and pathways involved in stress response (Dubey et al., 2019; Dormatey et al., 2020).

9.2 Integrating MAS with climate-resilient agriculture practices

Integrating MAS with climate-resilient agricultural practices is crucial for developing soybean varieties that can withstand the increasing frequency and severity of drought events due to climate change. Practices such as optimized irrigation management, soil health improvement, and the use of drought-tolerant crop rotations can complement the genetic improvements achieved through MAS. For instance, the use of Plant Growth-Promoting Rhizobacteria (PGPR) in conjunction with MAS can enhance root system architecture and water-use efficiency, further improving drought resilience (Dubey et al., 2019). Additionally, the introduction of underutilized drought-tolerant crops into existing cropping systems can provide a buffer against water scarcity, ensuring stable yields (Rosero et al., 2020).

9.3 Potential for further research and collaboration

The potential for further research and collaboration in the field of MAS for drought tolerance in soybean is vast. Collaborative efforts between geneticists, agronomists, and climate scientists can lead to the development of more robust and resilient soybean varieties. Research should focus on identifying new Quantitative Trait Loci (QTL) associated with drought tolerance and understanding the underlying genetic mechanisms (Ren et al., 2020; Singh et al., 2022). Moreover, international collaborations can facilitate the exchange of germplasm and knowledge, accelerating the breeding process and ensuring that the benefits of MAS are realized globally (Torres et al., 2010; Valliyodan et al., 2016). The integration of advanced phenotyping techniques and high-throughput genotyping platforms will also be essential in enhancing the efficiency and accuracy of MAS (Ribaut and Ragot, 2006).

10 Concluding Remarks

Marker-Assisted Selection (MAS) has shown significant promise in improving drought tolerance in various crops, including soybean. The application of MAS has led to the identification of Quantitative Trait Loci (QTL) and specific markers associated with drought resistance, which can be used to select high-performing genotypes early in the breeding process. For instance, in common bean, MAS was effective in identifying RAPD markers associated with yield under stress conditions, leading to improved performance in specific environments. Similarly, in maize, the introgression of favorable alleles through MAS significantly increased grain yield under drought conditions. In soybean, context-specific MAS (CSM) has been used to detect yield QTL within specific environments, resulting in statistically significant yield gains in selected sublines. These findings underscore the potential of MAS to enhance drought tolerance and yield stability in soybean and other crops.

The future role of MAS in addressing drought challenges in soybean is promising, given the advancements in genotyping technologies and the increasing understanding of the genetic basis of drought tolerance. The development of high-density genetic maps and the identification of specific markers linked to drought tolerance traits, such as those found in soybean, will facilitate more precise and efficient selection processes. Additionally, the integration of "omics" technologies, such as transcriptomics and proteomics, can further enhance the identification and characterization of genes involved in drought tolerance, leading to more targeted and effective breeding strategies. As genotyping becomes more cost-effective, the application of MAS in soybean breeding programs is likely to expand, enabling the development of drought-tolerant cultivars that can thrive in water-limited environments.

Continued research in MAS is crucial for the ongoing improvement of crop resilience to abiotic stresses, such as drought. The ability to select for desirable traits at the molecular level offers a significant advantage over traditional breeding methods, allowing for more rapid and precise development of stress-tolerant cultivars. The success of MAS in various crops, including soybean, common bean, maize, and wheat, highlights its potential to address the challenges posed by climate change and ensure food security. Future research should focus on expanding the genetic resources available for MAS, improving marker technologies, and integrating advanced genomic tools to enhance the efficiency and effectiveness of breeding programs. By continuing to invest in MAS research, we can develop crops that are better equipped to withstand environmental stresses, ultimately contributing to sustainable agricultural practices and global food security.

Acknowledgments

The authors extend heartfelt thanks to Dr. X. Fang, Director of the Hainan Institute of Tropical Agricultural Resources and an expert in soybean molecular genetics, for thoroughly reviewing the initial draft of this paper and providing comprehensive revision suggestions. The authors also express gratitude to the two anonymous peer reviewers for their valuable comments and suggestions on the manuscript.

Funding

This research is funded by Heilongjiang Province Agricultural Science and Technology Innovation Project, grant number 2020FJZX009.

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>
- Can L., 2011, QTL identification of drought tolerance to soybean in selection population, *Acta Agronomica Sinica*, 37(4): 603-611.
- Cattivelli L., Rizza F., Badeck F., Mazzucotelli E., Mastrangelo A., Francia E., Marè C., Tondelli A., and Stanca A., 2008, Drought tolerance improvement in crop plants: An integrated view from breeding to genomics, *Field Crops Research*, 105: 1-14.
<https://doi.org/10.1016/J.FCR.2007.07.004>
- Cerrudo D., Cao S., Yuan Y., Martínez C., Suarez E., Babu R., Zhang X., and Trachsel S., 2018, Genomic selection outperforms marker assisted selection for grain yield and physiological traits in a maize doubled haploid population across water treatments, *Frontiers in Plant Science*, 9: 366.
<https://doi.org/10.3389/fpls.2018.00366>
- 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>
- Dormatey R., Sun C., Ali K., Coulter J., Bi Z., and Bai J., 2020, Gene pyramiding for sustainable crop improvement against biotic and abiotic stresses, *Agronomy*, 10: 1255.
<https://doi.org/10.20944/preprints202008.0088.v1>
- Du W., Yu D., and Fu S., 2009, Detection of quantitative trait loci for yield and drought tolerance traits in soybean using a recombinant inbred line population, *Journal of Integrative Plant Biology*, 51(9): 868-878.
<https://doi.org/10.1111/j.1744-7909.2009.00855.x>
- 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>
- Eltaher S., Hashem M., Ahmed A., Baenziger P., Börner A., and Sallam A., 2023, Effectiveness of TaDreb-B1 and 1-FEH w3 KASP markers in spring and winter wheat populations for marker-assisted selection to improve drought tolerance, *International Journal of Molecular Sciences*, 24(10): 8986.
<https://doi.org/10.3390/ijms24108986>

- 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>
- Hassan M., Dahu N., Tong H., Qian Z., Yi Y., Yiru L., and Wang S., 2023, Drought stress in rice: morpho-physiological and molecular responses and marker-assisted breeding, *Frontiers in Plant Science*, 14: 1215371.
<https://doi.org/10.3389/fpls.2023.1215371>
- He J., Zhao X., Laroche A., Lu Z., Liu H., and Li Z., 2014, Genotyping-by-sequencing (GBS), an ultimate marker-assisted selection (MAS) tool to accelerate plant breeding, *Frontiers in Plant Science*, 5: 484.
<https://doi.org/10.3389/fpls.2014.00484>
- Manavalan L., Guttikonda S., Tran L., and Nguyen H., 2009, Physiological and molecular approaches to improve drought resistance in soybean, *Plant & Cell Physiology*, 50(7): 1260-1276.
<https://doi.org/10.1093/pcp/pcp082>
- Nugroho K., Kosmiatin M., Husni A., Tasma I., and Lestari P., 2020, Identification of soybean (*Glycine max* [L.] Merr.) mutants and improved varieties having diverse drought tolerance character using SSR marker, *IOP Conference Series: Earth and Environmental Science*, 482(1): 012014.
<https://doi.org/10.1088/1755-1315/482/1/012014>
- Ouyang W., Chen L., Ma J., Liu X., Chen H., Yang H., Guo W., Shan Z., Yang Z., Chen S., Zhan Y., Zhang H., Cao D., and Zhou X., 2022, Identification of quantitative trait locus and candidate genes for drought tolerance in a soybean recombinant inbred line population, *International Journal of Molecular Sciences*, 23(18): 10828.
<https://doi.org/10.3390/ijms231810828>
- Patil G., Do T., Vuong T., Valliyodan B., Lee J., Chaudhary J., Shannon J., and Nguyen H., 2016, Genomic-assisted haplotype analysis and the development of high-throughput SNP markers for salinity tolerance in soybean, *Scientific Reports*, 6(1): 19199.
<https://doi.org/10.1038/srep19199>
- Ren H., Jianan H., Wang X., Zhang B., Yu L., Gao H., Huilong H., Rujian S., Tian Y., Qi X., Liu Z., Wu X., and Qiu L., 2020, QTL mapping of drought tolerance traits in soybean with SLAF sequencing, *Crop Journal*, 8: 977-989.
<https://doi.org/10.1016/j.cj.2020.04.004>
- Ribaut J., and Ragot M., 2006, Marker-assisted selection to improve drought adaptation in maize: the backcross approach, perspectives, limitations, and alternatives, *Journal of Experimental Botany*, 58(2): 351-360.
<https://doi.org/10.1093/JXB/ERL214>
- Rosero A., Granda L., Berdugo-Cely J., Šamajová O., Šamaj J., and Cerkal R., 2020, A dual strategy of breeding for drought tolerance and introducing drought-tolerant, underutilized crops into production systems to enhance their resilience to water deficiency, *Plants*, 9(10): 1263.
<https://doi.org/10.3390/plants9101263>
- Sebastian S., Streit L., Stephens P., Thompson J., Hedges B., Fabrizio M., Soper J., Schmidt D., Kallem R., HindsMark A., Feng L., and Hoeck J., 2010, Context-specific marker-assisted selection for improved grain yield in elite soybean populations, *Crop Science*, 50: 1196-1206.
<https://doi.org/10.2135/CROPSCI2009.02.0078>
- Shaheen T., Rahman M., Riaz M., Zafar Y., and Rahman M., 2016, Soybean production and drought stress, *Abiotic and Biotic Stresses in Soybean Production*, 1: 177-196.
<https://doi.org/10.1016/B978-0-12-801536-0.00008-6>
- Singh L., Pierce C., Santantonio N., Steiner R., Miller D., Reich J., and Ray I., 2022, Validation of DNA marker-assisted selection for forage biomass productivity under deficit irrigation in alfalfa, *The Plant Genome*, 15(1): e20195.
<https://doi.org/10.1002/tpg2.20195>
- Sreenivasa V., Lal S., Babu P., Swamy H., Yadav R., Talukdar A., and Rathod D., 2020, Inheritance and mapping of drought tolerance in soybean at seedling stage using bulked segregant analysis, *Plant Genetic Resources: Characterization and Utilization*, 18: 63-70.
<https://doi.org/10.1017/S1479262120000052>
- Torres A., Ávila C., Gutiérrez N., Palomino C., Moreno M., and Cubero J., 2010, Marker-assisted selection in faba bean (*Vicia faba* L.), *Field Crops Research*, 115: 243-252.
<https://doi.org/10.1016/J.FCR.2008.12.002>
- Valliyodan B., Ye H., Song L., Murphy M., Shannon J., and Nguyen H., 2016, Genetic diversity and genomic strategies for improving drought and waterlogging tolerance in soybeans, *Journal of Experimental Botany*, 68: 1835-1849.
<https://doi.org/10.1093/jxb/erw433>
- Wang W., Zhou B., He J., Zhao J., Liu C., Chen X., Xing G., Chen S., Xing H., and Gai J., 2020, Comprehensive identification of drought tolerance QTL-allele and candidate gene systems in Chinese cultivated soybean population, *International Journal of Molecular Sciences*, 21(14): 4830.
<https://doi.org/10.3390/ijms21144830>
- Wang X., Wu Z., Zhou Q., Wang X., Song S., and Dong S., 2022, Physiological response of soybean plants to water deficit, *Frontiers in Plant Science*, 12: 809692.
<https://doi.org/10.3389/fpls.2021.809692>
- Xiong R., Liu S., Considine M., Siddique K., Lam H., and Chen Y., 2020, Root system architecture, physiological and transcriptional traits of soybean (*Glycine max* L.) in response to water deficit: a review, *Physiologia Plantarum*, 172(2): 405-418.
<https://doi.org/10.1111/ppl.13201>

Zhou X.X., and Guo T.X., 2024, Genomic tools in soybean breeding: innovations and impacts, Legume Genomics and Genetics, 15(3): 126-139.
<https://doi.org/10.5376/lgg.2024.15.0014>



Disclaimer/Publisher's Note

The statements, opinions, and data contained in all publications are solely those of the individual authors and contributors and do not represent the views of the publishing house and/or its editors. The publisher and/or its editors disclaim all responsibility for any harm or damage to persons or property that may result from the application of ideas, methods, instructions, or products discussed in the content. Publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
