Molecular Plant Breeding 2025, Vol.16, No.1, 73-81
http://genbreedpublisher.com/index.php/mpb

Case Study Open Access

Case Study on Molecular Breeding for Drought-Resistant Sweet Potato Varieties

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Molecular Plant Breeding, 2025 Vol.16, No.1 doi: 10.5376/mpb.2025.16.0008
Received: 11 Jan., 2025
Accepted: 17 Feb., 2025
Published: 25 Feb., 2025

Preferred citation for this article:
Ying X.B., Lu Y.J., Shi S.P., Zhao L., and Shi J., 2025, Case study on molecular breeding for drought-resistant sweet potato varieties, Molecular Plant Breeding, 16(1): 73-81 (doi: 10.5376/mpb.2025.16.0008)

Abstract This study clarifies the importance of drought resistance in sweet potato cultivation and systematically evaluates the application of molecular techniques, such as marker-assisted selection (MAS), genomic selection (GS), and quantitative trait loci (QTL) mapping, in enhancing drought tolerance in sweet potatoes. Several candidate genes associated with water retention, abscisic acid (ABA) signaling pathways, and key transcription factors were identified, which play a crucial role in improving drought resistance. Field trials validated that newly developed drought-resistant sweet potato varieties exhibited significantly enhanced water-use efficiency, optimized root architecture, and stable yield performance, outperforming traditional breeding methods. By focusing on molecular breeding for drought-resistant sweet potatoes, this study provides both technical support for breeding resilient varieties and essential insights for improving agricultural adaptability to climate change, thus contributing to food security.

Keywords Drought resistance; Molecular breeding; Marker-assisted selection (MAS); Genomic selection (GS); Quantitative trait loci (QTL)

1 Introduction

Sweet potato (*Ipomoea batatas* [L.] Lam.) is a crucial subsistence crop, particularly in regions like Sub-Saharan Africa, where it plays a significant role in food security (Sapakhova et al., 2023). However, drought stress poses a substantial threat to its yield and productivity, making the development of drought-resistant varieties a critical objective 3. Drought conditions can lead to severe osmotic stress, which negatively impacts the agronomic and economic productivity of sweet potato by inducing various morphological, physiological, and biochemical changes. Therefore, enhancing drought resistance in sweet potato is essential to ensure stable yields and food security under the increasingly unpredictable climate conditions predicted by global climate models (Sprenger et al., 2015).

Molecular breeding techniques have emerged as powerful tools in developing drought-resistant crops, including sweet potato. These techniques involve the identification and manipulation of specific genes associated with drought tolerance. For instance, transcriptomic analyses have identified candidate genes and differentially expressed genes (DEGs) that play crucial roles in drought response (Lau et al., 2018; Beketova et al., 2021). The overexpression of certain genes, such as IbMIPS1 and ItfWRKY70, has been shown to enhance drought tolerance by regulating various physiological and biochemical pathways (Zhai et al., 2016; Qin et al., 2019). Marker-assisted selection (MAS) and the use of transcript and metabolite markers have proven effective in predicting and selecting drought-tolerant genotypes. These molecular approaches enable the precise and efficient development of sweet potato varieties that can withstand drought stress.

This study will explore the application of molecular breeding techniques in developing drought-resistant sweet potato varieties, analyzing key genes and molecular markers associated with drought tolerance in sweet potatoes. It will assess the effectiveness of various molecular breeding strategies, including gene overexpression and marker-assisted selection, in enhancing drought resilience, as well as examine the physiological and biochemical responses of transgenic sweet potato varieties under drought conditions. The study aims to provide valuable insights into the potential of molecular breeding to improve drought tolerance in sweet potatoes, thereby contributing to sustainable agriculture and food security in drought-prone regions.

2 Background on Sweet Potato Genetics and Drought Tolerance

2.1 Genetic basis of drought tolerance in sweet potato

Sweet potato (*Ipomoea batatas* [L.] Lam.) is a vital crop for food security, particularly in regions prone to drought. The genetic basis of drought tolerance in sweet potato involves complex interactions between various genes and environmental factors. Transcriptomic studies have identified numerous differentially expressed genes (DEGs) in response to drought stress, highlighting the importance of genetic regulation in drought tolerance. For instance, a study on the sweet potato cultivars Beauregard and Tanzania revealed that between 4 000 to 6 000 genes were differentially expressed under drought conditions, with many of these genes being associated with known drought response pathways (Lau et al., 2018; You et al., 2022). The overexpression of specific genes such as *IbMIPS1* has been shown to enhance drought tolerance by regulating inositol biosynthesis and stress response pathways.

2.2 Key genes and pathways related to stress response

Several key genes and pathways have been identified as crucial for drought tolerance in sweet potato. The WRKY transcription factors, particularly ItfWRKY70, play a significant role in enhancing drought tolerance by regulating abscisic acid (ABA) biosynthesis, stress-response genes, and reactive oxygen species (ROS) scavenging systems (Alvarez-Morezuelas et al., 2023). The *IbMIPS1* gene is another critical gene that enhances drought tolerance by up-regulating genes involved in inositol biosynthesis, phosphatidylinositol (PI) signaling, and ABA signaling pathways (Zhai et al., 2016). Furthermore, transcriptomic analyses have identified genes involved in plant hormone transduction, MAPK signaling, and carbohydrate metabolism as essential for drought stress responses (Qin et al., 2022; Sun et al., 2022). These pathways collectively contribute to the plant's ability to withstand drought by modulating physiological and biochemical processes.

2.3 Natural variation and existing drought-resistant varieties

Natural variation in drought tolerance among sweet potato cultivars provides a valuable resource for breeding programs. Studies have classified sweet potato cultivars into different groups based on their drought tolerance performance. For example, a comprehensive study on seven sweet potato cultivars identified distinct drought tolerance mechanisms, with some cultivars showing minimal impact from drought stress while others exhibited significant physiological and metabolic changes. The cultivar Xuzi-8, for instance, demonstrated extreme drought tolerance by primarily regulating cell wall components, whereas other cultivars like Chaoshu-1 and Z15-1 regulated flavonoid and carbohydrate metabolism to cope with drought (Figure 1) (Liu et al., 2023). These findings underscore the importance of leveraging natural genetic variation to develop drought-resistant sweet potato varieties through molecular breeding techniques.

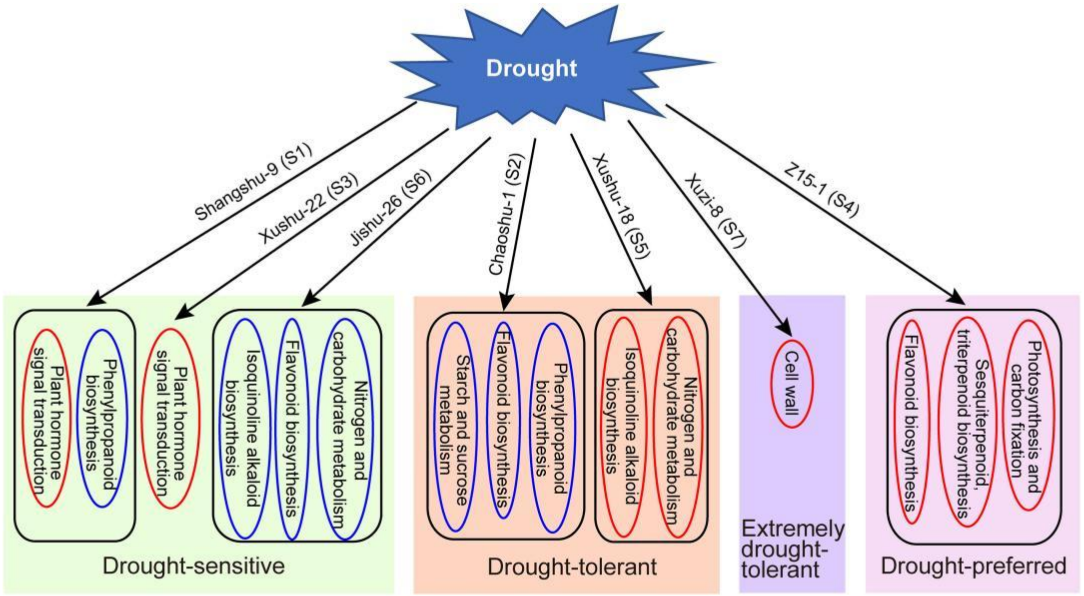

Figure 1 A corresponding working model of different drought tolerant sweet potato clutivars in response to drought. Red box indicate up-regulation, blue box indicate down-regulation (Adopted from Liu et al., 2023)

3 Methodology in Molecular Breeding for Drought Resistance

3.1 Marker-assisted selection (MAS) for drought tolerance

Marker-Assisted Selection (MAS) is a powerful tool in molecular breeding that leverages DNA markers to select plants with desirable traits, such as drought tolerance, at early stages of development. This method significantly reduces the time and resources required for breeding compared to traditional methods. MAS has been successfully applied in various crops to enhance traits like frost tolerance and disease resistance, demonstrating its potential for drought tolerance as well (Hasan et al., 2021; Tu et al., 2023; Wang and Zhang, 2024). By identifying and utilizing specific markers linked to drought tolerance, breeders can efficiently screen and select superior sweet potato varieties that are more resilient to water scarcity.

3.2 Genomic selection and quantitative trait loci (QTL) mapping

QTL mapping has been instrumental in identifying genetic loci associated with drought tolerance in various crops. For sweet potato, QTL-seq, a modified bulked segregant analysis using next-generation sequencing, has been employed to pinpoint clusters of SNPs linked to important traits (Yamakawa et al., 2021). This method allows for the rapid development of tightly linked DNA markers, facilitating the selection of drought-tolerant varieties. The identification of stable QTLs across different environments and genetic backgrounds is crucial for the success of breeding programs aimed at improving drought resilience (Selamat and Nadarajah, 2021; Wang et al., 2024).

Genomic selection (GS) involves predicting the genetic value of plants using genome-wide markers, which is particularly useful for complex traits like drought tolerance that are controlled by multiple genes. High-throughput genotyping (HTG) and phenotyping (HTP) platforms enhance the accuracy and efficiency of GS by providing comprehensive data on genetic and phenotypic variations (Bhat et al., 2020). These tools enable breeders to estimate genomic estimated breeding values (GEBVs) with high precision, accelerating the development of drought-tolerant sweet potato varieties (Halladakeri et al., 2023).

3.3 Genetic transformation and CRISPR applications

Genetic transformation and CRISPR/Cas9 technology offer innovative approaches to introduce or modify genes associated with drought tolerance in sweet potato. CRISPR/Cas9, a precise genome-editing tool, allows for targeted modifications in the plant genome, enabling the introduction of drought-resistance genes or the knockout of genes that negatively affect drought tolerance (Rosero et al., 2020; Raj and Nadarajah, 2022). This technology, combined with traditional breeding methods, can significantly enhance the development of drought-resistant sweet potato varieties by directly manipulating specific genetic pathways involved in drought response.

4 Case Study Overview: Breeding Drought-Resistant Sweet Potato

4.1 Selection of parental lines and breeding strategy

The selection of parental lines is a critical step in breeding drought-resistant sweet potato varieties. Parental lines are chosen based on their demonstrated drought tolerance and high yield potential. For instance, the study by Sapakhova et al. (2023) highlights the importance of selecting genotypes that exhibit physiological and biochemical traits conducive to drought resistance, such as antioxidant activity and stress protein production. Additionally, transcriptomic analyses, as discussed in Lau et al. (2018), can identify candidate genes associated with drought tolerance, which can be used to select and crossbreed suitable parental lines.

4.2 Development and testing of new varieties

Once the parental lines are selected, the breeding strategy involves crossing these lines and developing new varieties through traditional and molecular breeding methods. The use of marker-assisted selection (MAS) is particularly effective in this phase, as it allows for the identification of drought-tolerant traits at the genetic level, thereby accelerating the breeding process (Sprenger et al., 2015; 2017). The development of new varieties is followed by rigorous testing under controlled drought conditions to evaluate their performance. For example, Lau et al. (2018) utilized polyethylene glycol (PEG) to simulate drought conditions and assess the differential gene expression in sweet potato cultivars, providing insights into the genetic mechanisms underlying drought tolerance (Figure 2).

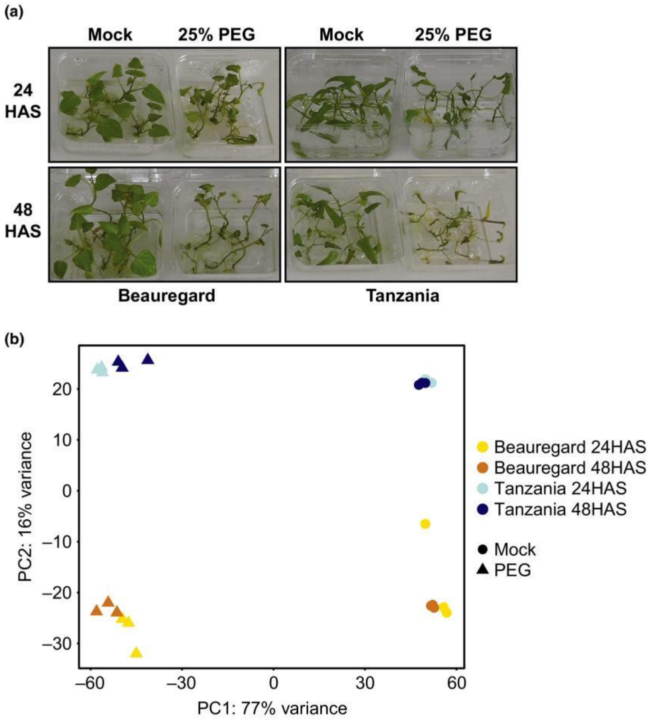

Figure 2 Phenotypic and genetic response of Beauregard and Tanzania sweet potato varieties to the application of polyethylene glycol (PEG) (Adopted from Lau et al., 2018)

Image caption: (a) Plantlets incubated in untreated media or 25% PEG media at 24 and 48 hr after stress (HAS). (b) Principal component analysis of individual biological replicates using variance stabilizing transformed read counts. Each point represents a biological replicate (Adopted from Lau et al., 2018)

4.3 Field trials and phenotypic assessments

Field trials are essential for validating the drought tolerance of newly developed sweet potato varieties under real-world conditions. These trials involve growing the new varieties in drought-prone areas and monitoring their phenotypic responses, such as yield, tuber quality, and physiological traits. The study by Qin et al. (2019) demonstrated the effectiveness of greenhouse methods to screen potato genotypes for drought tolerance, which can be adapted for sweet potatoes. Additionally, phenotypic assessments, including measurements of gas exchange, chlorophyll content, and specific leaf area, are conducted to correlate these traits with drought tolerance. The integration of high-throughput phenotyping platforms, as mentioned in Obidiegwu et al. (2015), can further enhance the precision and efficiency of these assessments.

5 Key Genes and Pathways Identified for Drought Resistance

5.1 Identification of candidate genes related to water retention

The identification of candidate genes related to water retention is crucial for developing drought-resistant sweet potato varieties. One such gene is IbMIPS1, which encodes myo-inositol-1-phosphate synthase. This gene has been shown to enhance drought tolerance by up-regulating genes involved in inositol biosynthesis, phosphatidylinositol (PI), and abscisic acid (ABA) signaling pathways. The overexpression of IbMIPS1 in transgenic sweet potato plants significantly increased the content of inositol, inositol-1,4,5-trisphosphate (IP3), phosphatidic acid (PA), Ca^{2+} , ABA, K^{+} , proline, and trehalose, which are critical for water retention and stress response (Zhai et al., 2016).

5.2 Role of abscisic acid (ABA) pathways in drought response

ABA plays a pivotal role in the drought response of sweet potato by mediating various physiological and molecular processes. The IbPYL8-IbbHLH66-IbbHLH118 complex is a key component in the ABA-dependent drought response. Under drought stress, ABA accumulates and promotes the formation of this complex, which interferes with the repression of ABA-responsive genes by IbbHLH118, thereby activating ABA responses and enhancing drought tolerance. The ItfWRKY70 transcription factor, induced by ABA, enhances drought tolerance by increasing ABA and proline content, and activating the ROS scavenging system. The suppression of StHAB1, a negative regulator of ABA signaling, also enhances drought tolerance by increasing ABA sensitivity (Liu et al., 2023). These findings underscore the importance of ABA pathways in modulating drought responses in sweet potato.

5.3 Transcription factors and their influence on drought tolerance

Transcription factors (TFs) are critical regulators of gene expression in response to drought stress. The WRKY family, particularly ItfWRKY70, has been shown to play a significant role in enhancing drought tolerance by regulating ABA biosynthesis and stress-response genes (Figure 3) (Sun et al., 2022). Another important group of TFs is the MYB family, including MYB33, MYB65, and MYB101, which regulate the ABA signaling pathway. Overexpression of these MYB TFs in transgenic plants leads to increased ABA sensitivity and improved drought tolerance (Wyrzykowska et al., 2021). Furthermore, the bHLH transcription factors, IbbHLH66 and IbbHLH118, have opposing roles in the ABA-mediated drought response, with IbbHLH66 enhancing and IbbHLH118 reducing drought tolerance (Xue et al., 2022). These TFs are crucial for fine-tuning the plant's response to drought stress, making them valuable targets for molecular breeding programs aimed at improving drought resistance in sweet potato.

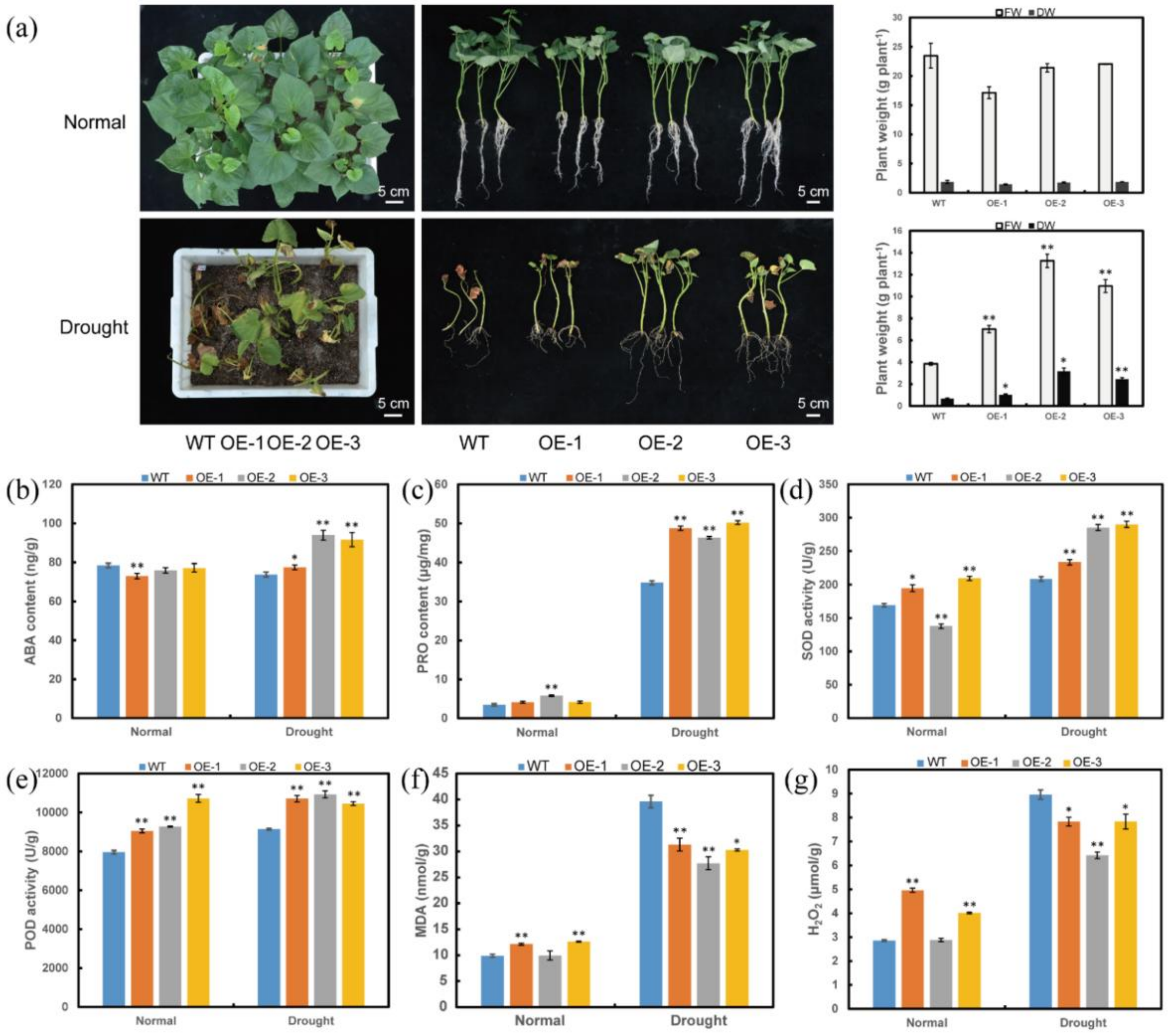

Figure 3 Responses of transgenic and WT sweet potato plants grown in a transplanting box with no stress (normal) and drought stress (Adopted from Sun et al., 2022))

Image caption: (a) Phenotypes, FW and DW, (b) ABA content, (c) Proline content, (d) SOD activity, (e) POD activity, (f) MDA content, (g) H_2O_2 content. The phenotypes are shown after drought treatment for 6 weeks. FW, fresh weight; DW, dry weight. Data are presented as the means ± SE (*n*=3) (Adopted from Sun et al., 2022)

6 Impact of Molecular Breeding on Crop Performance

6.1 Improved yield and water use efficiency in new varieties

Molecular breeding has significantly enhanced the yield and water use efficiency (WUE) of sweet potato varieties under drought conditions. By employing advanced genetic tools, researchers have been able to identify and incorporate drought-tolerant traits into new cultivars. For instance, the study of physiological and biochemical features has been crucial in selecting genotypes that maintain high yields despite water stress (Lee et al., 2015). The use of next-generation sequencing (NGS) and genome-wide association studies (GWAS) has facilitated the identification of key genetic markers associated with drought tolerance, thereby accelerating the breeding process. These molecular techniques have led to the development of sweet potato varieties that exhibit improved WUE, which is essential for maintaining productivity in arid and semi-arid regions (Gervais et al., 2021).

6.2 Enhanced root architecture for drought adaptation

One of the critical factors in drought resistance is the root architecture of the plant. Molecular breeding has enabled the modification of root traits to enhance water uptake and retention. For example, the expression of the *DEEPER ROOTING 1* (*DRO1*) gene in sweet potato has been shown to alter root architecture, resulting in increased lateral root number and improved drought tolerance (Sun et al., 2022). Comparative transcriptome analysis has also revealed that deep-rooting genotypes exhibit a more robust response to drought stress, with significant changes in gene expression related to root cell elongation and division. These modifications in root architecture not only improve the plant's ability to access water from deeper soil layers but also contribute to overall drought resilience (Ponce et al., 2022).

6.3 Comparison with conventional breeding methods

Molecular breeding offers several advantages over conventional breeding methods, particularly in the context of developing drought-resistant sweet potato varieties. Traditional breeding relies on phenotypic selection, which can be time-consuming and less precise. In contrast, molecular breeding utilizes genetic markers and advanced biotechnological tools to identify and incorporate desirable traits more efficiently (Sapakhova et al., 2023). Studies have shown that molecular breeding can significantly reduce the time required to develop new cultivars while also increasing the accuracy of trait selection (Saidi and Hajibarat, 2020). Moreover, the integration of high-throughput phenotyping platforms with molecular techniques allows for the precise screening and pyramiding of drought-related genes, which is less feasible with conventional methods (Obidiegwu et al., 2015). Molecular breeding represents a more effective and efficient approach to developing drought-resistant sweet potato varieties, ensuring better crop performance under challenging environmental conditions.

7 Breeding Strategies for Drought-Resistant Varieties

7.1 Crossbreeding and hybridization strategies

Crossbreeding and hybridization are fundamental strategies in developing drought-resistant sweet potato varieties. These methods involve the selection and crossing of parent plants with desirable traits to produce offspring that exhibit improved drought tolerance and high yield potential. The study of physiological and biochemical features of certain sweet potato varieties is crucial for implementing effective drought resistance measures (Aslam et al., 2022). By understanding the genetic basis of drought tolerance, breeders can select and improve genotypes adapted to specific growing conditions, making the creation of drought-resistant varieties more cost-effective for smallholder farmers (Sapakhova et al., 2023).

7.2 Backcrossing with elite varieties

Backcrossing is a breeding method where a hybrid organism is crossed with one of its parents or an organism genetically similar to its parent. This technique is used to introduce or maintain specific desirable traits, such as drought resistance, in elite varieties. For instance, marker-assisted backcross breeding has been successfully applied in rice to improve multiple biotic and abiotic stress tolerances, including drought, by introgressing specific genes and QTLs into elite varieties (Moon et al., 2018; Ramayya et al., 2021). This approach can be adapted for sweet potato breeding to enhance drought resistance while maintaining high yield and other agronomically important traits.

7.3 Use of molecular markers in field trials

The use of molecular markers in field trials is a powerful tool for accelerating the breeding process of drought-resistant varieties. Marker-assisted selection (MAS) allows for the identification and selection of plants with desired traits based on their genetic markers rather than solely on phenotypic traits. This method is cheaper, faster, and reduces classification errors caused by non-controlled environmental effects 1. For example, in potato breeding, metabolite and transcript markers have been used to predict drought tolerance with high accuracy, significantly reducing the prediction error when combined (Sprenger et al., 2017). Similarly, next-generation sequencing (NGS) technologies, such as GWAS and RNA-seq, have been employed to identify markers associated with drought tolerance, facilitating the genetic improvement of crops under drought stress conditions (Saidi and Hajibarat, 2020). These molecular tools can be applied to sweet potato breeding programs to enhance the efficiency and accuracy of selecting drought-resistant varieties.

8 Concluding Remarks

This study has provided significant insights into the molecular breeding of drought-resistant sweet potato varieties. Key findings include the identification of physiological, biochemical, and genetic traits that contribute to drought tolerance in sweet potatoes. Various studies have highlighted the importance of osmotic stress responses, including the activation of antioxidants, accumulation of suitable solutes, and stress proteins, which are critical for selecting drought-tolerant genotypes. Transcriptomic analyses have revealed differentially expressed genes and alternative splicing events that play crucial roles in drought response mechanisms. The study has identified specific genes and pathways, such as those involved in flavonoid and carbohydrate metabolism, that are pivotal for drought tolerance. These findings contribute to a deeper understanding of the genetic basis of drought tolerance and provide valuable resources for breeding programs aimed at developing drought-resistant sweet potato varieties.

By leveraging the identified genetic markers and physiological traits, breeders can more effectively select and develop drought-resistant varieties. This is particularly important for regions prone to drought, where sweet potatoes are a staple food crop. The development of drought-resistant varieties can significantly enhance food security by ensuring stable yields under adverse climatic conditions. Moreover, the integration of molecular breeding techniques, such as marker-assisted selection and genomic selection, can accelerate the breeding process, making it more efficient and cost-effective. This approach not only improves the resilience of sweet potato crops but also supports the livelihoods of smallholder farmers in drought-affected regions.

Advancing molecular breeding for climate resilience in sweet potatoes requires a multifaceted approach. Future research should focus on expanding the genetic diversity of breeding programs by incorporating wild relatives and landraces that possess natural drought tolerance traits. The use of advanced genomic tools, such as CRISPR technology, can further enhance the precision and efficiency of breeding efforts. It is essential to develop comprehensive phenotyping platforms to accurately assess drought tolerance traits in diverse environmental conditions. Collaborative efforts between researchers, breeders, and policymakers are crucial to ensure the successful implementation of these strategies. Through continuous innovation and application of molecular breeding techniques, sweet potato varieties that are not only drought tolerant but also high-yield and nutrient rich can be developed, thereby contributing to global food security and sustainable agriculture.

Acknowledgments

Thanks to the reviewers for their valuable feedback, which helped improve the manuscript.

Funding

This study was funded by Lin’an Tianmu Xiaoxiang Potato New Variety Research and Transformation Project, Hangzhou Science and Technology Commissioner Special Project-Variety Comparison Test and Demonstration Promotion of Fresh Sweet Potato New Varieties.

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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