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Breeding *Triticale* **for Stress-Prone Environments: Genetic Insights and Methodologies**

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Abstract *Triticale*, a hybrid cereal combining the qualities of wheat and rye, has become a key focus in crop breeding for stress-prone environments. This paper discusses recent advancements in *Triticale* breeding methodologies and improving stress tolerance, with a focus on the application of molecular genetics, biotechnology, and hybrid breeding techniques.Key topics covered include genomic tools such as genome-wide association studies (GWAS), marker-assisted selection (MAS), and the use of CRISPR/Cas9 gene editing to enhance resilience againstabiotic and biotic stresses. The integration of high-throughput phenotyping and multi-omics approaches has provided deeper insights into the physiological and molecular responses of *Triticale* to environmental challenges. Additionally, strategies to overcome genetic bottlenecks, balance trade-offs between yield, quality, and stress tolerance, and develop sustainable, low-input cropping systems are examined. The paper emphasizes the role of *Triticale* in ensuring food security in the context of climate change and highlights future directions for research and innovation in breeding programs to meet the demands of increasingly challenging agricultural environments.

Keywords *Triticale* breeding; Stress tolerance; CRISPR/Cas9 gene editing; Abiotic stress resilience; Hybrid breeding techniques

1 Introduction

The impact of global climate change and the resulting environmental pressures have profound effects on crop production. Rising temperatures, altered precipitation patterns, and the increased frequency of extreme weather events are causing crops to face both biotic (such as pests and diseases) and abiotic (such as drought, salinity, and temperature stress) challenges. These stresses significantly reduce crop yields, threatening global food security, particularly for key crops like wheat and *Triticale*. Research has shown that the combination of genetic factors and environmental changes underscores the critical need to develop crops with enhanced stress resilience, especially in the face of rapidly changing climatic conditions (Hossain et al., 2021; Robles-Zazueta et al., 2023). Breeding programs must prioritize the integration of stress-resistant traits to ensure that crop yields remain stable under adverse conditions (Kumar et al., 2021).

As a hybrid of wheat and rye, *Triticale* offers significant value in stress-resistant breeding. This man-made cereal combines the high grain quality and agronomic traits of wheat with the superior stress tolerance of rye, particularly under conditions of drought, salinity, and low soil fertility (Golebiowska-Paluch et al., 2023). Its genetic diversity and adaptability make it a highly valuable crop for stress-prone environments, where traditional crops may struggle to thrive. *Triticale*'s robust performance in these conditions has positioned it as an important focus for future breeding programs aiming to develop crops resilient to both biotic and abiotic stresses.

Addressing the challenges of breeding for abiotic and biotic stress resistance in black wheat remains a complex task. The multigenic nature of stress tolerance requires advanced breeding techniques, such as marker-assisted selection, genomic selection, and gene editing, to identify and incorporate resilient traits into elite varieties (Cooper and Messina, 2022). Despite the advancements in modern breeding technologies, the process of developing varieties that exhibit strong resistance to both abiotic and biotic stresses while maintaining high yield potential remains a significant challenge. Limited genetic diversity in some black wheat varieties further complicates this process, making the task of breeding stress-resilient crops even more critical (Bakala et al., 2021).

The study provides an overview of recent advances in the genetic improvement of *Triticale* and black wheat, particularly in stress-prone environments. This study will assess the impact of global climate change on crop productivity and highlight the role of stress-resistant breeding in mitigating these effects. It will also explore the genetic contributions of rye to *Triticale*, emphasizing its importance in breeding for abiotic and biotic stress tolerance. It will discuss current breeding challenges and opportunities, particularly in integrating modern genomic tools to address these stress factors. Finally, it will evaluate the progress and future directions in breeding *Triticale* and black wheat for enhanced resilience to environmental pressures.

2 Genetic Insights into *Triticale***'s Stress Tolerance**

2.1 Genomic composition of *Triticale* **and its influence on stress adaptation**

Triticale is a hybrid cereal, derived from the cross between wheat (*Triticum spp.*) and rye (*Secale cereale*), combining the genetic traits of both species. The genomic structure of *Triticale* plays a pivotal role in its adaptation to stress environments. The wheat genome contributes to high grain yield and superior quality, while the rye genome offers tolerance to various abiotic stresses such as drought, salinity, and freezing temperatures. This combination allows *Triticale* to outperform both wheat and rye in stress-prone environments, particularly in areas with poor soil fertility and water availability. The wheat-rye hybrid nature of *Triticale*, especially the presence of rye chromosomes like 4R and 5R, has been shown to enhance its resistance to environmental stress factors, such as low temperatures and salinity (Golebiowska-Paluch and Dyda, 2023).

2.2 Key quantitative trait loci (QTLs) associated with drought, salinity, and temperature stress tolerance

Several quantitative trait loci (QTLs) have been identified that are critical for *Triticale*'s stress resilience, particularly for tolerance to drought, salinity, and temperature extremes. Notably, QTLs located on rye chromosomes 4R, 5R, and 6R are associated with both abiotic and biotic stress tolerance. These loci influence traits such as drought resistance and freezing tolerance by controlling physiological processes like water retention, cell membrane stability, and osmotic balance.The QTLs on 5R and 6R are of particular importance for drought tolerance, as they regulate water use efficiency and enhance photosynthetic stability under water deficit conditions. Furthermore, QTLs for salinity tolerance have also been mapped to chromosomes 5R and 7R, indicating their role in ion regulation and osmotic adjustment under salt stress (Hura et al., 2017). Additionally, research has shown that specific QTLs located on the wheat-derived chromosome 7A are crucial for freezing tolerance, controlling the recovery and survival rates of plants exposed to freezing temperatures.

2.3 Genetic markers linked to disease resistance in *Triticale*

In terms of disease resistance, several genetic markers have been linked to QTLs associated with resistance to common diseases such as powdery mildew and snow mold. For example, QTLs on rye chromosome 5R are associated with enhanced resistance to biotic stresses, including fungal pathogens. These QTLs influence plant height and spike traits, which are indirectly related to resistance mechanisms by improving airflow and reducing humidity around the plant surface, thus limiting pathogen infection (Golebiowska-Paluch and Dyda, 2023). Furthermore, molecular markers have been identified that are linked to genes conferring resistance to powdery mildew and snow mold, particularly on the rye chromosomes 4R and 5R, providing opportunities for marker-assisted selection (MAS) in breeding programs aimed at improving disease resistance.

3 Progress in Molecular Breeding to Improve Stress Resistance of *Triticale*

3.1 Marker-assisted selection (MAS) for improving stress resilience in *Triticale*

Marker-assisted selection (MAS) is a powerful tool used in modern breeding programs to improve stress resistance by utilizing molecular markers associated with desirable traits. In *Triticale*, MAS has proven effective in transferring multiple stress resistance genes, particularly for abiotic stresses like drought and salinity. By selecting specific markers linked to stress tolerance genes, breeders can accelerate the incorporation of these traits into elite lines without the long timelines typically associated with traditional breeding methods. MAS also enables the combination of multiple stress-resistant traits in a single variety through gene pyramiding, a process used in crops like rice and maize, which can also be applied to *Triticale* (Das and Rao, 2015). Recent studies demonstrate that MAS has the potential to improve complex traits, such as drought and disease tolerance, in

triticale breeding by reducing the environmental variability that typically affects phenotypic selection (Figure 1) (Wani et al., 2018).

Figure 1 Various types of abiotic stresses affecting plant growth and the complex mechanisms involved leading to plant senescence (Adopted from Wani et al., 2018)

3.2 Use of genome-wide association studies(GWAS) to identify stress-tolerance loci

Genome-wide association studies (GWAS) have become a key approach in identifying loci linked to stress tolerance in crops, including triticale. GWAS scans the genome for associations between genetic markers and phenotypic traits, allowing breeders to pinpoint quantitative trait loci (QTLs) linked to importantstress tolerance mechanisms. Recent studies in triticale and related crops have used GWAS to identify significant QTLs for drought, salinity, and disease resistance, enabling the development of more resilient cultivars. For example, GWAS inwheat and triticale has identified multiple QTLs related to leaf rust, yellow rust, and powdery mildew resistance, facilitating marker-assisted breeding for these traits (Pang et al., 2021). The combination of GWAS with genomic prediction models has been shown to enhance the efficiency of breeding programs by providing more accurate predictions of stress tolerance traits across different environments (Mathew et al., 2019).

3.3 Role of genomic selection (GS) in accelerating breeding for complex traits

Genomic selection (GS) represents a significant advancement in plant breeding, particularly for complex traits like stress resistance, which are often controlled by multiple genes. GS uses genome-wide markers to estimate the breeding values of individuals, allowing for more accurate selection of desirable traits across generations. In triticale, GS has been employed to improve traits such as drought and heat tolerance by enabling breeders to select for multiple small-effect genes that contribute to overall stress resilience (Jim, 2024). Studies in crops such as alfalfa and wheat have demonstrated that GS outperforms traditional MAS, particularly for complex traits like biomass yield under stress conditions (Figure 2) (Medina et al., 2021). GS is particularly effective when combined with high-throughput phenotyping and multi-environment trials, which allow breeders to predict how new cultivars will perform under varying environmental conditions, thus accelerating the breeding cycle and improving genetic gains (Bhandari et al., 2018).

4 Integrating Multi-Omics Approaches to Enhance Stress Tolerance in Triticale 4.1 Transcriptomic studies identifying stress-responsive genes in triticale

Transcriptomics has become an essential tool in identifying stress-responsive genes in triticale, allowing for a deeper understanding of the molecular mechanisms underpinning stress tolerance. Transcriptomic analyses provide insights into gene expression profiles under various stress conditions, such as drought, salinity, and heat stress. These studies have revealed that key genes involved in stress responses, including those regulating osmotic adjustment, antioxidant defense, and hormonal signaling, are upregulated under stress conditions. By identifying specific stress-responsive transcripts, breeders can focus on incorporating these genes into elite triticale lines through advanced breeding techniques like marker-assisted selection (MAS) (Bjornson et al., 2017). Furthermore, the integration of transcriptomic data with other omics platforms, such as proteomics and metabolomics, enhances the accuracy of identifying critical genes involved in stress adaptation.

Figure 2 Schematic representation of various steps involved markerassisted selection for abiotic stress tolerance in plants (Adopted from Wani et al., 2018).

4.2 Metabolomics and proteomics insights into physiological adaptations under stress

Metabolomics and proteomics provide complementary insights into the physiological adaptations of triticale under stress. Proteomic analyses have uncovered stress-induced changes in protein expression, particularly those involved in photosynthesis, energy metabolism, and oxidative stress responses. These proteins help the plant maintain cellular homeostasis and energy production under adverse environmental conditions (Nakayasu et al., 2016). Metabolomic studies have shown that stress conditions lead to the accumulation of osmoprotectants, such as proline and sugars, which play vital roles in osmotic regulation and protection against oxidative damage. Together, metabolomic and proteomic data highlight the importance of both metabolic and protein-level adjustments in enhancing stress tolerance in triticale (Pinu et al., 2019). These findings can guide the development of new triticale varieties with improved stress resilience.

4.3 Multi-omics integration for a comprehensive understanding of stress responses

The integration of multiple omics approaches, including transcriptomics, proteomics, and metabolomics, offers a comprehensive understanding of the molecular responses to stress in triticale. Multi-omics integration allows for the identification of interactions between different molecular layers, providing a more holistic view of the plant's stress response mechanisms. For example, integrating transcriptomic and proteomic data can reveal post-transcriptional modifications that regulate protein abundance under stress, while combining metabolomics and transcriptomics can highlight the biochemical pathways most affected by stress conditions. Recent advances in computational tools and data analysis methods have facilitated the integration of multi-omics data, enabling more accurate predictions of stress tolerance traits and guiding future breeding programs (Jamil et al., 2020). This comprehensive approach will be crucial in developing stress-resilient triticale varieties for increasingly challenging environmental conditions.

5 Application of Hybrid Breeding and Heterosis in Triticale for Stress-Prone Environments 5.1 Development of hybrid triticale varieties with enhanced stress tolerance

Hybrid breeding in triticale has been increasingly explored as a means to develop varieties with enhanced stress tolerance. By utilizing the heterosis (hybrid vigor) effect, hybrid triticale varieties have shown improved resilience to a range of environmental stresses, including drought, salinity, and extreme temperatures. The genomic composition of triticale, which combines wheat and rye genomes, allows hybrid varieties to harness the stress tolerance traits of both parental species. This has been particularly useful in stress-prone regions, where abiotic

stresses are major yield-limiting factors. Recent advances in hybrid breeding techniques, including the use of marker-assisted selection (MAS) and genomic selection (GS), have enabled breeders to select for key stress-tolerance traits more efficiently (Losert et al., 2016; Prasanna et al., 2021).

5.2 Exploiting heterosis to improve yield and resilience in challenging conditions

Heterosis plays a critical role in improving both yield and resilience in triticale under challenging environmental conditions. The ability of hybrid triticale varieties to outperform inbred lines in terms of biomass production, grain yield, and stress tolerance is well documented. Studies have shown that heterosis in triticale hybrids can lead to significant improvements in traits such as water-use efficiency, heat tolerance, and disease resistance, making these hybrids ideal for cultivation in regions with unpredictable weather patterns or poor soil conditions (Makumbi et al., 2018). For example, heterosis has been successfully exploited in wheat and maize breeding programs to improve grain yield under heat and drought stress, and similar approaches have been applied to triticale (Menkir et al., 2020).

5.3 Case studies demonstrating the success ofhybrid breeding in stress-prone regions

Several case studies have demonstrated the success of hybrid breeding in triticale for stress-prone regions. In Europe, hybrid triticale varieties have been developed to enhance biomass yield and adaptability to drought-prone areas. A study conducted on 91 hybrid triticale genotypes in Germany revealed that hybrid varieties exhibited higher biomass yields and improved stress tolerance compared to inbred varieties, showcasing the potential of hybrid breeding in low-inputenvironments (Losert et al., 2016). Similarly, in Africa, hybrid maize varieties bred for drought and Striga tolerance have shown remarkable success, with hybrids out-yielding commercial varieties under stress conditions (Menkir et al., 2020). These examples highlight the potential of hybrid triticale varieties to offer solutions for improving food security in stress-prone regions globally.

6 Biotechnological Tools in Triticale Breeding

6.1 Application of CRISPR/Cas9 and other gene-editing technologies to enhance stress tolerance

The use of CRISPR/Cas9 technology has revolutionized plant breeding by enabling precise genome modifications to improve stress tolerance traits in crops, including triticale. CRISPR/Cas9 is widely applied to introduce targeted mutations in genes associated with drought, salinity, and temperature tolerance. This technology allows for the rapid development of stress-resistant triticale varieties by editing specific genes related to osmotic regulation, hormone signaling, and antioxidant defense pathways (Kumar et al., 2023). Moreover, recent advancements in CRISPR technologies, such as prime editing and base editing, provide even greater precision, further enhancing the potential for improving abiotic stress tolerance in triticale.

6.2 Genetic modification strategies for improving abiotic stress resistance

Genetic modification techniques, including transgenic approaches, have been employed to enhance abiotic stress resistance in crops. In triticale, genetic engineering strategies focus on manipulating genes that regulate water-use efficiency, root architecture, and ion transport under stress conditions. By incorporating genes responsible for the synthesis of osmoprotectants such as proline and trehalose, transgenic triticale can better tolerate drought and salinity (Wang et al., 2022). Additionally, these approaches help modify key signaling pathways, such as abscisic acid (ABA) signaling, which plays a crucial role in stress perception and response, thereby improving the resilience of genetically modified triticale to challenging environments (Biswas et al., 2021).

6.3 Potential and challenges oftransgenic approaches in triticale breeding

While transgenic approaches offer significant potential for improving stress tolerance in triticale, they also face several challenges. Regulatory hurdles and public acceptance of genetically modified organisms (GMOs) remain major barriers to the widespread adoption of transgenic triticale.Additionally, concerns about the potential for gene flow and unintended environmental impacts further complicate the use of transgenic crops (Abdelrahman et al., 2018). Despite these challenges, transgenic approaches continue to show promise, especially with the development of transgene-free gene-editing techniques such as CRISPR/Cas9, which may alleviate some regulatory and public concerns. These tools could provide a more acceptable pathway for enhancing stress tolerance in triticale without the stigma associated with traditional transgenics.

7 Field-Based Phenotyping and High-Throughput Screening in Triticale

7.1 Advances in high-throughput phenotyping for assessing stress tolerance in the field

High-throughput phenotyping (HTP) technologies have advanced significantly, providing breeders with powerful tools to assess stress tolerance in crops like triticale.These technologies leverage non-invasive sensors, such as RGB cameras, multispectral, and hyperspectral imaging, to capture detailed data on traits such as canopy cover, plant height, and biomass under stress conditions. This allows for the rapid evaluation of large breeding populations across different environmental conditions, aiding in the selection of varieties with improved stress tolerance. UAVs (unmanned aerial vehicles) have been particularly effective, offering high-resolution, cost-efficient phenotyping platforms that can monitor crops over large areas and multiple time points, providing a comprehensive understanding of their stress response (Xie and Yang, 2020).

7.2 Role of remote sensing and UAVs in large-scale screening for stress-adaptive traits

UAV-based remote sensing technologies play a crucial role in large-scale phenotypic screening, allowing for the assessment of stress-adaptive traits such as drought tolerance and water-use efficiency. These systems utilize various sensors, including thermal, multispectral, and hyperspectral cameras, to monitor physiological changes in crops in response to stress. For instance, thermal imaging can detect changes in canopy temperature, which is indicative of water stress, while multispectral imaging can capture data related to plant health and vigor, such as normalized difference vegetation index (NDVI). UAVs have proven to be highly effective in capturing these data across extensive fields, reducing the need for manual measurements and allowing breeders to screen large numbers of genotypes rapidly (Ludovisi et al., 2017).

7.3 Integrating field phenotyping data with genomic information for precision breeding

The integration of phenotyping data with genomic information is essential for precision breeding in triticale.By combining HTP data from field trials with genomic tools such as genome-wide association studies (GWAS) and genomic selection (GS), breeders can more accurately identify and select for stress tolerance traits (Jiang, 2024). This approach enhances the efficiency of breeding programs by enabling the identification of quantitative trait loci (QTLs) linked to stress tolerance and the prediction of plant performance under various environmental conditions. The use of machine learning and advanced data analysis techniques further enhances the integration of phenotypic and genomic data, allowing breeders to make more informed decisions when selecting genotypes for breeding (Li et al., 2022).

8 Sustainable Breeding Strategies for Stress-Prone Environments

8.1 Breeding for climate resilience: strategies for adapting to changing environmental conditions

In the face of global climate change, breeding strategies for climate resilience have become paramount in ensuring stable crop production, particularly in stress-prone environments. Climate-resilient breeding focuses on developing triticale varieties that can withstand the adverse effects of drought, heat, and other environmental stresses. One successful approach has been the application of the breeder's equation to predict the response of crops under different environmental conditions, allowing breeders to optimize the selection of traits for resilience (Cooper and Messina, 2022). Genetic improvements in root system architecture, for example, enhance water and nutrient uptake, which can be crucial in regions affected by erratic rainfalland poor soil quality (Ober et al., 2021). Through the integration of advanced molecular and genomic tools, breeders can more efficiently select for complex traits like drought and heat tolerance.

8.2 Enhancing water-use efficiency and nutrient uptake in triticale varieties

Improving water-use efficiency (WUE) and nutrient uptake is a central goal in breeding triticale for sustainable agriculture. Enhanced root traits, such as deeper root systems and increased root hair density, have shown promise in boosting water and nutrient acquisition in low-input systems. For instance, studies have demonstrated that optimized root systems in cereals like wheat and triticale improve resilience by facilitating better access to water and nutrients in dry conditions (Figure 3) (Ndoye et al., 2022). Moreover, incorporating traits related to efficient nutrient use, particularly nitrogen and phosphorus, helps minimize the environmental footprint of farming by reducing the need for excessive fertilizer application. These advancements contribute to the development of triticale varieties that are more sustainable and resource-efficient.

Figure 3 Crops grown in low-input environments require specific root traits to optimize water and nutrient acquisition (Adopted from Ndoye et al., 2022)

8.3 Developing sustainable, low-input triticale cropping systems

Sustainable cropping systems emphasize low-input agricultural practices, which rely on efficient resource use rather than external inputs like chemical fertilizers and irrigation. In low-inputsystems, breeding efforts focus on improving crop traits that enhance resilience and reduce dependency on external resources. Triticale, due to its inherent tolerance to abiotic stresses, is well-suited to these systems. Root traits, such as greater root length and density, have been targeted in breeding programs to improve adaptation to low-inputenvironments (Ndoye et al., 2021). Additionally, symbiotic relationships with soil microbes, such as mycorrhizal associations, can enhance nutrient uptake, making triticale more suitable for sustainable agriculture. These strategies can create triticale varieties that thrive in marginal environments, contributing to both food security and environmental sustainability.

9 Challenges and Future Directions in Triticale Breeding

9.1 Overcoming genetic bottlenecks and limited genetic diversity in breeding programs

One of the greatest challenges in triticale breeding is the limited genetic diversity available within the species, which constrains the ability of breeders to respond to new environmental stresses and agronomic needs. The genetic bottlenecks that arose during the domestication and modern breeding processes have reduced the variability necessary for long-term improvement. However, recent advances in genomic tools, such as genome-wide association studies (GWAS) and genomic selection, have made it possible to introduce novel diversity into breeding programs by accessing the genetic resources of related species and wild relatives. These approaches allow breeders to identify and incorporate new alleles that can help restore diversity and improve adaptation to a variety of stressors (Kapazoglou et al., 2023).

9.2 Addressing the trade-offs between yield, quality, and stress tolerance

Another major challenge in triticale breeding is balancing the trade-offs between high yield, grain quality, and stress tolerance. Breeding for stress resistance often leads to compromises in yield or grain quality due to the complex genetic interactions involved. For instance, increased stress tolerance traits, such as drought resistance, may be associated with reduced grain size or yield potential. To address these trade-offs, breeders are increasingly turning to genomic selection and marker-assisted breeding, which enable the selection of favorable combinations of genes that maximize both yield and stress resilience. These methods improve precision in selection, allowing breeders to more effectively manage the balance between yield, quality, and stress adaptation traits (Golebiowska-Paluch and Dyda, 2023).

9.3 Future prospects for incorporating novel genetic and breeding technologies into triticale breeding

Looking to the future, the integration of novel genetic technologies such as CRISPR/Cas9 gene editing, precision genome editing, and high-throughput phenotyping holds significant potential for advancing triticale breeding.

Genome editing offers the ability to make targeted modifications to genes responsible for stress tolerance, yield, and quality traits without introducing foreign DNA, which could help address public and regulatory concerns over genetically modified organisms (GMOs) (Nerkar et al., 2022). Furthermore, advances in phenotyping technologies, including remote sensing and UAV-based data collection, combined with genomic information, are expected to accelerate breeding cycles and improve the accuracy of trait selection (Bentley et al., 2022). These tools will enhance breeders' capacity to develop high-yielding, stress-resilient triticale varieties that meet the demands of future agricultural systems.

10 Concluding Remarks

The current state of genetic research and methodologies in triticale breeding highlights remarkable advancements in both traditional and modern approaches to enhance the crop' s adaptability to stress-prone environments. Significant progress has been made in the identification of key genetic loci associated with abiotic and biotic stress tolerance, aided by advanced tools such as genome-wide association studies (GWAS), marker-assisted selection (MAS), and genomic selection (GS). Additionally, the incorporation of biotechnological tools like CRISPR/Cas9 has accelerated the ability to precisely edit genes related to stress adaptation, enabling faster and more targeted breeding strategies. However, challenges such as genetic bottlenecks and balancing trade-offs between yield, quality, and stress tolerance remain areas where further research is needed.

Triticale's role in ensuring food security, especially in stress-prone environments, is increasingly significant as the effects of climate change continue to intensify. Its unique genomic composition, which combines the stress tolerance of rye and the high yield potential of wheat, positions it as a crucial crop for marginal lands and regions susceptible to drought, salinity, and extreme temperatures. This hybrid crop offers a sustainable solution for improving food production while reducing reliance on high-input agricultural practices.

To address future challenges, continuous research and innovation are imperative. New breeding technologies, including genome editing, high-throughput phenotyping, and multi-omics integration, offer promising pathways to develop triticale varieties that can thrive in increasingly hostile environments. Moreover, the integration of data-driven approaches, such as machine learning and big data analytics, with field-based phenotyping will enhance precision breeding, ensuring the development of resilient, high-yielding varieties that meet global food demands. As agricultural pressures continue to rise, investing in these cutting-edge technologies and interdisciplinary research will be critical for the future of triticale breeding and its contribution to global food security.

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Conflict of Interest Disclosure

The author affirms 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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