

Marker-Assisted Introgression of Terminal Heat Tolerance and High-Zinc Quantitative Trait Loci (QTLs) in Bread Wheat (*Triticum aestivum* L.) for Climate-Resilient Nutritional Security

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Abstract

Terminal heat stress during the grain-filling stage and widespread micronutrient (especially zinc) deficiency poses major threats to global wheat productivity and nutritional security. This study focuses on the marker-assisted introgression of QTLs conferring terminal heat tolerance (early anthesis via Xbarc186 on 4AL, kernel weight via Xgwm190 on 1BS) and high grain zinc concentration (including Gpc-B1/NAM-B1 on 6BS and stable QTLs on 4DS, 6AS, and 7BL) into elite bread wheat backgrounds using marker-assisted backcross breeding (MABB). Foreground selection with tightly linked SSR/SNP/KASP markers, recombinant selection to minimize linkage drag, and background selection for high recurrent parent genome recovery (90–98%) enabled the development of improved lines with enhanced heat resilience and elevated grain zinc (target >45 mg/kg) without compromising yield potential. Physiological mechanisms such as maintained photosynthetic efficiency, antioxidant defense, stay-green trait, and efficient nutrient remobilization underpin the tolerance, while biofortification leverages wild emmer and synthetic hexaploid diversity. Multi-environment evaluations confirm significant improvements in thousand-grain weight, yield stability under late-sown conditions, and grain zinc content. Integration with speed breeding and genomic selection accelerates the development of climate-resilient, nutritionally enriched wheat varieties, offering a sustainable pathway to combat hidden hunger and ensure food security in heat-prone regions.

Keywords: Marker-Assisted Backcrossing, Terminal Heat Tolerance, High-Zinc QTLs, Biofortification, Bread Wheat, Gpc-B1, Early Anthesis, Thousand-Grain Weight, Climate-Resilient Breeding, Genomic Selection, Kasp Markers, Nutritional Security

1. Introduction

The global agricultural landscape is currently navigating a period of unprecedented volatility, driven by the intersecting crises of climate change and systemic nutritional deficiencies. Bread wheat (*Triticum aestivum* L.) serves as the primary staple for over 40% of the world's population, providing approximately 20% of the total caloric and protein intake globally (Fu et al., 2023). As the most widely cultivated cereal, its productivity is a fundamental determinant of international food security and economic stability. However, the Sixth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC) underscores a harrowing trajectory, predicting an increase in the frequency and

intensity of extreme temperature events that threaten to destabilize major wheat-producing regions (IPCC, 2022).

Terminal heat stress, specifically defined as temperatures exceeding 31°C during the critical grain-filling phase, has emerged as a primary constraint to wheat yields, particularly in the semi-arid and subtropical regions of South Asia (Assad & Paulsen, 2002). In the Indo-Gangetic Plains, the late harvest of rice frequently delays wheat sowing until late November or early December, pushing the reproductive phase of the crop into the high-temperature window of March and April (Mondal et al., 2013). This temporal mismatch results in a catastrophic decline in productivity, with estimates suggesting a 6% loss in global wheat yield for every 1°C rise in seasonal temperature (Asseng et al., 2015). In India, the 2021-22 growing season witnessed an unprecedented rise in March temperatures, leading to a production decline of approximately 5.7% to 10%, causing significant distress in the North Western and North Eastern Plains Zones (Gupta et al., 2022).

Parallel to these production challenges is the persistent issue of “hidden hunger” the deficiency of essential micronutrients like zinc (Zn^{2+}) and iron (Fe^{2+}) in human diets. Over two billion people worldwide suffer from micronutrient malnutrition, which leads to impaired immune function, stunted growth in children, and cognitive developmental delays (White & Broadley, 2009). Modern wheat varieties, while optimized for yield during the Green Revolution, often exhibit significantly lower micronutrient concentrations compared to their wild relatives and landraces, a phenomenon known as the “dilution effect” (Hao et al., 2022). The objective of breeding for “Climate-Resilient Nutritional Security” thus represents a sophisticated integration of traits: the ability to maintain yield under thermal stress while simultaneously accumulating higher levels of bioavailable zinc in the grain (Velu et al., 2016).

2. Physiological and Biochemical Mechanisms of Terminal Heat Stress

Understanding the physiological impact of terminal heat stress is essential for identifying the genetic loci that can confer resilience. Wheat is a cool-season C_3 cereal that achieves optimal physiological performance at temperatures between 12°C and 22°C during anthesis and grain filling. When environmental temperatures surpass these thresholds, a cascade of deleterious effects is initiated at both the cellular and whole-plant levels (Yadav et al., 2025).

2.1. Disruption of Photosynthetic Integrity and Source-Sink Balance

The primary target of high-temperature stress is the photosynthetic machinery. Heat stress accelerates the degradation of chlorophyll a and b and inhibits the biosynthesis of new pigments by destroying enzymes like 5-aminolevulinic acid dehydratase (Wang et al., 2024). The thylakoid membranes, which house the photosynthetic apparatus, become excessively fluid and unstable under heat stress, leading to the dissociation of Photosystem II (PSII). This disruption reduces the quantum efficiency of photosynthesis and triggers premature leaf senescence, thereby shortening the effective grain-filling duration (Lowe, 2021).

Furthermore, high temperatures inhibit Ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) activase (RCA), which is essential for maintaining Rubisco in its active state. Above 30°C, RCA becomes unstable, leading to a decline in CO_2 fixation and an increase in photorespiration (Gahlaut et al., 2021). This imbalance significantly reduces the pool of photo-assimilates available for translocation to the developing grains. Under terminal heat, the duration of grain-filling can be reduced by as much as three weeks, leading to shriveled grains and reduced thousand-grain weight (TGW) (Narendra et al., 2021). The physiological cascade triggered by terminal heat stress is summarized in Figure 1. This schematic illustrates how heat-induced damage to photosynthesis and cellular stability ultimately leads to reduced grain yield.

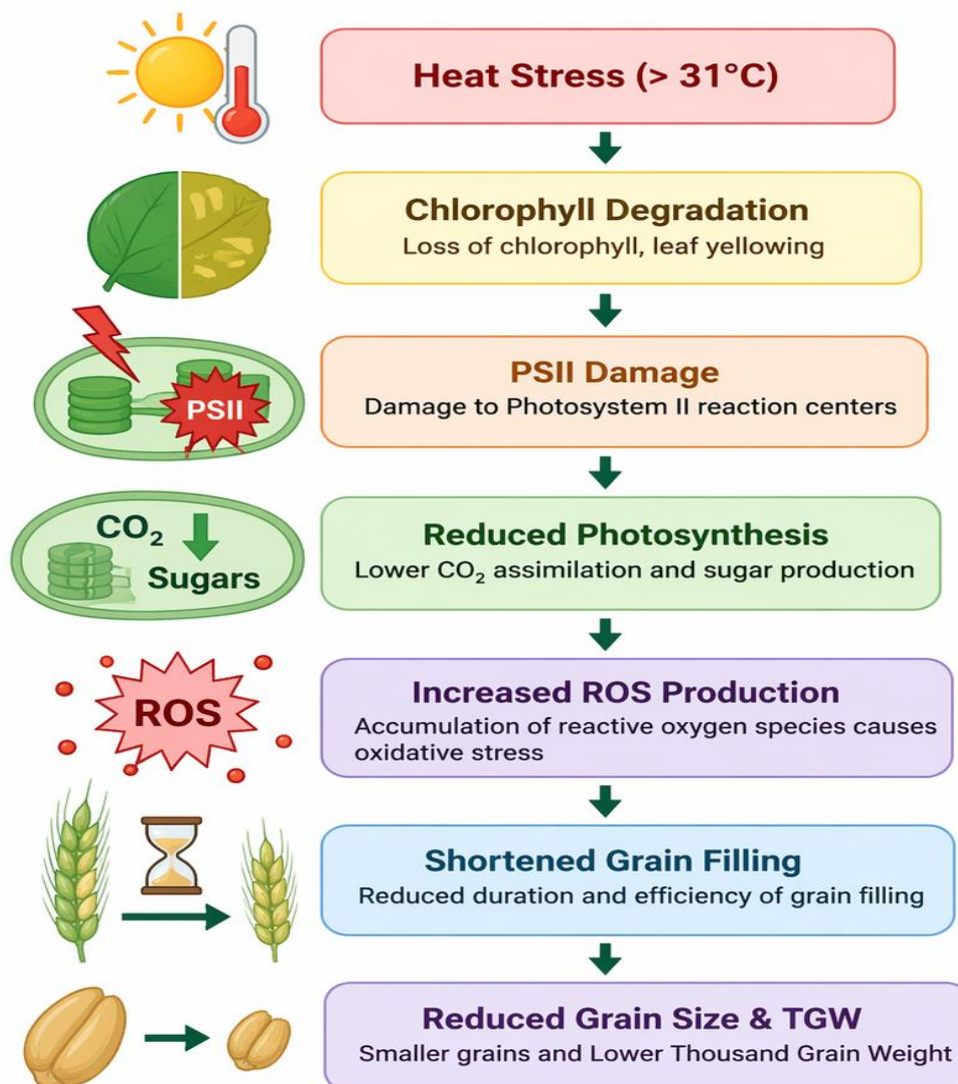


Figure 1: Impact of Terminal Heat Stress on Wheat Physiology and Yield Formation

2.2. Oxidative Damage and Antioxidant Defense Systems

The biochemical hallmark of heat stress is the overproduction of reactive oxygen species (ROS), including superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH\bullet$). These molecules are highly reactive and cause extensive lipid peroxidation, which damages cellular membranes and impairs the function of nucleic acids and proteins (Garvin et al., 2006). To survive these episodes, heat-tolerant genotypes activate a suite of antioxidant enzymes. Superoxide dismutase (SOD) acts as the primary defense by converting O_2^- to H_2O_2 , while catalase (CAT) and peroxidase (POX) further neutralize H_2O_2 into water and oxygen (Cakmak, 2008).

Table 1: Physiological Responses to Heat Stress and Their Consequences for Crop Performance

Trait	Physiological Response to Heat Stress	Consequence for Crop Performance
Canopy Temperature	Increased due to stomatal closure or low transpirational cooling	Heat-sensitive plants suffer from tissue scorching
Chlorophyll Stability	Rapid degradation of pigments in the flag leaf	Shorter grain-filling duration and lower GFR

Membrane Stability	Increased lipid peroxidation and electrolyte leakage	Loss of cellular homeostasis and nutrient transport
Antioxidant Capacity	Upregulation of SOD, CAT, and POX in tolerant lines	Protection of photosynthetic apparatus from ROS
Grain Filling Rate	Initial increase followed by premature cessation	Reduced final grain size and shriveled appearance

In addition to enzymatic defenses, tolerant plants accumulate osmoprotectants such as proline and glycine betaine, which stabilize proteins and maintain osmotic balance under heat and water stress. The "stay-green" trait, which is the ability of certain genotypes to maintain green leaf area and photosynthetic activity longer under stress, is a critical component of terminal heat tolerance (Shiferaw et al., 2013).

3. Genetic Architecture of Heat Tolerance: Avoidance and Tolerance Strategies

The genetic control of heat tolerance in wheat is predominantly polygenic, involving numerous Quantitative Trait Loci (QTLs) with varying effects. Breeders distinguish between heat avoidance where the plant escapes the stress through phenological adjustments and true heat tolerance where the plant maintains productivity despite the stress (Devasirvatham et al., 2016).

3.1. Phenological Escape through Early Anthesis

One of the most effective strategies for managing terminal heat in regions like the North Eastern Plains Zone of India is the manipulation of flowering time. Early anthesis allows the plant to complete the sensitive reproductive stages and a significant portion of grain-filling before the temperatures peak in late spring (Mondal et al., 2013).

A major, validated QTL for early anthesis has been identified on chromosome 4A, linked to the Simple Sequence Repeat (SSR) marker Xbarc186 (Paliwal et al., 2008). In breeding programs using the donor WH730, this QTL consistently reduced the number of days to anthesis by 5-10 days, providing a critical buffer against late-season heatwaves. Another stable QTL for days to heading has been mapped to chromosome 7D, which explained up to 19.6% of the phenotypic variation in yield under heat and drought stress (Tahmasebi et al., 2017).

3.2. QTLs for Yield Components and Physiological Resilience

While avoidance is critical, improving the intrinsic tolerance of the grain-filling process is equally important. Significant QTLs for thousand-grain weight (TGW) and grain yield (GY) under heat stress have been identified across the wheat genome. For example, a major QTL linked with marker Xgwm190 was targeted for its association with high kernel weight and yield stability under terminal heat stress in Indian wheat backgrounds (Bellundagi et al., 2022).

Table 2: Validated QTL Regions and Associated Markers for Heat Tolerance in Wheat

QTL Region	Associated Marker	Trait	Environment/Condition
4AL	Xbarc186	Early Anthesis	Late-sown (Heat Stress)
1BS	Xgwm190	Kernel Weight/Yield	Late-sown (Heat Stress)
7DS	AX-94637211	Thousand Grain Weight	Heat/Drought Combined
3BL	AX-110010996	Canopy Temperature	Terminal Heat
2BS	AX-94537892	Soluble Sugars	Heat Stress Quality

Recent Genome-Wide Association Studies (GWAS) have expanded this repertoire, identifying 23 marker-trait associations (MTAs) for seedling traits and over 150 MTAs for reproductive stage heat tolerance in diverse emmer-based populations. Regions on chromosomes 2B and 3A have been

specifically noted for their positive impact on grain yield under stress, contributing up to a 7% allelic effect on productivity (Ullah et al., 2023).

4. Principles of Genetic Biofortification for Grain Zinc and Iron

Biofortification represents a sustainable approach to addressing micronutrient deficiencies by enhancing the natural ability of plants to uptake, translocate, and accumulate minerals in the edible portions. In wheat, the primary objective is to increase grain zinc (GZn) from the baseline of 25-30 mg/kg to a target of 45 mg/kg (Police Patil et al., 2025).

4.1. Harnessing Diversity from the Wheat Gene Pool

The domestication of bread wheat led to a significant loss of genetic diversity for micronutrient accumulation. However, wild relatives like *Triticum sp. dicoccoides* (wild emmer) and *Aegilops tauschii* (the D-genome donor) possess exceptionally high levels of grain Zn and Fe, often exceeding 60-100 mg/kg. CIMMYT has successfully utilized synthetic hexaploid wheats (SHW) and emmer wheat derivatives to introduce these "high-zinc" alleles into modern bread wheat (Velu et al., 2016).

A landmark discovery in wheat biofortification is the *Gpc-B1* locus on chromosome 6BS, derived from *T. sp. dicoccoides*. This 250 kb locus encodes a NAC transcription factor (*SNAM-B1*) that accelerates leaf senescence and enhances the remobilization of nutrients (N, Zn, and Fe) from vegetative tissues to the grain (Nasim et al., 2025). Most modern hexaploid and tetraploid wheats possess a non-functional allele of this gene, making its re-introgression a high priority for nutritional enhancement (Saini et al., 2020).

4.2. Mapping and Characterization of Zinc QTLs

Advancements in high-throughput genotyping have facilitated the identification of stable QTLs for GZn across multiple environments. These QTLs are often located on chromosomes 1B, 2A, 3B, 4D, 6A, 7B, and 7D (Liu et al., 2021). The chromosomal distribution of major zinc-associated QTLs is illustrated in Figure 2. These loci represent key targets for marker-assisted biofortification in wheat breeding programs.

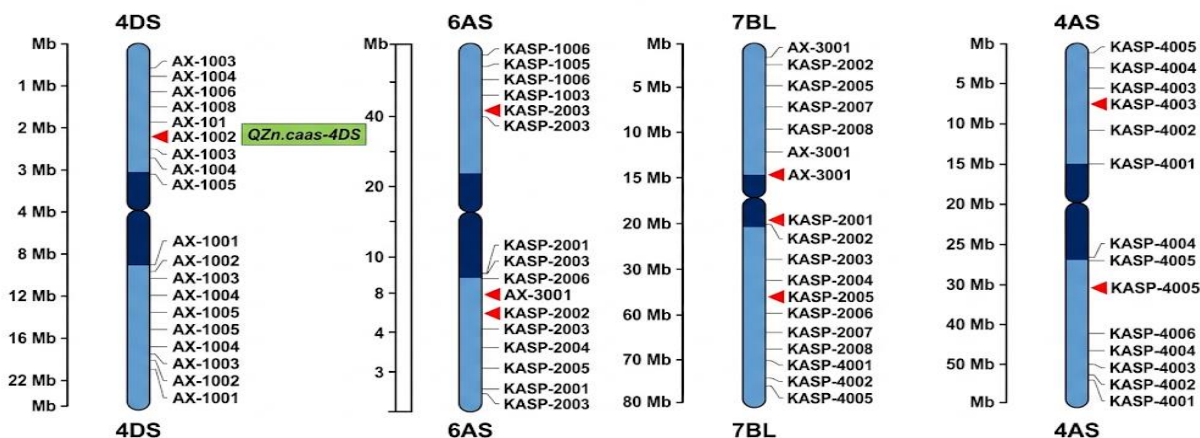


Figure 2: Major QTLs Associated with Grain Zinc Concentration in Wheat Genome

One of the most stable zinc-related loci identified in recent studies is *QZn.caas-4DS*, located near the Rht-D1 (reduced height) locus on chromosome 4D. This QTL explained up to 25.1% of the phenotypic variance and was stable across eight different environments (Tong et al., 2022). Another significant QTL, *QGrZnc.sau-4AS*, was identified in tetraploid wheat crosses and validated for its lack of negative effects on yield-related traits, making it an ideal candidate for introgression without "dilution" penalties (Sun et al., 2023).

Table 3: Identified Major Zinc and Iron QTLs and Tightly Linked Markers in Wheat

Chromosome	Major Zinc/Iron QTL	Linked Marker	Key Finding/Stability
4DS	<i>QZn.caas-4DS</i>	AX-89703298	Stable across 8 environments; pleiotropic for Fe
6AS	<i>QZn.caas-6AS</i>	AX-110640576	Major locus at 124.3 Mb; highly heritable
7BL	<i>QZn.caas-7BL</i>	AX-89745787	Stable locus at 725.4 Mb; validated in panels
7DS	<i>Qql.iari-7D</i>	AX-95220192	Linked to Oligopeptide transporter gene
4AS	<i>QGrZnc.sau-4AS</i>	KASP-AX-108829087	Novel locus from wild emmer; no dilution effect

5. Interactions between Terminal Heat Stress and Zinc Biofortification

A critical aspect of climate-resilient nutritional security is the interaction between thermal stress and mineral accumulation. While heat stress is fundamentally deleterious to yield, its impact on nutrient concentration is nuanced and highly dependent on the metabolic stability of the genotype (Kumar et al., 2023).

5.1. Metabolic Trade-offs and the "Concentration Effect"

Several studies have reported that terminal heat stress can paradoxically lead to an increase in grain zinc and protein concentrations. This occurs because high temperatures reduce starch accumulation more drastically than mineral translocation, leading to a "concentration effect" where the mineral density appears higher in the smaller, shriveled grains (Police Patil et al., 2025).

Conversely, some micronutrients are highly vulnerable to thermal degradation. For instance, in trials of 25 diverse wheat genotypes, grain iron (Fe) content was reduced by an average of 31.98% under terminal heat, while zinc (Zn) was relatively more stable, showing only a 5.91% reduction (Narendra et al., 2021). This indicates that the mechanisms governing Zn translocation are more resilient than those for Fe, though both are ultimately limited by the heat-induced premature senescence of tissues (Beyer, 2025).

5.2. Zinc as a Mitigation Agent for Thermal Stress

Evidence suggests that the relationship between zinc and heat is not merely one-way. Adequate zinc nutrition plays a proactive role in conferring heat tolerance to the wheat plant. Zinc is a structural component of SOD enzymes and is essential for maintaining the stability of cellular membranes under high temperatures (Peck & McDonald, 2010).

Research has shown that wheat plants grown under zinc-deficient conditions suffer significantly more from heat shocks compared to those with sufficient zinc. Low zinc levels exacerbate heat-induced damage to the chloroplast ultrastructure and accelerate the loss of photosynthetic pigments (Mosavian

et al., 2021). Therefore, the introgression of high-zinc QTLs not only serves to improve human nutrition but also functions as a biological insurance policy, enhancing the plant's innate ability to withstand terminal heat waves (Mued et al., 2025).

6. Marker-Assisted Backcross Breeding (MABB): Strategies for Precision Introgression

Marker-Assisted Backcross Breeding (MABB) has revolutionized the transfer of complex traits from donors to adapted elite varieties. Unlike conventional breeding, which relies on phenotypic selection and multiple generations of selfing, MABB allows for the precise tracking of target genomic regions while simultaneously screening for the recovery of the recurrent parent genome (RPG) (Bellundagi et al., 2022).

6.1. Workflow of Precision Introgression

The MABB process involves three distinct levels of selection:

1. **Foreground Selection:** Using markers tightly linked to the target QTL (Xbarc186 for early anthesis) to ensure that the donor alleles are present in each generation (Cobb et al., 2019).
2. **Recombinant Selection:** Using flanking markers to minimize the size of the donor chromosome segment, thereby reducing linkage drag the carryover of undesirable wild alleles (Huang et al., 2025).
3. **Background Selection:** Using a genome-wide array of polymorphic markers (typically SSRs or SNPs) to select individuals that have the highest percentage of the recurrent parent's DNA (Ahmad et al., 2018).
- 4.

Table 4: Selection Levels in Marker-Assisted Backcross Breeding (MABB) Workflow

Selection Type	Purpose	Markers Used	Impact on Variety Development
Foreground	Identify target QTLs	Xbarc186, Xgwm190, KASP-SNPs	Guarantees presence of tolerance/Zn genes
Background	Recover elite parent genome	124 polymorphic SSRs or 35K SNPs	Maintains yield and local adaptation
Recombinant	Reduce linkage drag	Flanking markers within 1-5 cM	Eliminates undesirable traits from donor

6.2. Case Study: Improving HD2733 for Heat Resilience

HD2733 is an elite bread wheat variety widely grown in India. While it possesses high yield potential, it is significantly affected by late-season heat stress. In a targeted MABB program, HD2733 was used as the recurrent parent, and the heat-tolerant line WH730 was used as the donor (Singh et al., 2023). The breeding team targeted two primary QTLs: one for early anthesis (Xbarc186) and one for kernel weight under stress (Xgwm190). Through two backcrosses (BC₂) followed by selfing, researchers identified families with 90.9% to 97.9% RPG recovery (Krishna et al., 2022). These improved lines were evaluated in multi-location trials under late-sown conditions. The results were highly significant: the introgressed lines flowered earlier and maintained higher grain weight, outperforming the original HD2733 by substantial margins in heat-stressed environments (Kumar et al., 2023).

7. Identification and Validation of High-Zinc KASP Markers

For marker-assisted breeding to be effective at a commercial scale, markers must be both reliable and cost-effective. The conversion of SNP (Single Nucleotide Polymorphism) markers identified via GWAS into Kompetitive Allele Specific PCR (KASP) markers represents a significant milestone in high-throughput selection (Liu et al., 2021).

7.1. The KASP Advantage

KASP markers are highly reproducible and can be used to screen thousands of plants in early generations. Tightly linked SNPs for grain zinc on chromosomes 4DS, 6AS, and 7BL were converted to KASP assays (K-AX-89703298, K-AX-110640576, and K-AX-89745787). These markers have been validated across diverse wheat germplasm panels, confirming that genotypes carrying the favorable alleles consistently show significantly higher zinc concentrations (Ramya et al., 2022). Furthermore, novel markers like AX-95220192 on chromosome 7D have been linked to candidate genes encoding Oligopeptide Transporters (OPT family). These transporters are critical for moving Zn-chelates through the phloem during the final stages of grain maturation. The identification of such "biological markers" enhances the precision of breeding for nutritional traits (Police Patil et al., 2025).

8. Managing Linkage Drag and Genetic Diversity Loss

A recurring challenge in the introgression of traits from wild relatives is the introduction of undesirable characteristics, such as small grain size or poor threshability. This "linkage drag" is particularly problematic when transferring high-zinc alleles (Jain et al., 2022).

8.1. Precision Mapping to Minimize Donor Segments

High-depth genome sequencing can be used to reduce the introgressed wild fragment from several megabases to much smaller intervals (Huang et al., 2025). A similar approach is being applied in wheat to isolate high-zinc QTLs from their unfavorable neighboring genes. For example, the Gpc-B1 locus was fine-mapped to a 250 kb region, allowing for its successful transfer into varieties like 'Zinc Shakti' without compromising yield (Velu et al., 2016).

8.2. Root Biomass and Thermal Adaptation

An often-overlooked aspect of climate resilience is the root system. Research into European bread wheat has shown that strong selection for specific flowering time haplotypes inadvertently eliminated genetic diversity for root biomass due to linkage drag (Beyer et al., 2017). Recovering this root diversity is essential for future climate adaptation, as more robust root systems are better equipped to extract water and nutrients (including Zn and Fe) during heat and drought episodes (Krishnappa et al., 2022).

9. Integration of Speed Breeding and Genomic Selection

To keep pace with the accelerating rate of climate change, wheat breeders are integrating MABB with other rapid-breeding technologies (Prashanth Babu et al., 2022).

9.1. Accelerating Generation Turnover

Speed breeding (SB) utilizes manipulated photoperiods (22-hour light) and temperature-controlled glasshouses to achieve up to six generations of wheat per year. By combining SB with marker-assisted selection, the time required to develop a near-isogenic line can be reduced from 5-7 years to just 2-3 years (Watson, 2019). In South Korea, this synergized breeding strategy was used to introgress the Glu-B1i allele for high grain quality, reducing the development time by 53% (Anilkumar et al., 2022).

9.2. Genomic Selection (GS) for Complex Polygenic Traits

While MABB is effective for major QTLs, many components of heat tolerance are controlled by hundreds of minor-effect loci. Genomic Selection (GS) uses high-density marker data from a training population to predict the genomic estimated breeding values (GEBVs) of individuals in a breeding population (Yunus et al., 2025). This allows for the selection of superior lines based on their genetic potential for both yield and micronutrient density, even before they are tested in the field (Krishnappa et al., 2022).

10. Performance of Stress-Tolerant Genotypes in Diverse Environments

The validation of introgressed lines under real-world conditions is the ultimate test of any breeding program. Studies evaluating CGIAR Research Program (CRP) lines have identified several genotypes that maintain high yield stability and grain micronutrient levels across both normal and heat-stressed environments (Krishna et al., 2023).

Table 5: Performance and Stability of Diverse Wheat Genotypes under Normal and Heat-Stressed Environments

Genotype	Stability Performance	Grain Zn (ppm) - Normal	Grain Zn (ppm) - Heat	Key Advantage
CRP7	Highly Stable	55.4	52.1	Yield stability in NEPZ
CRP48	High Nutrient	65.6	55.1	Highest Zn across years
HD-2967	Moderate	25.2	23.5	Popular Indian check
RAJ4546	Climate Resilient	46.7	45.2	High TGW and Zn
BRW3863	Biofortified	50.1	48.4	Resistant to terminal heat

Data from multiple Indian wheat breeding centers highlight that genotypes like RAJ4546 and DBW296 excel in maintaining both thousand-grain weight (TGW) and grain zinc content under terminal heat and restricted irrigation (Sindhushree et al., 2025).

11. Future Outlook: CRISPR/Cas9 and Advanced Gene Editing

The next frontier in developing climate-resilient, high-zinc wheat involves the use of precision gene editing tools like CRISPR/Cas9. Rather than introgressing large chromosomal segments, CRISPR allows for the targeted modification of specific genes to enhance their expression or function (Fu et al., 2023).

For instance, the \$TaZFP13D\$ gene, which encodes a Zinc Finger Protein, has been identified as a key regulator of the oxidative stress response during photosynthesis. Overexpressing this gene or similar regulators could theoretically create wheat varieties that are "pre-adapted" to high temperatures without the need for traditional backcrossing (Gahlaut et al., 2021). Similarly, editing the promoter regions of metal transporters could increase the loading of zinc into the grain endosperm (Nasim et al., 2026).

12. Conclusions

Marker-assisted introgression of QTLs for terminal heat tolerance and high grain zinc concentration represents a powerful strategy to develop climate-resilient, nutritionally enriched bread wheat varieties capable of sustaining productivity and combating hidden hunger under rising temperatures. By precisely transferring major QTLs for early anthesis, kernel weight stability, and enhanced zinc uptake/remobilization (including the valuable Gpc-B1 locus) into elite backgrounds through MABB, breeders have successfully combined heat resilience with biofortification while recovering high proportions of the recurrent parent genome and minimizing linkage drag. These improved lines maintain superior photosynthetic efficiency, antioxidant capacity, stay-green traits, and efficient nutrient partitioning under stress, resulting in higher thousand-grain weight, yield stability, and grain zinc content (>45 mg/kg target) across diverse environments. The integration of high-throughput KASP markers, speed breeding, and genomic selection further shortens the breeding cycle and enhances selection accuracy for complex polygenic traits. Future efforts should focus on pyramiding multiple heat and zinc QTLs, expanding validation in farmer fields, exploring CRISPR-based editing of key regulators (e.g., NAM-B1, metal transporters), and addressing genotype × environment interactions. Ultimately, the widespread adoption of these climate-smart, biofortified wheat varieties

will be instrumental in achieving sustainable food and nutritional security for millions in heat-vulnerable regions of South Asia and beyond.

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