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Integrating genetic tools for enhancing abiotic stress resilience in wheat

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Abstract
Abiotic stresses such as heat and drought present significant challenges to global wheat production, threatening food security. Wheat breeders have traditionally relied on empirical selection to develop stress-tolerant cultivars, but recent advancements in genetic tools are enhancing the efficiency of breeding for abiotic stress resilience. This paper reviews the integration of modern genetic technologies like marker-assisted selection (MAS), marker-assisted backcrossing (MABC), quantitative trait loci (QTL) mapping, and genomic selection (GS) into wheat breeding. These methods enable the identification and introgression of key genes associated with stress tolerance. Traits such as efficient root systems, osmotic adjustment, and antioxidant defenses are essential for improving wheat's ability to endure heat and drought stress. This review also highlights the potential of genetic engineering and transgenic approaches to accelerate the development of climate-resilient wheat varieties. Advanced phenotyping technologies, combined with a comprehensive understanding of stress tolerance mechanisms, can further optimize breeding strategies, ensuring sustainable wheat production in the face of climate change.

Keywords: Wheat, abiotic stress, genetic tools, drought tolerance, heat tolerance

Introduction
Globally, agriculture faces significant challenges concerning various types of abiotic stresses. Abiotic stresses in wheat encompass a wide range of non-biological, external factors, such as temperature fluctuations, water scarcity, soil salinity, and exposure to various chemicals (Abhinandan, K *et al*., 2018) [26]. These stresses can disrupt the delicate balance of wheat's physiological processes, leading to reduced yields, lower quality grain, and increased susceptibility to pests and diseases. particularly heat and drought pose major obstacles to agricultural production and threaten food security. To address these challenges with limited resources, the development of climate-resilient cultivars is considered a practical and cost-effective approach. Plants have evolved adaptive strategies to mitigate the impact of stress, leading to changes in various morphological traits. Bread wheat is one among them.

Wheat breeders have been able to improve the tolerance of cultivars to abiotic stresses through empirical selection in the environment. However, new phenotyping and genetic technologies and strategies can significantly improve rates of genetic gain (Reynolds, M. P *et al*., 2022) [35]. The integration of new tools and knowledge in the plant breeding process can lead to better breeding targets, improved choice of genetic diversity, more efficient phenotyping methods and strategies, and optimized integration of genetic technologies in the context of commonly used wheat breeding strategies (Reynolds, M. P *et al*., 2022) [35].

Bread wheat, scientifically known as *Triticum aestivum L.* (with a genetic composition of $2n = 6x = 42$, AABBDD), occupies a paramount position in the realm of agriculture and nutrition (Shewry, P. R., 2003) [40]. This cereal crop has emerged as a basis in ensuring global food security and promoting human health. The significance of wheat is underscored by its substantial contribution to both the quantity and quality of the world's food supply.

Nutritional status of wheat
Wheat provides approximately 20% of calories and protein to 4.5 billion people in various forms of food products (FAOSTAT 2022; Shiferaw *et al*., 2013) [42]. One of the most striking aspects of bread wheat is its rich nutritional value.

It is a source of vital nutrients, carbohydrates (70 g), proteins (13-15 g), including dietary fiber (12 g), fats (less than 2 g) B vitamins, such as thiamine (B1), riboflavin (B2), niacin (B3) and folate (B9), and essential minerals like iron, magnesium, phosphorus, and zinc (MacRitchie, F. 2012) [27]. The presence of dietary fiber is particularly noteworthy, as it plays a critical role in promoting digestive health and reducing the risk of chronic diseases. And it contains gluten, which is a protein that can be problematic for individuals with celiac disease or gluten sensitivity. Gluten-free alternatives are available for those who need to avoid it. These nutritional attributes make wheat a staple that contributes to a balanced diet and a healthy lifestyle.

Wheat's versatility in cooking applications is another witness to its significance. From the classic loaf of bread to pasta, breakfast cereals, and a multitude of baked goods, wheat forms the foundation of a wide range of food products. Its adaptability and ability to combine with various ingredients and flavors make it a cornerstone of global cuisine, catering to diverse tastes and cultural preferences. Furthermore, the importance of wheat extends beyond its nutritional and culinary roles. It has deep-rooted cultural and historical significance in many societies. Wheat has been a symbol of sustenance, fertility, and prosperity in various cultures throughout history. In religious rituals and traditional ceremonies, wheat often holds a central place, signifying its enduring importance in the human experience.

However, increased occurrence of abiotic stress like heat and drought in the area of wheat cultivation led to threatening food security and quality food product to the growing population of the world. Various attempts have taken place to develop climate-resilient varieties.

Importance of Abiotic Stresses in Wheat

Overview of Abiotic Stresses

Abiotic stresses pose formidable challenges to global agricultural production and the overarching goal of ensuring food security worldwide (Bailey-Serres, J *et al.*, 2006) [5]. Within this spectrum of challenges, heat stress (HS) and drought stress (DS) stand out as the most pivotal stressors, exerting profound and far-reaching impacts on crop growth, development, and productivity.

Drought stress

Drought stress, for instance, arises when a plant faces an inadequate supply of water to fulfill its physiological needs (Rizhsky, L *et al.*, 2002) [36]. It casts a shadow over a substantial portion of the global wheat-growing regions, precipitating yield reductions that can vary from 10% to a staggering 50%, contingent on factors like the duration of the stress, its severity, and the specific crop variety (Lobell, D. B *et al.*, 2012) [25]. Drought stress can be defined as a shortage of water that induces dramatic morphological, biochemical, physiological, and molecular changes in wheat (Sallam, A *et al.*, 2019) [37]. Drought limits photosynthetic efficiency due to stomatal closure, which reduces CO2 intake and increases leaf temperature, reduced soil moisture affects the mobility of nutrients, leading to deficiencies that can further impair plant growth. (Farooq *et al.*, 2009) [12]. The impact of drought stress tends to increase in some critical regions (Adel, S *et al.*, 2023) [1]. Wheat cultivars have adapted various drought tolerance mechanisms, including the formation of deeper roots, accumulation of higher biomass, better stomatal control over transpiration, enhancement of osmoprotective and antioxidant response, and better coordination of positive and negative regulation of gene expression (Kulkarni, M *et al.*, 2017) [22]. Drought stress triggers a diminishment in the water turgor potential, reverberating through to hamper cellular growth and expansion. This adversity reaches deeper still, touching upon pivotal plant physiological and biochemical processes, encompassing photosynthetic activity, respiration, stomatal conductance, chlorophyll content, metabolic pathways, ion conduction, the accumulation of reactive oxygen species (ROS), and the transport of sugars, to name a few. The repercussions of drought stress ripple through multiple dimensions of plant functioning (Rizhsky, L *et al.*, 2002) [36].

Some studies related to drought stress were during the stress condition which can induce differential expression of cytochrome P450, heat shock proteins, dehydrins, glutathione transferase, and other genes. Additionally, Microarray and RNA sequencing-based gene expression analyses have been used to understand wheat responses to various abiotic stresses, including drought stress (Kulkarni, M *et al.*, 2017) [22]. The tolerance ability of wheat genotypes to drought stress has been evaluated through culm-reserves contribution and grain filling physiology (Islam, M. A *et al.*, 2021) [17].

Heat stress

On the other hand, heat stress manifests when plants endure prolonged exposure to temperatures surpassing their critical threshold levels. This protracted exposure leads to the continual or irreparable impairment of growth and development. High temperatures disrupt an array of fundamental physiological and biochemical processes within the plant, leading to compromised growth, decreased photosynthetic activity, and, ultimately, diminished crop yield and quality (Bailey-Serres, J *et al.*, 2006) [5]. Heat stress can also cause biochemical disruptions in wheat, including changes in the activity of enzymes involved in photosynthesis, respiration, and other metabolic processes (Akter, N *et al.*, 2017) [3]. Heat stress can have an impact on wheat production from the seedling stage to the maturing of the grain, but it is most likely to reduce yields during the reproductive and maturing phases (Farhad, M *et al.*, 2023) [11]. Mitigation of abiotic stress tolerance in wheat through conventional breeding has been successful in improving the tolerance of cultivars to these stresses. There is a need for new strategies to improve the tolerance of wheat to heat stress, including the use of new breeding targets, improved choice of genetic diversity, more efficient phenotyping methods and strategies, and optimized integration of genetic technologies (Yadav, M. R *et al.*, 2022) [46].

These formidable stressors, DS and HS, have been proven to inflict substantial reductions in both crop yields and quality, resulting in tangible economic losses and exacerbating the issue of food scarcity on a global scale (Lobell, D. B *et al.*, 2012) [25]. Considering these far-reaching implications, it becomes abundantly clear that mitigating the adverse effects of heat stress and drought stress is of paramount importance. Achieving food security goals in the agricultural sector hinges upon our ability to address and manage these formidable challenges effectively.

Significance of Drought and Heat Stress on wheat

The era of global warming and climate change creates unusual rainfall and drastic increase in atmospheric temperature. Due to this, there is an occurrence of drought and heat stress to the crop plants including wheat. In wheat, reduction in yield (1000 grain weight) and quality (protein quality) due to HS and DS has been observed. Heat and drought stress impact wheat mainly during the blooming and grain filling stages of the crop (Farooq, M *et al*., 2014) [12]. Heat stress causes an increase in evapotranspiration rate, and it can enhance the water absorption from the soil which leads to terminal stress conditions for the crop (Farhad, M *et al*., 2023) [11]. Heat stress mainly coincides with the flowering stage. Similarly, drought stress creates lower water potential, and turgor potential in the leaf, and it affects various physio-bio-chemical mechanisms in the plant system (Farhad, M *et al*., 2023) [11].

Drought and heat stress are significant challenges to wheat production, and understanding the mechanisms of stress tolerance in wheat is essential for developing stress-tolerant cultivars. The integration of new tools and knowledge in the plant breeding process can lead to better breeding targets, improved choice of genetic diversity, more efficient phenotyping methods and strategies, and optimized integration of genetic technologies in the context of commonly used wheat breeding strategies (Akter, N *et al*., 2017; Farhad, M *et al*., 2023) [3, 11].

Tolerance Mechanisms for Abiotic Stress

In crop plants, there are two types of tolerance mechanisms: *viz.*, avoidance or escape mechanisms and tolerance mechanisms. The escape includes earlier flowering and early crop maturity. The avoidance and tolerance mechanism's are leaf rolling, change in leaf angle or leaf orientation, transpirational cooling, accumulation of osmoprotents, antioxidant defense, signaling mechanism *etc.*, (Akter, N *et al*., 2017; Farhad, M *et al*., 2023) [3, 11]

Component Traits for Stress Tolerance

Component traits of drought stress in wheat are deep and efficient deep root systems, increased water-use efficiency (WUE), reduced leaf area, early maturity, long awns, improved photosynthetic efficiency, osmotic adjustment (OS) to maintain the cell turgor potential transpiration efficiency (TE), which regulates the water flow and is mainly dependent on the root architecture and relative water content (RWC), which is a main physiological trait under stress to increase the grain yield and it showed positive correlation, therefore the RWC is a main physiological trait under stress to increase the grain yield. The drought impacts on chlorophyll apparatus and the chlorophyll content in the leaf shows variable responses with respect to the duration of drought stress. Initial 3 days of stress condition slight increase in the chlorophyll content and duration between 5 to 7 days which shows slight significant decrease and 13-15% reduction in 7th day onwards and goes on (Nikolaeva *et al*., 2010) [30]. The stomatal conductance is the major trait that is affected due to DS (Yang, X *et al*., 2021) [47]. The water scarcity condition reduces the stomatal conductance. Stomatal conductance plays a crucial role in the response of wheat plants to drought stress. Under drought conditions, wheat plants often reduce stomatal conductance to minimize water loss through transpiration. Stomatal regulation optimizes water use and minimizes water loss (Saradadevi, R *et al*., 2017) [38]. At the time of drought stress, various osmoregulatory solutes accumulate in the cell *viz.*, amino acids (proline), poly amines and anthocyanins *etc.* which acts as osmo protectants. The ROS level in the cell also hinders plant growth. Antioxidant defence systems protect against oxidative damage, while hormonal signaling pathways regulate stress responses (Kettani, R *et al*., 2023) [20].

Additionally, the component traits related to heat stress also more or less same, and some are cooler canopy, waxy leaf, and modifications of leaf morphology. The physiological traits associated with heat stress tolerance were grouped mainly into heat stress escape, heat stress avoidance and tolerance. The escape mechanisms include early blooming and early maturity. The stress avoidance mechanisms include the change in the leaf orientation (leaf angle), transcriptional cooling, leaf rolling and alteration of lipid membrane composition *etc.*, The perpendicular or vertically oriented leaf experiences less transcriptional water loss and leaf maintain the better water content (Jha, U. C *et al*., 2014; Devi, J *et al*., 2023) [19, 10]. The transcriptional cooling is the important avoidance mechanism. Efficient transpiration cooling occurs when there is a relatively higher evaporative demand due to significant heating effects, coupled with sufficient soil moisture to support evapotranspiration. Additionally, leaf rolling mechanism causes close of stomata and reducing the loss of water by avoid the exposure of whole leaf surface to heat stress. Furthermore, heat stress can affect the composition of lipids in membrane. Which induces changes in the membrane fluidity and permeability (Akter, N *et al*., 2017; Farhad, M *et al*., 2023; Devi, J *et al*., 2023) [3, 11, 10].

The accumulation of Osmo protectants, antioxidant defense mechanism and expression of stress proteins grouped under tolerance mechanisms. The predominate osmolytes such as proline, poly amines, glycine-betaine and sugars are the accumulated during the stress. These are playing a crucial role in protecting the cells from the oxidative damage caused by super oxide (02), hydrogen peroxide (H202) and other hydroxyl free radicles (OH) (Yang, X *et al*., 2021; Hill, C. B *et al*., 2022) [47, 16]. During the stress period various types of stress proteins are expressed due the concerned signaling genes. Especially, the five types of Heat shock proteins (HSPs) are produced and these acts as molecular chaperones and control protein folding. Additionally, the other component traits are NDVI (Normalized difference vegetation index), canopy temperature (CT) and stem reserve mobilization (Hill, C. B *et al*., 2022) [16]. The NDVI measures the different patterns of chlorophyll loss in the genotypes and stay green traits across the genotypes over the growth period of plant. The CT shows a positive correlation between grain yield and a deeper root system. Stem reserves are mobilized during the grain filling time, it associates with the remobilization of water-soluble carbohydrates (Jha, U. C *et al*., 2014; Hill, C. B *et al*., 2022) [19, 16].

Advanced Breeding Methods for Stress Breeding in Self-pollinated Crops

For successful breeding strategies for enhancing the stress breeding varieties achieving by both conventional breeding methods and advanced breeding approaches. The conventional breeding approaches includes basic level of hybridization and selection methods. Although these are time consuming and low throughput methods. Today's growing world these methods are not feasible to enhance the food production. Therefore, we are using advanced breeding strategies like RS, MAS, MARS, MABB, QTLs identification and mapping approach, GWAS, GS and transgenic approaches (Anand, A *et al*., 2023) [4].

Pedigree Method

The pedigree breeding method is a conventional breeding approach that involves selecting and crossing plants based on their desired traits to improve a particular crop variety. This method can also be applied to breeding for abiotic stress tolerance in wheat, such as drought and heat stress (Ahmar, S *et al*., 2020; Anand, A *et al*., 2023) [2, 4]. Performing systematically by selecting, crossing, evaluating, and selecting superior individuals, breeders can gradually enhance the tolerance of wheat varieties to these stresses, contributing to increased agricultural productivity and resilience in the face of challenging environmental conditions. Although the pedigree method is a time-consuming and low-throughput method, it has been successful in improving the genetic characteristics of crops.

Single Seed Descent (SSD)

SSD involves advancing one seed per generation from each selected plant, allowing for rapid fixation of desired traits. The SSD method allows for the efficient selection of individual plants with desired traits while preserving genetic purity within each selected line. This breeding approach enables the development of wheat varieties with improved abiotic stress tolerance, as individual plants are evaluated and selected based on their performance under stress conditions (Jeon, D *et al*., 2023) [18]. The SSD method can be a valuable tool in breeding for abiotic stress tolerance in wheat, contributing to the development of more resilient and productive varieties.

Recurrent Selection (RS)

Recurrent selection schemes can be optimized for parental selection, maintenance of genetic variation, and optimal cross-prediction (Merrick, L. F *et al*., 2022) [28]. The RS aims to improve stress tolerance by recurrently crossing selected plants and recombining desirable genes. It involves multiple cycles of selection to enhance stress resilience in wheat populations. Recurrent selection allows for the continuous recombination of genetic material and the accumulation of desirable traits over successive generations (Ramya, P *et al*., 2016) [34]. It offers the advantage of maintaining genetic diversity while gradually improving the population's tolerance to abiotic stress. By utilizing recurrent selection, breeders can develop wheat varieties that exhibit enhanced resilience to drought and heat stress, contributing to increased agricultural productivity in challenging environments. A recurrent selection program was conducted to improve the drought tolerance and yield of bread wheat using drought tolerant advanced breeding lines from a drought tolerant x susceptible cross (HI 1500 x HUW 510). The parental lines were evaluated for yield, biomass, and harvest index (HI) in addition to the drought adaptive traits like Canopy Temperature (CT), chlorophyll content, and Normalized Difference Vegetative Index (NDVI) (Ramya, P *et al*., 2016) [34].

Marker-Assisted Recurrent Selection (MARS)

Marker-assisted recurrent selection enables breeders to combine the advantages of traditional recurrent selection with the precision and efficiency of molecular markers. By integrating marker analysis and phenotypic evaluation, this method facilitates the selection of plants with desirable genotypes and phenotypes, accelerating the breeding process and enhancing the development of wheat varieties with improved abiotic stress tolerance. MARS involves evaluating plant performance across multiple environments and selecting individuals with superior performance under different stress conditions. It enhances the adaptability of wheat varieties to diverse stress environments.

Marker-Assisted Backcrossing (MABC)

Marker-assisted backcrossing (MABC) is a breeding method that enables selective insertion of targeted traits into the genome to improve yield, quality, and stress tolerance in wheat (Bellundagi, A *et al*., 2022; Gokidi *et al*., 2016) [6, 15]. MABC involves the transfer of a specific gene or trait from a donor parent to a recurrent parent through backcrossing and make the use of molecular markers in MABC allows breeders to select for specific traits and reduce the time required for the development of new varieties. Therefore, transferred four drought tolerance QTLs controlling traits *viz* canopy temperature, NDVI, chlorophyll content, and grain yield from drought tolerant donor line C306 into a high yielding, drought stress sensitive popular variety HD2733 (Kumar, P. K *et al*., 2023) [23]. Marker-assisted selection coupled with stringent.

Phenotypic selection was followed to advance each generation. It allows breeders to develop improved wheat varieties with enhanced abiotic stress tolerance more efficiently and accurately. By combining marker analysis with phenotypic evaluation, MAB provides a powerful tool for precise trait introgression and the development of stress-tolerant wheat cultivars. This approach accelerates the development of stress-resistant varieties by facilitating targeted gene transfer (Bellundagi, A *et al*., 2022) [6].

QTLs identification and mapping

One of the important focuses of biotechnology research in these days is to figure out the genetic basis of quantitative traits in crop plant by using various approaches. Various QTL mapping studies have been carried out in this regard to improve our understanding about the nature of inheritance of quantitative traits. It has been observed that numerous crucial agronomic traits, such as drought stress tolerance, exhibit a quantitative nature, governed by the influence of multiple genes, making them polygenic in essence. To investigate and pinpoint the chromosomal locations (loci) of genes responsible for these quantitative traits, specific molecular markers, such as SSR, SNP, DaRT, EST, AFLP, and RFLP, are employed. Moreover, a range of statistical tools and software applications, including MAPMAKER/QTL, QTL Network, QTL Cartographer, QGene, MQTL, MapQTL, QTLMapper, and PLABQTL, are used to analyze the data generated by these studies. The outcomes of these investigations yield the identification of loci known as "QTLs." These QTLs represent genomic regions that may encompass a single gene or a cluster of genes influencing the traits of interest.

QTL mapping studies have successfully revealed diverse traits, including the capacity of crop plants to withstand abiotic and biotic stresses, such as drought. The insights gleaned from QTL analyses hold significant promise for the long-term development of stress-tolerant crops. This potential is harnessed through the application of marker-assisted selection (MAS) and advanced molecular breeding strategies.

Genome-Wide Association Studies (GWAS)

Identification of linked markers to the trait of interest is the prime objective before marker assisted breeding. GWAS is one among the most important method of identifying MTAs using high-throughput genome-wide markers. To enhance the precision of improving complex quantitative traits for adaptation to specific environmental conditions such as drought and heat, it is crucial to pinpoint relevant genomic regions, known as Quantitative Trait Loci (QTLs). Linkage Disequilibrium (LD), which measures the non-random association of alleles at different genetic loci, is a valuable concept for identifying these genes or QTLs. Genome-Wide Association Studies (GWAS) have emerged as an effective method for this purpose. GWAS has gained widespread popularity for predicting candidate genes associated with complex traits in various crops. This method relies on utilizing densely distributed markers across the entire genome. The advantages of GWAS are manifold. It offers the capability to identify QTLs associated with multiple traits simultaneously and with high precision.

Notably, it stands out for its efficiency and cost-effectiveness when compared to traditional bi- parental QTL mapping methods, as exemplified by the work of Nonetheless, it is essential to recognize that the resolution and power of association studies hinge on the extent of LD across the genome. LD varies between populations due to factors such as historical relatedness, recombination frequency, and mating systems. Consequently, LD should be assessed in each study to ensure the validity and relevance of GWAS results, as highlighted by Edae *et al*. (2014). Such studies for drought and heat tolerance were done to find out putative regions of the genome governing stress tolerant activity. These findings can guide future breeding efforts to develop wheat varieties with improved abiotic stress tolerance through marker-assisted selection or gene editing techniques.

Genomic Selection (GS)

Marker-Assisted Selection (MAS) traditionally focuses on major effect Quantitative Trait Loci (QTLs) while often overlooking minor QTLs with meaningful impacts. To address this limitation, Genomic Selection (GS) offers a promising alternative. GS harnesses sophisticated genomic prediction models to estimate the genetic worth of individuals based on their genetic profiles. This approach streamlines the selection process for traits related to stress tolerance, thereby bolstering the accuracy and expediency of breeding programs. GS empowers breeders to make more informed choices by capitalizing on the wealth of genetic information furnished by genotyping technologies. Notably, it enables the evaluation and selection of individuals based on their genetic potential, referred to as Genomic Estimated Breeding Value (GEBV), even before their phenotypic traits are observable. In this approach, we focus on estimating the specific impact of each genetic marker. The cumulative effect of all these markers is then employed to calculate the Genomic-Estimated Breeding Values (GEBV) foreach individual. Given the current challenges posed by climate change, Genomic Selection (GS) emerges as a promising tool to enhance the genetic progress of individuals within breeding programs.

The fundamental process of any Genomic Selection strategy commences with the formation of a training population, comprising individuals for whom both genetic and phenotypic data are available. This combined information is used to construct a model in which the phenotype serves as the outcome, while the genotype acts as the predictor. Subsequently, the insights derived from this model are applied to predict the GEBV of the breeding population, which consists of individuals possessing solely genetic information. Genomic Selection (GS) offers a significant advantage in terms of substantially shortening the breeding cycle duration when compared to traditional breeding methods. It also effectively reduces the expenses associated with extensive phenotyping. As a result, this acceleration of genetic progress plays a pivotal role in ensuring food and nutritional security. Nonetheless, several key factors can impact the accuracy of genomic predictions. These include the size of the training and breeding populations, the genetic diversity within the breeding population, the heritability of the trait in question, the influence of genotype-environment interactions (GxE), marker density, and the genetic relatedness between the training population and the breeding population or selection candidates. For stress tolerance traits, such as abiotic stress tolerance, GS is particularly advantageous due to its capacity to encompass the polygenic nature of these traits. This elevates the efficiency and precision of breeding efforts by merging genomics with advanced statistical modeling techniques.

Genomic Selection (GS) for abiotic stress tolerance

Drought, high-temperature stress during crop growth phases, flood, and other natural disasters are on the rise as a result of climate change, resulting in considerable crop losses. Wheat yield reductions of up to 6.4 percent are expected with a 1°C increase in global temperature (Liu *et al*., 2016) [24]. In such cases, the most sustainable and cost-effective ways to compensate losses are to change cropping patterns or produce abiotic stress-tolerant cultivars. Identification of tolerant genotypes from germplasm and their use in breeding programmes have become critical requirements for the production of such kinds. The main challenge in breeding for abiotic stress resistance is their complex inheritance, poor heritability, and significant environmental impact.

Traditional breeding strategies for abiotic stressors have accuracy and repeatability limits. Though molecular markers have been used to identify and transfer yield QTLs under abiotic stress conditions (Almeida *et al*., 2013), they may not be effective because QTL from limited genetic resources explain little variation for grain yield under stress and are also highly influenced by genetic background as well as the environment and their interactions (Semagn *et al*., 2013). GS outperforms MAS in terms of prediction efficiency for abiotic stress tolerance (Cerrudo *et al*., 2018). Drought and heat tolerance have been demonstrated in wheat, maize, and rice using GS.

Genetic Engineering and transgenic approach

Drought and heat stress–tolerant germplasm might be developed quickly via genetic engineering. Although phenotyping has mostly been done under greenhouse conditions, there is a substantial literature on candidate genes and the examination of transgenic line performance under drought stress. Transgenic and genetic engineering approaches provide precise control over the introduction of specific genes into the wheat genome, offering the potential to enhance abiotic stress tolerance and other desirable traits.

Genetic engineering and the transgenic approach offer innovative solutions to address the pressing issue of climate resilience in wheat, a vital staple crop worldwide.

In an era of climate change characterized by rising temperatures, changing precipitation patterns, and an increase in extreme weather events, the need for crops that can adapt to these challenges is paramount. Genetic engineering, a technology that involves modifying an organism's genetic material, and the transgenic approach, which introduces specific genes from one organism into another, have become powerful tools in the quest to enhance the climate resilience of wheat. Wheat, a fundamental source of nutrition for billions of people, is susceptible to the adverse impacts of climate change. Droughts, heatwaves, shifting pest and disease pressures, and alterations in soil conditions are among the challenges wheat faces. Genetic engineering and transgenic approaches hold the promise of creating wheat varieties that can better withstand these environmental stresses, ultimately ensuring food security and sustainable agriculture in a changing world.

Success Stories in Breeding for Stress Resistance

The use of a wide range of germplasm, including wild forms and relatives, is critical for a successful breeding effort. Germplasm resources were examined and assessed to identify donor genotypes for drought and heat tolerance features. The evaluation of genotypes for stable grain production under diverse stress situations is a primary technique for identifying better wheat genetic stock for increased tolerance. *Triticum dicoccum* and *Aegilops geniculata* are possible gene pool sources for wheat heat and drought resistance development. Furthermore, *Triticum dicoccoides* and *Triticum monococcu*m have been discovered as potential germplasm sources for enhancing bread wheat heat tolerance. The heat tolerance of *Aegilops speltoides*, *Aegilops longissima*, and *Aegilops searsii* varied. Based on physiological and biochemical features, BARI GOM-28 was determined to be superior for heat stress endurance in a previous study. ALTAR 84/*Aegilops squarrosa* (192), 68.111/RGB-U/WARD/3/FGO/4/RABI/5/*Aegilops squarrosa* (629), 68.111/RGB- U/WARD/3/22 FGO/4/RABI/5/*Aegilops squarrosa* (878), and LCK59.6′1/*Aegilops squarrosa* (313). C306 and HI1136 (hexaploid wheat), *Triticum dicoccoides*, *Triticum monococcum* acc. BSP1 and *Aegilops speltoides ssp. liqustica* were discovered to be highly heat resistant in terms of grain yield (Sareen *et al*., 2012) [39].

The elite Indian variety GW322 was transplanted with QTLs relevant to drought and heat tolerance features from HI1500 using the MABB technique. Similarly, another Indian cultivar, HD2733, was enhanced with drought-tolerant QTL by MABB (Todkar *et al*., 2020; Rai *et al*., 2018) [45, 33]. To create a high-yielding drought tolerant genotype, four wheat cultivars, HUW468, HUW234, DBW17, and K307, were introgressed with Qyld.csdh.7AL (Gautam *et al*., 2020) [14]. Seven stable drought-related QTLs for DH (QDh. iari 6D), GWPS (QGWPS.iari 5B), PH (QPh.iari 4B-2, QPh.iari 4B-3) and NDVI (QNdvi1.iari 5D, QNdvi3.iari 5A) were discovered using SSR and SNP markers in a backcross inbred line population established from HD2733/2 (Puttamadanayaka *et al*., 2020) [31]. Drought-tolerant QTLs were transferred from the doners HI1500 and DBW43 to the elite variety HD3086 at IARI, New Delhi. This hybrid produced improved lines with drought tolerance and rust resistance, including as HD3470 and HD3471, which were submitted to AICRP testing (Sunilkumar *et al*., 2022) [43].

The MABB technique was used to transfer four drought tolerance QTLs controlling canopy temperature, normalized difference vegetative index (NDVI), chlorophyll content, and grain yield from a drought-tolerant donor line, C306, into a popular high-yielding, drought-sensitive variety, HD2733, resulting in improved 23 lines with a range of 85.35 percent -95.79 percent background recovery (Kumar *et al*., 2023) [23]. Using 251 recombinant inbred lines (RILs) derived from a hybrid HD 2808/HUW510, 40 QTLs associated with heat-tolerant characteristics were discovered under terminal heat stress (Bhusal *et al*., 2018) [7]. Heat tolerant QTLs related with early anthesis, and high kernel weight were transferred from the donar parent (WH730) to the high yielding variety HD2733 using the MABB method (Bellundagi *et al*., 2022) [6].

The MARS strategy, which uses marker effects for a few minor or large QTLs, followed by two or three rounds of recombination, is another method for simultaneous identification and pyramiding QTLs. MARS was used to test drought and heat tolerance by conducting multi-location phenotyping under drought (limited irrigation) and irrigated conditions, and progenies containing favorable QTLs were found. To find the best progenies, multi-location data and genotyping with SSR markers linked with stress adaptation features were used.

Table 1: QTLs identified across the different populations, *NOTE*: DH=drought and heat stress; D=drought; DH=double haploid; RILs=Recombinant inbred lines; DMA=days to maturity; YLD=yield traits; TGW=thousand grain weight.

Stress type	Mapping population	Parents	traits	Number of QTLs	PVE (%)	References
DH	RILs	SeriM82/Babax	DMA	6	19.6	Tahmasebi *et al.* (2016) [44]
D	DH	Kukri/Excalibur	Yield traits	98		Gahlaut *et al.* (2017) [13]
DH	RILs	SYND / Weebill1	YLD traits	5	26.6	Liu *et al.* (2019) [24]
DH	RILs	Ningchun4 / Ningchun27	TGW	5	27.53	Shi *et al.* (2021) [41]
D	RILs	Reeder/albaney	YLD	1 major	13.94	Rabbi *et al.* (2021) [32]
H	RILs	GW322/KAUZ	YLD	6	13.84	Manjunath *et al.* (2022) [9, 43]
H	RILs	GW322/KAUZ	Days to heading	5	10.57	Manjunath *et al.* (2023) [9, 43]

The genome-wide association study (GWAS) for drought and heat tolerance was conducted to identify potential regions of the genome that influence stress tolerance. A GWAS was used to study flowering time (FT), leaf relative water content (RWC), osmotic adjustment (OA), chlorophyll content (SPAD), and leaf rolling (LR) in durum wheat, which discovered 15 significant QTLs for OA (global R2 = 63.6 percent) (Condorell *et al*., 2022) [8]. DArTseq data from 93 diverse bread wheat lines evaluated under normal and drought stress conditions revealed 62 significant marker-trait associations (MTAs) for traits such as days-to-heading (DTH), number of days-to-maturity

(DTM), plant height (PHT), spike length (SPL), number of kernels per spike (KPS), thousand kernel weight (TKW), and grain yield (GYLD). The GWAS study looked at the genetic areas that influence grain protein content (GPC), 1000 kernel weight (TKW), and normalized differential vegetation index in 280 bread wheat genotypes (NDVI).

A total of 26 marker-trait associations (MTAs) for various essential features in bread wheat were discovered across 18 chromosomes (Krishnappa *et al*., 2023) [21]. The GWAS mapping approach was used for advanced breeding lines from IARI, and phenotyping for yield and component traits was done across five locations. The study discovered 57 distinct markers related with diverse qualities across places, with 23 of them proving to be stable (Devate *et al*., 2022) [9]. As a continuation of the research, the GS technique was used in the IARI abiotic stress breeding programme using 13 different GS models. Selection of lines, creation of a magic population, and application of GWAS data in GS to identify superior drought and heat tolerance lines.

When challenged to water stress, transgenic wheat lines expressing the barley (*Hordeum vulgare* L.) gene *HVA1*, which encodes for a series of late embryogenesis abundant proteins that function as Osmo protectants, displayed enhanced water consumption efficiency, biomass accumulation, and root weight. The proline inducing gene (*P5CS*) increased drought tolerance, most likely due to proline's antioxidant protection of cells from oxidative damage caused by oxygen free radicals. Furthermore, an E. coli mannitol biosynthesis (*mtlD*) gene that served as an Osmo protectant boosted wheat drought tolerance.

Conclusion

Abiotic stresses, particularly drought and heat stress, significantly impact wheat production and global food security. As climate change intensifies, understanding these stresses and their mechanisms becomes increasingly crucial. Integrating genetic tools to enhance abiotic stress resilience in wheat is vital for developing varieties that can thrive under adverse conditions. To effectively combat drought and heat stress, it is essential to incorporate stress tolerance mechanisms into breeding programs. This includes understanding the genetic basis of stress responses, which involves identifying key genes and pathways associated with tolerance. Genomic approaches, such as Genome-Wide Association Studies (GWAS) and quantitative trait locus (QTL) mapping, have proven invaluable in elucidating the complex genetic architecture of stress resilience. By pinpointing loci associated with heat and drought tolerance, breeders can make informed decisions in their selection processes.

In addition to identifying candidate genes, it is important to explore novel traits that confer resilience. For instance, traits such as deep root systems, efficient stomatal regulation, and enhanced photosynthetic capacity can significantly improve a plant's ability to withstand heat and drought stress. Advances in phenomics—high-throughput phenotyping technologies—allow for the rapid assessment of these traits in diverse wheat genotypes, facilitating the selection of superior candidates. The integration of advanced breeding methods, such as genomic selection and marker-assisted breeding, will expedite the development of stress-resistant wheat varieties. Genomic selection leverages predictive modeling based on genomic data to estimate the breeding value of individuals, allowing for more precise and efficient selection. Meanwhile, marker-assisted breeding can assist in tracking desirable alleles associated with stress resilience, speeding up the breeding cycle and reducing the time to market for new varieties. Furthermore, collaboration between plant biologists, geneticists, and breeders is essential for translating genomic findings into practical breeding strategies. This multidisciplinary approach will enhance our understanding of the interplay between genetic, physiological, and environmental factors influencing stress tolerance.

Ultimately, the integration of genetic tools, coupled with a comprehensive understanding of stress tolerance mechanisms, will not only enhance wheat resilience to heat and drought but also contribute to sustainable agricultural practices. As we strive to secure global food systems in the face of climate challenges, prioritizing research in these areas will be crucial for ensuring the production of robust, high-yielding wheat varieties that can withstand the rigors of a changing environment.

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