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Advances in Molecular Breeding Techniques for Pitaya (*Hylocereus*)

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Abstract Dragon fruit (*Hylocereus* spp.) has attracted much attention due to its nutritional value and economic benefits. The advancement of molecular breeding technology has promoted the genetic improvement of dragon fruit. This study reviews the applications of chromosome level genome sequencing, transcriptomics, and functional genomics in revealing the evolutionary history and key metabolic pathways of dragon fruit, such as beetroot biosynthesis. Marker assisted selection (MAS) and quantitative trait locus (QTL) mapping have been used to identify genes related to fruit quality, stress resistance, and yield, while gene editing techniques such as CRISPR Cas systems provide new possibilities for precision breeding. Meanwhile, epigenomic research has revealed the response mechanism of dragon fruit under abiotic stress, laying the foundation for cultivating varieties with stronger stress resistance. Case studies have shown that the combination of traditional breeding and molecular technology has improved the disease resistance, fruit quality, and breeding efficiency of dragon fruit. This study emphasizes that utilizing genetic diversity, expanding genomic resources, and applying modern biotechnology are of great significance for improving the cultivation level of dragon fruit, responding to environmental changes, and meeting market demand. Through sustainable breeding strategies, the global competitiveness and environmental adaptability of dragon fruit cultivation can be further improved.

Keywords Pitaya; Molecular breeding; Genomics; Gene editing; QTL mapping

1 Introduction

Pitaya, commonly known as dragon fruit, belongs to the *Hylocereus* genus and is a member of the cactus family. It is native to Central and South America but has gained global popularity due to its nutritional, ornamental, and economic value (Shah et al., 2023). The fruit is particularly valued for its betalain-rich content, which contributes to its vibrant color and potential health benefits (Chen et al., 2021). Major producers of pitaya include countries like Colombia and Mexico, where the fruit is cultivated on diverse soil types, including gravel and rocky terrains, due to its rusticity (Junqueira et al., 2010). The increasing demand for pitaya, driven by its high consumption and industrial applications, underscores the need for advanced breeding techniques to enhance its genetic potential and adaptability (Shah et al., 2023).

Despite its growing popularity, pitaya breeding faces several challenges. One significant issue is the lack of a comprehensive reference genome, which hampers genetic improvement efforts (Chen et al., 2021). Additionally, there is a need for varieties that meet specific climatic and consumer demands, particularly in regions like Brazil, where no suitable varieties have been released yet (Junqueira et al., 2010). The genetic variability within pitaya species, as revealed by molecular markers, presents both a challenge and an opportunity for breeding programs (Junqueira et al., 2010; Nashima et al., 2021). Furthermore, technical challenges such as DNA isolation, chromosome doubling, and embryo rescue require sophisticated protocols to overcome (Tel-Zur, 2022).

This study focuses on the cutting-edge progress of molecular breeding technology for dragon fruit, with a particular emphasis on the innovation and application of genomic tools and biotechnology methods. It attempts to clarify the focus and development path of future exploration, and also evaluates the role of molecular markers, genetic mapping, and genome sequencing technologies in enhancing the genetic diversity of dragon fruit and optimizing the breeding process. The aim is to provide profound insights and assist researchers and breeding experts in promoting the sustainable development and variety improvement of dragon fruit cultivation.

2 Genomic Resources for Pitaya

2.1 Genome sequencing projects

Recent advancements in genome sequencing have significantly contributed to the understanding of pitaya (*Hylocereus* spp.) genetics. A notable project involved the assembly of a chromosome-level genome for *Hylocereus* undatus using various sequencing technologies, including PacBio-SMRT and Illumina HiSeq, which provided a comprehensive genomic resource for studying genome evolution and betalain biosynthesis (Chen et al., 2021). This genome assembly revealed a whole-genome triplication and a recent whole-genome duplication, offering insights into the evolutionary history of pitaya (Chen et al., 2021). Additionally, a high-density genetic map was constructed using whole genome resequencing, which aids in quantitative trait mapping and marker-assisted selection (Wu et al., 2021).

2.2 Transcriptomics and gene expression studies

Transcriptomic analyses have been pivotal in understanding gene expression during pitaya fruit development and stress responses. For instance, RNA sequencing has been employed to identify differentially expressed genes during the ripening of red pitaya (*Hylocereus polyrhizus*), providing a basis for selecting reference genes for qRT-PCR studies (Zheng et al., 2020). Moreover, transcriptomic data have facilitated the construction of gene regulatory networks involved in betalain biosynthesis, enhancing our understanding of the molecular mechanisms underlying this process (Chen et al., 2021; Zhao et al., 2023).

2.3 Functional genomics approaches

Functional genomics approaches have been utilized to explore gene functions in pitaya, particularly in stress tolerance. The HuERF1 gene, an ethylene response factor from *Hylocereus undatus*, was identified and shown to enhance salt tolerance when overexpressed in Arabidopsis, indicating its role in abiotic stress regulation (Qu et al., 2020). Additionally, the development of SSR markers has enabled the assessment of genetic diversity and identification of pitaya accessions, which is crucial for breeding programs (Nashima et al., 2021). These functional genomics tools are essential for advancing molecular breeding and improving pitaya cultivars.

3 Marker-Assisted Breeding in Pitaya

3.1 Development of molecular markers

The development of molecular markers is a crucial step in marker-assisted breeding, providing the tools necessary for genetic analysis and selection. In pitaya (*Hylocereus* spp.), various types of molecular markers have been developed to assess genetic diversity and assist in breeding programs. For instance, Random Amplified Polymorphic DNA (RAPD) markers have been used to study genetic variability among pitaya accessions, revealing significant genetic diversity even within the same species (Junqueira et al., 2010). Additionally, simple sequence repeat (SSR) markers have been developed using next-generation sequencing technologies, which have proven effective in distinguishing genetic differences among pitaya accessions and assessing genetic diversity parameters such as heterozygosity and polymorphic information content (Nashima et al., 2021). These markers are essential for identifying and selecting desirable traits in breeding programs.

3.2 QTL mapping for key traits

Quantitative Trait Loci (QTL) mapping is a powerful tool for identifying the genetic basis of important traits in plants. In pitaya, the construction of a high-density genetic map using whole genome resequencing has facilitated QTL mapping for key traits. This map, which includes 6 434 single nucleotide polymorphism (SNP) markers, provides a comprehensive framework for locating QTLs associated with economically important traits in pitaya (Wu et al., 2021). The availability of such detailed genetic maps allows for the precise identification of QTLs, which can be targeted in breeding programs to improve traits such as fruit quality, yield, and stress tolerance.

3.3 Application in breeding programs

The application of marker-assisted selection (MAS) in pitaya breeding programs has significantly enhanced the efficiency and accuracy of developing new varieties. By utilizing molecular markers linked to desirable traits, breeders can select plants with the best genetic potential early in the breeding process, thus reducing the time and resources required for traditional breeding methods (Thomson et al., 2009; Hasan et al., 2021). For example, the use of SSR markers has facilitated the genetic identification and selection of pitaya accessions with superior traits, aiding in the development of improved cultivars (Nashima et al., 2021). Furthermore, the integration of QTL mapping and MAS allows for the targeted improvement of specific traits, such as fruit size, color, and stress resistance, ultimately leading to the production of high-quality pitaya varieties that meet market demands (Chen et al., 2021; Wu et al., 2021).

4 Genetic Transformation Techniques

4.1 Overview of transformation methods

Genetic transformation techniques are pivotal in enhancing the genetic makeup of pitaya (*Hylocereus* spp.), facilitating the introduction of desirable traits such as improved stress tolerance and enhanced nutritional content. Various biotechnological tools have been employed, including cell and tissue culture, micropropagation, and molecular marker technology, which have been instrumental in the development of pitaya germplasm (Shah et al., 2023). These methods enable the manipulation of genetic material to produce new varieties with improved characteristics, thereby supporting the breeding programs aimed at optimizing pitaya's horticultural potential (Xi et al., 2019; Tel-Zur, 2022).

4.2 CRISPR-Cas systems

The CRISPR-Cas system represents a revolutionary tool in genetic engineering, offering precise genome editing capabilities. Although specific applications of CRISPR-Cas in pitaya are not extensively documented in the current literature, the system's potential for targeted gene modification could significantly advance pitaya breeding. This technology could be used to enhance traits such as betalain biosynthesis, which is crucial for the fruit's coloration and nutritional value (Figure 1) (Zhang et al., 2021). The development of a chromosome-scale genome sequence for pitaya provides a foundational resource that could facilitate the application of CRISPR-Cas systems in future breeding efforts (Chen et al., 2021).

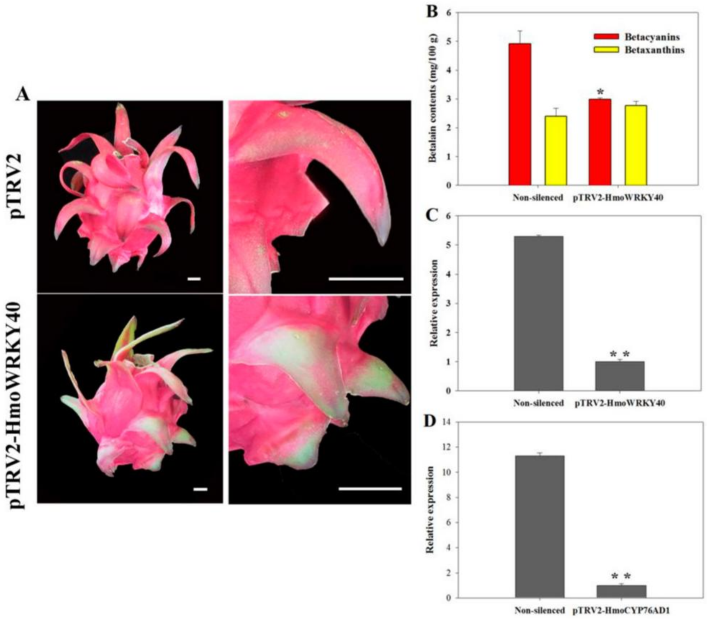

Figure 1 Silencing of HmoWRKY40 inhibits betalain production: (A) Virus-induced gene silencing of HmoWRKY40 in red scales. Bars = 2 cm, (B) Betalain contents in pitaya scales after virus-induced silencing of HmoWRKY40 (* indicates $p < 0.05$). Three independent experiments were conducted (n= 3). The error bars indicate one standard error, (C) RT-qPCR analyses of HmoWRKY40 in virus-induced gene silencing (VIGS) treatment scales. The expression level of pTRV2-HmoWRKY40 was used as the calibrator (set as 1). The data represent mean values from three biological replicates (±S.D.). ** indicates significant differences at p value < 0.01 using a two-tailed t-test and (D) RT-qPCR analyses of HmoCYP76AD1 in VIGS treatment scales. The expression level of pTRV2-HmoCYP76AD1 was used as the calibrator (set as 1).The data represent mean values from three biological replicates (±S.D.). ** indicates significant differences at p value < 0.01 using a two-tailed *t*-test (Adopted from Zhang et al., 2021)

4.3 Stable vs. transient transformation

In the context of genetic transformation, stable and transient transformations are two distinct approaches. Stable transformation involves the integration of foreign DNA into the host genome, resulting in heritable genetic changes. This method is crucial for developing new pitaya varieties with permanent trait enhancements. On the other hand, transient transformation allows for temporary expression of introduced genes without integration into the genome, which is useful for functional studies and rapid trait assessment (Figure 2) (Shah et al., 2023). The choice between stable and transient transformation depends on the specific objectives of the breeding program and the desired outcomes in terms of trait stability and expression.

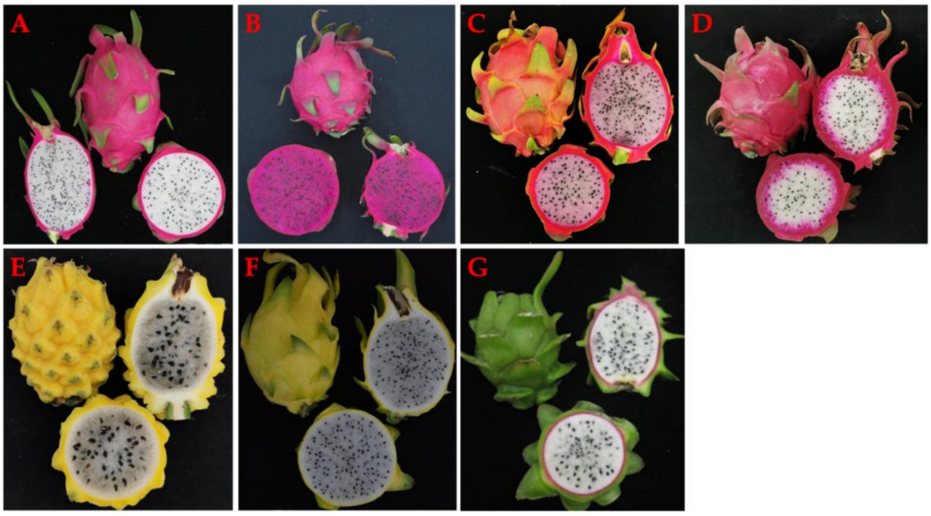

Figure 2 Fruit peel, pulp, and scale and areoles phenotype found in different *Hylocereus* species. (A) *H. undatus*; (B) *H. polyrhizus*; (C, D) hybridization offspring of *H. polyrhizus* and *H. undatus*; (E) *H. megalanthus*; (F) Golden pitaya (yellow clone of *H. undatus*), (G) *H. stenopterus* (Adopted from Shah et al., 2023)

5 Genomic Selection and Prediction

5.1 Principles of genomic selection

Genomic selection (GS) is a modern breeding approach that utilizes genome-wide markers to predict the genetic value of individuals in a breeding population. This method leverages the entire genome to make predictions about complex traits, which is particularly useful for crops like pitaya where traditional phenotypic selection can be challenging due to long generation times and complex trait inheritance (Chen et al., 2021; Tel-Zur, 2022). The principle of GS is to use a training population with known phenotypes and genotypes to develop a prediction model, which can then be applied to a selection population to predict their genetic potential without phenotypic evaluation (Figure 3) (Zheng et al., 2020; Hua et al., 2021; Wu et al., 2021; Xu and Wang, 2024).

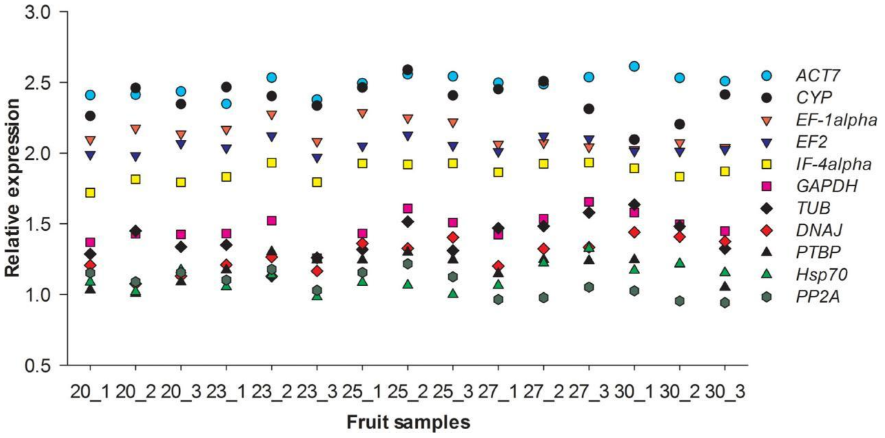

Figure 3 Relative expression patterns of 11 candidate reference genes across fruit samples of red pitaya, based on the RNA-seq data. The relative expression level of each candidate was represented by the $\log_{10}$ (Adopted from Zheng et al., 2020)

5.2 Prediction models and accuracy

Prediction models in genomic selection are crucial for determining the accuracy and effectiveness of the selection process. Common models include Best Linear Unbiased Prediction (BLUP) and Bayesian approaches, which are used to estimate the effects of markers across the genome (Chen et al., 2021; Wu et al., 2021). The accuracy of these models depends on several factors, including the size of the training population, the heritability of the trait, and the density of the markers used. In pitaya, the construction of high-density genetic maps using whole genome resequencing has enhanced the precision of these models, allowing for more accurate predictions of traits such as fruit quality and yield (Figure 3) (Zheng et al., 2020; Wu et al., 2021).

5.3 Implementation in pitaya breeding

The implementation of genomic selection in pitaya breeding programs is facilitated by recent advancements in genomic resources, such as the development of a chromosome-scale genome sequence and high-density genetic maps (Chen et al., 2021; Wu et al., 2021). These resources provide a comprehensive framework for identifying and utilizing genetic markers associated with desirable traits. The integration of genomic selection into pitaya breeding can accelerate the development of new cultivars with improved traits, such as disease resistance and enhanced nutritional content, by enabling breeders to make more informed decisions based on genetic predictions rather than solely on phenotypic observations (Chen et al., 2021; Wu et al., 2021; Tel-Zur, 2022).

6 Epigenomics and Stress Tolerance

6.1 Role of epigenetics in pitaya development

Epigenetics plays a crucial role in the development and growth of pitaya (*Hylocereus* spp.), influencing various physiological and morphological traits. The application of molecular tools, such as DNA isolation and flow cytometry, has facilitated the understanding of genetic relationships and dominant/recessive traits in pitaya, which are essential for breeding programs (Zerpa-Catanho et al., 2019; Tel-Zur, 2022). These tools help in identifying epigenetic modifications that can affect gene expression without altering the DNA sequence, thereby contributing to the plant’s adaptability and development.

6.2 Epigenomics of abiotic stress

The study of epigenomics in pitaya has provided insights into how this plant responds to abiotic stresses, such as cold temperatures. The identification of NAC transcription factors, particularly HuNAC20 and HuNAC25, has shown that these genes are highly induced under cold stress conditions. These transcription factors play a significant role in enhancing cold tolerance by regulating stress-responsive genes, thereby reducing ion leakage and oxidative damage (Hu et al., 2022). This epigenomic regulation is crucial for pitaya's survival and productivity in varying environmental conditions.

6.3 Applications in breeding programs

The integration of epigenomic insights into breeding programs can significantly enhance the development of pitaya cultivars with improved stress tolerance and desirable traits. The availability of a chromosome-scale genome sequence of pitaya provides a valuable resource for molecular breeding, allowing for the identification and manipulation of genes involved in stress responses and other important traits (Chen et al., 2021). By leveraging epigenetic modifications, breeders can develop new pitaya varieties that are better equipped to withstand environmental stresses, thereby improving yield and quality. Additionally, the use of biotechnological tools such as somatic embryogenesis and molecular marker technology further supports the enhancement of pitaya germplasm (Shah et al., 2023; Khokhar et al., 2024).

7 Case Study Sharing

7.1 Molecular breeding for disease resistance

Molecular breeding techniques have been pivotal in enhancing disease resistance in pitaya (*Hylocereus* spp.). The development of a high-density genetic map using whole genome resequencing has facilitated the identification of single nucleotide polymorphisms (SNPs) that are crucial for marker-assisted selection, which can be used to breed disease-resistant varieties (Wu et al., 2021). Additionally, the use of RAPD markers has revealed significant genetic variability among pitaya accessions, which is essential for selecting disease-resistant traits (Junqueira et al., 2010). These molecular tools enable breeders to efficiently select and propagate pitaya varieties with enhanced resistance to diseases, thereby improving crop resilience and yield.

7.2 Enhancing fruit quality traits

Advancements in molecular breeding have also focused on improving fruit quality traits in pitaya. The chromosome-scale genome sequence of *Hylocereus* undatus has provided insights into the regulation of betalain biosynthesis, a key determinant of fruit color and nutritional value (Chen et al., 2021). This genomic information, combined with transcriptomic analyses, has identified gene networks involved in fruit quality traits, allowing for targeted breeding strategies to enhance these characteristics. Furthermore, the development of SSR markers has facilitated the genetic identification and selection of pitaya accessions with desirable fruit quality traits, such as size, taste, and nutritional content (Nashima et al., 2021; Demi̇rkaplan and Gübbük, 2023).

7.3 Integrating techniques for comprehensive improvement

Integrating various molecular breeding techniques has led to comprehensive improvements in pitaya cultivation. The combination of interspecific and interploid crosses, along with embryo rescue procedures, has resulted in the creation of improved hybrids with enhanced horticultural traits (Tel-Zur, 2022). These hybrids have been further refined using molecular tools such as SSR and RAPD markers to ensure the selection of superior genotypes (Junqueira et al., 2010; Nashima et al., 2021). Additionally, the construction of high-density genetic maps and the availability of a draft genome have provided a robust framework for ongoing breeding programs aimed at improving both disease resistance and fruit quality in pitaya (Chen et al., 2021; Wu et al., 2021). This integrated approach ensures that breeding efforts are both efficient and effective, leading to the development of pitaya varieties that meet both agricultural and consumer demands.

8 Future Directions

8.1 Emerging technologies

The future of pitaya breeding is poised to benefit significantly from emerging technologies in genomics and biotechnology. The development of a chromosome-scale genome sequence for *Hylocereus* undatus has provided a comprehensive resource for understanding the genetic basis of important traits, such as betalain biosynthesis, which can be leveraged for molecular breeding programs (Chen et al., 2021). Additionally, the use of high-density genetic maps constructed through whole genome resequencing offers a powerful tool for quantitative trait mapping and marker-assisted selection, facilitating the identification of desirable traits for breeding (Wu et al., 2021). The application of biotechnological tools, including cell and tissue culture, micropropagation, and molecular marker technology, has already shown promise in enhancing pitaya germplasm and will continue to play a crucial role in future breeding efforts (Shah et al., 2023).

8.2 Collaborative efforts

Collaborative efforts among researchers, breeders, and institutions are essential to advance pitaya breeding. The integration of genetic resources from diverse geographical regions, such as those collected in Okinawa, Japan, can enhance the genetic diversity available for breeding programs (Nashima et al., 2021). International collaborations can also facilitate the sharing of genomic data and breeding techniques, accelerating the development of improved pitaya varieties. Programs like Embrapa Cerrados' pitaya selection and improvement initiative demonstrate the potential of coordinated efforts to address specific regional needs and consumer preferences (Junqueira et al., 2010).

8.3 Vision for sustainable pitaya breeding

Sustainable pitaya breeding aims to develop varieties that are not only high-yielding and nutritionally superior but also resilient to environmental stresses. The use of molecular tools to understand genetic relationships and dominant/recessive traits can guide the creation of hybrids with enhanced horticultural potential (Tel-Zur, 2022). Moreover, the exploration of genetic variability within pitaya species using techniques like RAPD markers can identify plants with desirable production traits, contributing to the sustainability of breeding programs (Junqueira et al., 2010). By focusing on both genetic improvement and environmental adaptability, future breeding efforts can ensure the long-term viability and success of pitaya cultivation worldwide.

9 Concluding Remarks

Recent advancements in molecular breeding techniques for pitaya (*Hylocereus* spp.) have significantly enhanced our understanding and ability to improve this economically important fruit crop. Key advances include the development of interspecific and interploid hybrids through techniques such as embryo rescue and chromosome doubling, which have led to improved hybrids with desirable traits. The construction of a high-density genetic map using whole genome resequencing has provided a robust framework for quantitative trait mapping and marker-assisted selection, facilitating the genetic improvement of pitaya. Additionally, the sequencing of a chromosome-scale genome of *Hylocereus* undatus has offered novel insights into genome evolution and the regulation of betalain biosynthesis, which is crucial for the fruit's coloration and nutritional value. The use of molecular markers, such as RAPD and SSR, has further enabled the assessment of genetic variability and diversity, aiding in the selection and breeding of superior pitaya varieties.

Case studies have highlighted the importance of integrating traditional breeding methods with modern molecular tools to achieve significant improvements in pitaya breeding programs. The successful development of intergeneric hybrids between *Hylocereus* and Selenicereus species demonstrates the potential of leveraging genetic diversity from related species to enhance desirable traits. The application of biotechnological tools, such as micropropagation and molecular marker technology, has proven essential in conserving and utilizing genetic resources effectively. Moreover, the challenges encountered in developing protocols for DNA isolation and chromosome doubling underscore the need for continued innovation and refinement of molecular techniques to overcome technical barriers in breeding programs.

The advances in molecular breeding techniques for pitaya underscore the potential for significant improvements in fruit quality, yield, and adaptability. However, to fully realize this potential, there is a need for continued research and collaboration among scientists, breeders, and industry stakeholders. Future efforts should focus on expanding genomic resources, refining breeding techniques, and exploring the genetic basis of key traits to accelerate the development of superior pitaya cultivars. A concerted effort to integrate these advances into practical breeding programs will ensure the sustainable growth and competitiveness of the pitaya industry in global markets.

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Conflict of Interest Disclosure

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

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