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Advancements in the Molecular Mechanisms of Heat Tolerance in Maize: Gene Regulation and Physiological Responses

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Abstract This study reviews recent advancements in the genetic regulation and physiological adaptations of maize under heat stress. It focuses on key regulatory networks, including heat shock proteins (HSPs), transcription factors (HSFs, AP2/ERF, and bZIP), and signal transduction pathways such as calcium signaling, MAPK cascades, and ABA-mediated responses. The study also analyzes the physiological and biochemical adaptation mechanisms of maize to heat stress, including antioxidant defense systems, osmolyte accumulation, and photosynthetic regulation. It highlights the genetic progress in breeding for heat tolerance, such as QTL mapping, genomic selection, and CRISPR-based gene editing. Through case studies of heat-tolerant maize varieties and field trials, this study provides practical insights into integrating molecular breeding and agronomic strategies. This study emphasizes the combination of molecular breeding and precision agriculture to cultivate maize varieties that adapt to climate change, thereby ensuring sustainable production under high temperature environments.

Keywords Maize (*Zea mays* L.); Heat tolerance; Gene regulation; Genetic breeding; Physiological response

1 Introduction

Maize (*Zea mays* L.) is an annual herbaceous plant of the genus Zea in the Poaceae family. It is native to Central and South America and is now cultivated all over the world. It is an important food crop and feed crop, and is also the crop with the highest total output in the world. Its planting area and total output are second only to rice and wheat. As a crop mainly grown in tropical and subtropical regions, maize is particularly sensitive to environmental stress, among which heat stress has become a key factor restricting its sustained high yield. High temperature environment, especially during key growth periods such as flowering and filling, can seriously interfere with the physiological processes of maize, destroy cell homeostasis, and ultimately lead to a decrease in yield (Wu et al., 2020; Liu et al., 2022; Chen et al., 2023).

In recent years, the negative impact of climate change on agricultural production has become increasingly significant. In the long run, extreme climate events (such as natural disasters such as droughts and floods) have occurred frequently, directly leading to a reduction in crop yields. As a crop that is sensitive to temperature and water conditions, corn requires suitable environmental conditions for its growth and development. When climate conditions deviate from the optimal range, corn yields will be significantly affected. Specifically, when the temperature exceeds the optimal growth range of corn (usually 30 °C~35 °C), plant photosynthesis is inhibited, and problems such as premature leaf aging, reproductive development disorders, and poor grain development occur frequently. These adverse effects often work in synergy with abiotic stress factors such as drought, further exacerbating yield losses (Saini et al., 2021; El-Sappah et al., 2022). Rising temperatures may also induce outbreaks of corn pests and diseases (such as borers and diseases), posing a double threat to corn production.

This study will systematically review the research progress on the molecular mechanism of corn heat tolerance in recent years, focusing on the regulation of heat-responsive genes, the mechanism of action of transcription factors and signaling pathways, and the adaptive changes of corn at the physiological and biochemical levels, and introduce how new technologies in genomics and functional analysis can accelerate the discovery of key heat-resistant genes. By integrating the research results at the molecular and physiological levels, this study is expected to provide a theoretical basis for the development of corn varieties that adapt to climate warming, thereby promoting sustainable agricultural production.

2 Impact of Heat Stress on Maize Growth and Development

2.1 Effects on morphological traits

High temperature and heat damage is a natural disaster that may occur in corn production. If there are disastrous climatic conditions such as continuous high temperature and drought or cloudy and rainy weather with little sunshine, it will seriously affect the growth and development, resulting in reduced yield or even total crop failure. When corn is in a meteorological condition where the daily average temperature is greater than or equal to 35 ℃ and lasts for more than 5 days, and ineffective rainfall lasts for more than 8 days, high temperature and heat damage will inevitably occur.

High temperature stress will significantly change the morphological development of corn plants, usually manifested as reduced plant height, reduced leaf area and inhibited root growth. During the flowering and pollination period of corn, drought and high temperature delay the development of anthers, missing the mature stage of female spike filaments, that is, the flowering period is not met; the improper use of herbicides causes herbicide damage or other reasons that lead to uneven development of plants in the field, which will undoubtedly affect the quality of pollination; the lack of phosphorus in plants causes poor anther development. High temperature during the vegetative growth period will inhibit the expansion and elongation of cells, resulting in short plants and reduced biomass accumulation. Common high temperature stress responses also include leaf curling and premature senescence, which further reduce the photosynthetic area and affect the photosynthetic efficiency and yield of crops. For example, maize hybrids such as ZD309 exhibit phenotypes such as plant dwarfing and leaf curling under heat stress conditions, indicating that their growth is significantly inhibited (Liu et al., 2022). High temperatures also inhibit root development, manifested as reduced root length and root volume, thereby limiting the absorption of water and nutrients (Xue et al., 2024). These adverse morphological changes not only affect the growth and development of individual plants, but also lead to weakened group growth and reduced overall field performance.

2.2 Physiological and biochemical responses to heat stress

Under high temperature stress, corn will initiate a series of physiological and biochemical reactions to alleviate damage and maintain cell homeostasis. The impact of high temperature stress on corn is not limited to changes in morphological characteristics, but also triggers a series of complex physiological and biochemical reactions to maintain cell homeostasis and improve stress resistance. Studies by Xi et al. (2022) and Xie et al. (2022) showed that the photosynthesis system is one of the most significant physiological processes affected by heat stress. High temperature can lead to a decrease in chlorophyll content, destroy the thylakoid membrane structure, reduce the activity of photosystem II (PSII), and thus inhibit the electron transport chain and carbon assimilation process. The decrease in Rubisco enzyme activity and the reduction in carbon dioxide supply caused by stomatal closure further weaken the photosynthetic efficiency.

At the same time, heat stress will cause a large amount of reactive oxygen species (ROS) such as O_2^- and H_2O_2 to accumulate in plants, inducing oxidative stress. To reduce the toxic effects of ROS, corn activates the antioxidant defense system, including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR), which effectively remove free radicals and protect cell membrane structure and enzyme system. Plants also alleviate cell dehydration and membrane lipid peroxidation and enhance cell stability by accumulating osmotic regulating substances such as proline, soluble sugar and betaine. Heat shock proteins (HSPs) are also an important component of corn's response to high temperature stress. For example, HSP70, HSP90 and small molecule heat shock proteins (sHSPs) can assist in the correct folding and repair of proteins, prevent heat-induced protein aggregation, and improve cell heat resistance.

In response to these changes, corn plants activate antioxidant defense systems, including enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) to remove excess reactive oxygen species (ROS) (Wu et al., 2020). At the same time, plants also synthesize osmotic regulating substances such as heat shock proteins (HSPs), proline, and soluble sugars to stabilize cell structure and protect protein function.

2.3 Effects on reproductive development and grain yield

The reproductive development stage of corn, especially the pollination and filling stages, is extremely sensitive to high temperature stress and is a critical period for determining the final grain yield. Djalović et al. (2023) pointed out that high temperature stress seriously interferes with the pollen formation, pollination process and grain filling mechanism of corn, thereby significantly reducing yield and quality. High temperature can directly damage the anther development process, resulting in decreased pollen vitality, reduced pollen grain number and reduced germination rate. The sensitivity of filaments also decreases with increasing temperature, resulting in delayed silking or drying of filaments, which in turn causes male and female asynchrony and affects the success rate of fertilization.

Heat stress can also have a negative impact on pollen tube growth and ovule development, reducing the efficiency of endosperm and embryo formation. During the grain formation stage, high temperature interferes with the transport and distribution of photosynthetic products, causing a decrease in the grain filling rate and a shortened filling duration, resulting in small grains, poor plumpness, and decreased thousand-grain weight. They further emphasized that heat stress not only affects the direct physiological process of yield formation, but also involves imbalances in hormone signals (such as auxin, abscisic acid, and ethylene) and abnormal expression of heat stress response genes. There are obvious differences in the response of different genotypes of corn to high temperature stress during the reproductive stage. Some heat-resistant materials show strong pollen heat tolerance, timely silking ability, and good grain filling stability. The use of genetic variation mining and breeding strategies, such as genomic selection, phenotypic precision screening, and QTL positioning, has become an effective way to improve the heat tolerance of corn in the reproductive stage.

3 Heat-Responsive Genes and Regulatory Networks in Maize

3.1 Heat shock proteins (HSPs) and their role in protein stability

Heat shock proteins (HSPs) are a class of highly conserved molecular chaperones that play a key role in maintaining protein stability under high temperature stress conditions. High temperatures often cause protein denaturation or misfolding, leading to cellular dysfunction. HSPs are rapidly induced to express at this time, assisting the correct folding of new proteins, the refolding of damaged proteins, and preventing their aggregation and inactivation. The main HSP families include HSP70, HSP90, HSP60, HSP100 and small molecule heat shock proteins (sHSPs), which play their own unique roles in cell protection. In maize, HSP genes are highly upregulated under high temperature stress, and their expression levels are closely related to the crop's heat tolerance. These proteins not only maintain protein homeostasis, but also interact with membrane systems and organelles to enhance cell structural stability. For example, overexpression of the maize heat shock transcription factor ZmHsf04 in *Arabidopsis* can significantly upregulate the expression of heat-specific HSP genes, thereby improving the heat tolerance of plants (Jiang et al., 2017). HSPs can also work synergistically with heat shock transcription factors (HSFs) to regulate the expression of other heat stress response genes, forming an important part of the maize heat stress regulatory network. The transcription factor ZmHsf17 further promotes the maintenance of protein stability and membrane integrity under heat stress conditions by regulating phosphatidic acid phosphohydrolases involved in lipid metabolism.

3.2 Transcription factors in heat stress response

Transcription factors (TFs) play a key regulatory role in the process of plant response to high temperature stress, and can activate a series of physiological and molecular defense mechanisms by regulating the expression of a large number of stress response genes. Various transcription factor families have important functions in improving plant heat tolerance, mainly including heat shock transcription factors (HSFs), WRKY, NAC, MYB, DREB and bZIP. They provide plants with multi-level heat stress adaptation capabilities by regulating pathways such as antioxidant defense, osmotic regulation, hormone signaling, and protein homeostasis. Among them, HSFs are the most intensively studied class of heat-responsive transcription factors, which can recognize and bind to heat shock elements (HSEs) and rapidly induce the expression of defense genes such as heat shock proteins (HSPs).

In maize, members such as ZmHSFA2, ZmHSFB1, and ZmHSFC1 have been shown to play an important role in the heat stress regulatory network. In addition to HSFs, WRKY transcription factors regulate ROS scavenging and antioxidant enzyme expression by binding to W-box elements, and participate in the regulation of hormone signaling pathways. NAC transcription factors not only regulate plant development, but also regulate programmed cell death and stress response gene expression in heat stress (Xi et al., 2022). MYB family members, especially R2R3-type MYBs, are involved in regulating secondary metabolism, stomatal regulation, and antioxidant defense systems. DREB transcription factors activate gene expression related to osmotic protection and stress signaling pathways by binding to DRE/CRT elements. bZIP transcription factors are widely involved in ABA-mediated signal regulation, promoting protein folding, ROS homeostasis maintenance and stress defense response. Heat stress response is a complex network composed of coordinated regulation of multiple transcription factors, signal interactions and feedback regulation. The integration of transcriptomics and functional genomics research helps to identify key transcription factor "regulatory hubs" in maize, providing targets and theoretical support for molecular breeding of heat-resistant varieties (Kumar et al., 2023; Li et al., 2024).

3.3 Signal transduction pathways in heat stress response

Heat shock factors are not only key transcriptional regulatory factors themselves, but also form a complex regulatory network with other signal molecules. For example, the transcription factor ZmHsf17 can interact with members of the HsfA2 subclass and jointly participate in the transcriptional regulation of genes related to lipid metabolism and membrane stability, playing an important role in ensuring the integrity of cell membrane structure under high temperature conditions (Zhang et al., 2024). The bZIP signaling pathway also plays an important role in the heat stress response of maize. In particular, the Unfolded Protein Response (UPR) mediated by bZIP60 is activated under high temperature stress conditions, which can alleviate heat-induced protein misfolding and photosynthetic system damage in cells by regulating the expression of heat shock protein genes and chlorophyll decomposition processes (Li et al., 2015; 2020).

Heat stress signal transduction pathways usually also include other classic mechanisms, such as calcium ion signaling (Ca^{2+} signaling), reactive oxygen species (ROS) signaling, and plant hormone signaling networks (such as ABA, ethylene, JA, etc.). These signaling systems enhance the heat tolerance of plants as a whole by rapidly transmitting heat stress information, activating antioxidant enzyme systems, and regulating stomatal behavior and cellular metabolic processes.

4 Physiological and Biochemical Adaptations to Heat Stress

4.1 Antioxidant defense mechanisms

Reactive oxygen species (ROS) are key signaling molecules that enable cells to respond quickly to different stimuli. High temperature stress often leads to oxidative stress, manifested by the accumulation of reactive oxygen species (ROS) such as superoxide anion radicals (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH·), which ultimately lead to biofilm peroxidation, protein structure destruction, and DNA damage. The production of ROS in plants is precisely regulated by an antioxidant enzyme system composed of catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX), and peroxidase (GPX), as well as a non-enzyme system composed of antioxidants such as ascorbic acid (ASA) and glutathione (CSH), so that ROS in plants is maintained at a physiological level that plants can tolerate.

Corn effectively alleviates the damage caused by ROS by enhancing enzymatic and non-enzymatic antioxidant mechanisms. The study by Yang et al. (2021) found that ZmRPP13-LK3 responded significantly to high temperature stress and was regulated by ABA. The cAMP catalyzed by it significantly increased the activity of antioxidant protection enzymes, heat shock proteins (HSPs), ABA-related transcription factors and other gene expressions in corn leaves; it proved that ZmRPP13-LK3 interacted with ABC2, suggesting that the cAMP catalyzed by ZmRPP13-LK3 may be transported out of the mitochondria through ABC2 and regulate the reverse signaling pathway from mitochondria to the nucleus; cAMP pretreatment significantly improved the survival rate of corn plants under high temperature stress conditions.

4.2 Osmolyte accumulation and membrane stability

Heat stress can cause an imbalance in cell osmotic pressure and have a significant impact on the stability of membrane structure. In response to this, corn accumulates osmotic regulating substances (such as proline, betaine, and soluble sugars) to maintain cell turgor, protect the integrity of macromolecular structures, and stabilize the cell membrane system. These osmotic regulating substances can not only act as molecular chaperones to prevent protein denaturation and aggregation under high temperature conditions, but also play an important role in cell osmotic regulation. Heat stress can also induce changes in membrane lipid composition, manifested as an increase in the proportion of saturated fatty acids, thereby reducing membrane fluidity and enhancing membrane stability (Chen et al., 2023). Under high temperature stress conditions, maintaining the integrity of the cell membrane is of vital importance to ensuring the normal physiological function of cells and preventing electrolyte extravasation.

4.3 Heat-induced modifications in photosynthesis and carbon metabolism

4.3.1 Downregulation of photosystem II efficiency and RuBisCO activity

Photosystem II (PSII) is very sensitive to high temperatures. Heat stress can damage its oxygen supply complex and reduce electron transfer efficiency, thereby causing a decrease in chlorophyll fluorescence and a decrease in photochemical efficiency (Fv/Fm ratio). At the same time, the activity and stability of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), which is responsible for carbon fixation, are also significantly reduced at high temperatures (Singh et al., 2020; Wu et al., 2020). Heat shock proteins (HSPs) and molecular chaperones play an important role in maintaining the structural stability of these photosynthetic proteins, helping to alleviate heat damage.

4.3.2 Role of C_4 metabolism in enhancing heat tolerance

C_4 plants have shown higher adaptability in evolution. Compared with C_3 plants, they have significant advantages in growth rate, carbon dioxide use efficiency and water use efficiency. As a typical C_4 plant, corn has a natural physiological advantage in photosynthetic adaptation mechanism. It can fix carbon dioxide more efficiently through the C_4 photosynthetic pathway, while significantly reducing water consumption, thereby synthesizing more carbohydrates in a shorter time. Under heat stress conditions, the expression levels of key enzymes in the C_4 pathway (such as phosphoenolpyruvate carboxylase PEPC, NADP-malic enzyme and pyruvate phosphate dikinase PPDK) are dynamically adjusted to optimize the carbon assimilation process. This metabolic flexibility enables corn to maintain high photosynthetic efficiency and biomass accumulation capacity in high temperature environments (Djalović et al., 2023; Li et al., 2024).

4.3.3 Regulation of sugar transport and source-sink dynamics

Heat stress also affects the distribution and transport of photosynthetic products. Corn maintains an effective source-sink relationship by regulating the expression of sugar transporter genes and phloem loading mechanisms. The enhanced activity of sucrose transporters (SUTs) and SWEETs family members helps transport photosynthetic carbohydrates from leaves to developing grains and roots. Trehalose metabolism also shows an important role in stress signal transduction and energy homeostasis regulation (Liu et al., 2022; Jagtap et al., 2023).

5 Progress in the Inheritance and Breeding of Heat Tolerance in Maize

5.1 Accurately explore heat-resistant germplasm resources and gene loci

According to statistics, for every 1 °C rise in global temperature, corn yield will decrease by 7.4%, which is significantly higher than that of rice (reduction of 3.2%). When high temperature stress occurs at the same time as the flowering period of corn, its negative impact will be further aggravated. A large number of studies have focused on the multi-faceted effects of high temperature stress on corn growth and development, including key stages such as flowering pattern, pollen vitality, pollination and fertilization process, and early seed development. The screening of heat-resistant germplasm resources is the primary link in heat-resistant breeding. Technical means such as quantitative trait loci (QTL) positioning and genome-wide association analysis (GWAS) have played an important role in identifying potential QTLs and dominant genes, which can be introduced into excellent varieties to enhance their heat tolerance (El-Sappah et al., 2022; Djalović et al., 2023). For example, the study by Seetharam et al. (2021) identified important single nucleotide polymorphisms (SNPs) associated with grain yield under heat stress, highlighting the genetic diversity in tropical corn germplasm and providing valuable genetic resources for heat-resistant breeding. In addition, the study by Inghelandt et al. (2019) showed that in temperate maize, QTL mapping technology has been used to evaluate phenotypic and genotypic diversity related to heat tolerance, revealing multiple specific QTLs related to heat tolerance, providing important molecular markers for heat-resistant breeding.

5.2 Integration of genomic tools and molecular breeding technology

The latest research of Jiang Haiyang's team in the National and Local Joint Engineering Laboratory for Crop Stress Breeding and Disaster Reduction, College of Life Sciences, Anhui Agricultural University on high temperature resistant corn breeding highlights the integrated application value of genomics and modern biotechnology. The research team used high-throughput sequencing technology to conduct genome-wide association analysis (GWAS) and discovered multiple key genes that regulate heat stress response, such as encoding heat shock proteins (HSPs), antioxidant enzymes and osmotic regulatory proteins. Gene editing technologies (such as CRISPR/Cas9) have been applied to the precise regulation of functional genes, and multiple heat-resistant mutants have been successfully obtained, which has improved the survival rate and photosynthetic efficiency of materials under high temperature conditions. These studies not only promoted the analysis of the genetic basis of maize heat tolerance, but also provided technical support for directional breeding.

5.3 Hybrid breeding and germplasm innovation

Hybrid breeding is a traditional method of using hybrid vigor in corn, which can improve corn yield and pest and disease resistance. In hybrid breeding, parents need to be selected for hybridization to obtain excellent offspring. The key to hybrid breeding is to select suitable parents. In general, varieties with excellent economic traits should be selected as parents. Among the hybrid offspring, some hybrids with excellent traits can be obtained, which usually have higher yields and stronger resistance than purebreds. Stacked heterosis refers to the production of more hybrids through multiple hybridizations on the basis of hybrid breeding, thereby improving yield and quality. This method can further improve hybrid vigor, thereby improving corn yield and quality. The key to stacking heterosis is to select suitable parents for hybridization, and at the same time pay attention to mating protection to avoid adverse effects. The development of heat-resistant hybrids (such as ZD309) shows that combining the genetic advantages of parental lines can enhance adaptability to heat stress (Liu et al., 2022).

Germplasm improvement is the genetic improvement of a certain type of germplasm to form better germplasm materials, which has a broader meaning. Germplasm innovation emphasizes innovation and creates new traits or new groups. For example, high-oil corn is a classic germplasm innovation work. There was no high-oil corn in the world, but it was formed through continuous gene aggregation. By collecting domestic and foreign germplasm resources, we carry out precise identification targeting production needs and industrial needs, and screen out breeding materials that are urgently needed for breeding, such as high salt-alkali tolerance materials, high protein, and high oil materials. Using modern breeding methods such as key genes or gene editing, we can create some new variants or new materials that are difficult to obtain in nature. The development of modern breeding technology provides such opportunities.

6 Case Studies of Heat Tolerance in Maize

6.1 High-temperature resilience in tropical and temperate maize varieties

In recent years, the Chongqing Academy of Agricultural Sciences has been deeply promoting agricultural breeding research projects, strengthening the construction of the core germplasm resource bank of corn, and focusing on screening local varieties and breeding materials that perform well in high temperature environments. In 2024, Dong Xin, an associate researcher at the Institute of Corn and Specialty Crops of the Academy, participated in a major breakthrough in the study of corn heat resistance, successfully determining the key threshold of the number of corn spikelets under high temperature stress, which can provide a basis for breeding and seed selection in nearly half of the world's corn-growing areas. He and Dr. Zhang Yingjun of China Agricultural University conducted precise identification of the backbone corn varieties of different ages in China and the United States, systematically analyzed the evolutionary trends of the male and female ears, yield characteristics and heat resistance of corn germplasm resources, and determined that under high temperature climate stress, the size of the corn male ear is indeed the key to affecting yield, and concluded that high temperature-resistant corn varieties require the number of male spikelets to be ≥ 700 spikelets and flowering to ensure normal pollination of corn and achieve high and stable yields.

6.2 Field trials of heat-tolerant hybrids and their performance evaluation

Field trials are an important part of maize heat tolerance research and breeding practice. Through planting and measurement in real agricultural ecological environments, the physiological, agronomic and yield performance of varieties under high temperature stress can be comprehensively evaluated (Longmei et al., 2021; Elmyhun et al., 2024). Li et al. (2024) from the Institute of Botany, Chinese Academy of Sciences, constructed a transcriptome map of maize under heat stress and used co-expression network analysis to identify that the HSF and ERF transcription factor families were highly enriched in the "heat response" module, and further locked in the core heat shock transcription factor ZmHSF20. Functional verification showed that under high temperature stress conditions, the Zmhsf20 mutant had stronger heat resistance than the wild type, while the ZmHSF20 overexpression line showed higher high temperature sensitivity, indicating that ZmHSF20 plays a negative regulatory role in regulating maize heat tolerance (Figure 1).

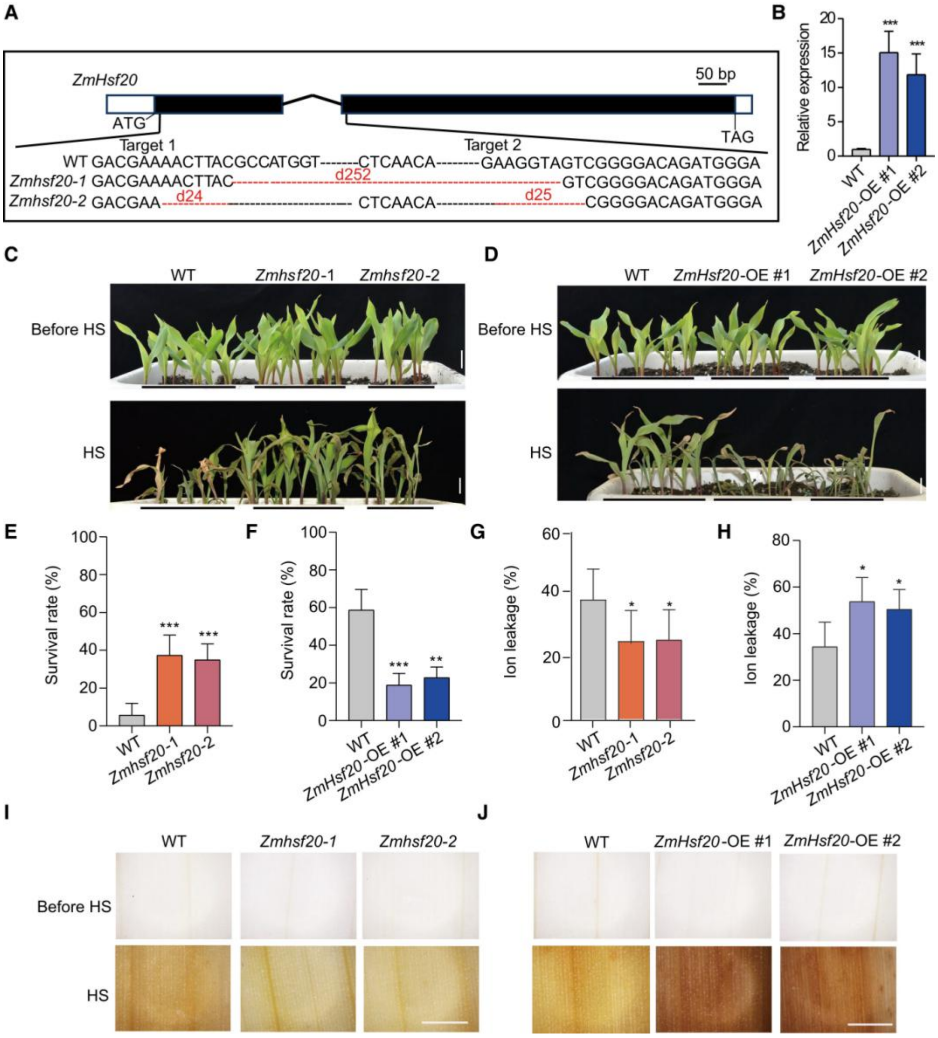

Figure 1 Heat tolerance is modulated by ZmHsf20. A) Construction of CRISPR/Cas9-mediated Zmhsf20 knockout lines (Adopted from Li et al., 2024)

In order to verify the performance of the ZmHSF20 regulatory mechanism in actual agricultural environments, the researchers subjected Zmhsf20 mutants, ZmHSF20 overexpression lines and wild types under different genetic backgrounds to field heat stress treatment and systematic evaluation. The results showed that under continuous high temperature environment, the performance of Zmhsf20 mutants was significantly better than that of wild types, and their pollen vigor, fruit set rate, 1000-grain weight and yield retention ability were significantly enhanced. At the same time, physiological parameters such as leaf photosynthetic efficiency, antioxidant enzyme activity and cell membrane integrity also showed that the mutants had stronger high temperature adaptability.

Further studies revealed that ZmHSF20 indirectly affected the expression of cell wall construction-related genes such as *ZmPAL1* by inhibiting the expression of its downstream regulatory factors ZmHsf4 and cellulose synthase gene *ZmCesA2*, thereby regulating cell wall stability. Enhanced expression of ZmHSF4 and *ZmCesA2* can improve cell wall stability and heat resistance. Double mutant experiments (Zmhsf20-1/Zmhsf4-1) verified the key role of ZmHsf4 downstream of ZmHSF20, which manifested as heat-resistant growth advantage in the field.

6.3 Integration of molecular and agronomic strategies for climate-resilient maize

In the study of high temperature stress, identifying and regulating stress response genes and their signaling pathways has become an important means to improve heat tolerance. ZmNAC074 is a typical stress-responsive gene in the maize NAC transcription factor family. Xi et al. (2022) found that it encodes a membrane-bound transcription factor (MTF) that may be located on the membrane. Under various abiotic stress conditions, the expression level of ZmNAC074 in leaves was significantly upregulated, especially under high temperature stress. This suggests that ZmNAC074 may play a core role in plant heat stress signal transduction and defense system. Through functional verification experiments of transgenic *Arabidopsis*, the researchers further revealed the regulatory pathway of ZmNAC074: ZmNAC074 overexpressing plants showed stronger heat tolerance under heat stress, and this phenomenon was closely related to changes in the content of a series of stress metabolites. Overexpression plants accumulated more antioxidants (such as proline, soluble proteins, and carotenoids), while reducing the accumulation of ROS and membrane lipid peroxidation products (MDA), thereby improving cell membrane stability and antioxidant capacity.

Real-time quantitative PCR analysis showed that overexpression of ZmNAC074 could significantly activate multiple genes related to heat stress response (HSR) and endoplasmic reticulum stress response (UPR), especially ROS scavenging-related genes (such as GPX and APX), indicating that ZmNAC074, as a positive regulator of heat stress, plays a core regulatory role in enhancing plant heat resistance. Wild relatives are plant species that are highly similar to the maize genome and can even hybridize with each other. They play an important role in maize breeding. First, in terms of increasing the germplasm diversity of maize, wild relatives can provide new unlimited potential for maize breeding. Wild relatives also have good disease resistance and stress resistance, and have broad application prospects in maize variety improvement (Jamil et al., 2024). By combining these molecular insights with traditional breeding techniques, it may be possible to develop maize varieties that are more resilient to the challenges of climate change.

7 Technological Innovations for Enhancing Heat Tolerance in Maize

7.1 Activation of endogenous regulatory mechanisms

In the technical path of improving the heat tolerance of corn, activating the endogenous regulatory mechanism of plants is becoming a new research and application focus. Compared with traditional exogenous physical protection or cultivation adjustment strategies, endogenous signal molecule regulation has the advantages of systematic, continuous, low-cost and high-efficiency. Among them, salicylic acid (SA), as one of the core signal molecules in the response of plants to abiotic stress, has been increasingly valued in regulating the heat stress defense mechanism.

The latest research results of Yangzhou University took glutinous corn as the research object, and for the first time systematically revealed the regulatory mechanism of salicylic acid in improving the heat tolerance of corn through the combined analysis technology of transcriptome and metabolome. The study found that salicylic acid treatment can significantly induce the expression of a series of genes related to high temperature stress response, including key genes involved in antioxidant defense, osmotic regulation, heat shock protein synthesis and energy metabolism. At the metabolome level, salicylic acid significantly promoted the accumulation of key stress relief metabolites such as proline, soluble sugar, glutathione and carotenoids, and enhanced the plant's self-protection ability against high temperature-induced cell damage.

7.2 Precision nutritional intervention

In the comprehensive technical system for coping with high temperature stress, targeted nutritional intervention, as a fast, efficient and universally applicable strategy, is gradually becoming an important technical path for heat-resistant cultivation of corn. On the one hand, high temperature often leads to reduced root activity and decreased mineral nutrient absorption efficiency, especially the lack of key trace elements such as calcium, boron, zinc, and magnesium, which will further weaken the stability of corn cell membranes, photosynthetic efficiency, and normal development of reproductive organs. On the other hand, high temperature will also aggravate the accumulation of reactive oxygen species (ROS) and lipid peroxidation in the body, causing cell damage. In response to this problem, researchers promote targeted nutritional intervention measures to build a "nutritional heat-resistant barrier" by rationally supplementing physiologically active nutrients and antioxidants during key growth periods (such as jointing and tasseling).

Biostimulants such as seaweed extracts, humic acids, and amino acid complexes can significantly improve plant antioxidant capacity, membrane stability, and heat-resistant enzyme expression. Trace elements such as zinc, boron, and calcium play an important role in cell signal regulation, heat stress relief, and pollen vitality maintenance. Studies have shown that foliar spraying of nutrients rich in heat-resistant ingredients can improve the heat resistance of plants during the tasseling and silking periods, and reduce the proportion of empty stalks and bald tips (Saini et al., 2021). In combination with modern agricultural technology, some areas have also begun to use chelated micro-fertilizers (such as EDTA-Ca, Zn-EDTA) and plant biostimulants (such as humic acid, alginic acid, amino acid compound liquid, etc.) for foliar spraying, which can not only directly improve the plant's stress resistance metabolism level, but also stimulate the activation of endogenous heat resistance pathways, forming a dual heat resistance mechanism of "nutrition + regulation". This nutritional intervention technology has shown significant advantages in the field. It can alleviate premature leaf senescence during high temperatures, improve pollination quality, prolong the filling period and maintain the photosynthetic efficiency of the group, effectively reduce yield losses in high temperature years, and is the "soft technology support" for the current prevention and control of corn heat damage.

7.3 Optimization of integrated cultivation strategies

In the context of global climate change, relying solely on genetic improvement can no longer fully cope with the yield risks brought about by high temperature stress. At present, integrated cultivation technology has gradually become a key supplementary path to improve the heat resistance and stable yield of corn. In particular, the close planting precision control technology, as the core content of the integrated agronomic management system, has been widely used in high-density planting areas to synergistically improve the output per unit area and environmental adaptability. According to the latest research and practical experience, the close planting precision control technology is not only an effective way to release high-yield potential, but also plays an important role in corn's response to high temperature stress. The core of this technology is to systematically configure and dynamically adjust the planting density, row spacing, sowing period and field management measures based on variety characteristics, soil conditions and climate factors. By optimizing the planting pattern, not only can the photosynthetic efficiency of the group be improved, but also the competitive pressure and heat accumulation problems caused by the density can be effectively alleviated, thereby reducing the impact of heat damage on reproductive growth.

Specific measures include: selecting high-density tolerant varieties to strengthen the stability of the population structure; implementing precision sowing technology to ensure uniform density through mechanized strip sowing and GPS positioning technology; regulating the population structure and canopy ventilation to improve field permeability and reduce ear temperature; combining water-fertilizer integration and micro-spray irrigation technology to optimize soil moisture and alleviate thermal transpiration; implementing a phased density control strategy to achieve a dynamic balance between the vegetative growth and reproductive growth stages. Precise control of dense planting can promote the efficient use of photosynthetic resources, and effectively alleviate the adverse effects of high temperature on pollen vitality, grain filling process and grain formation by improving the leaf area index (LAI), ear microclimate and root distribution. At the same time, this technology forms a synergistic effect with heat-resistant varieties, heat-resistant biostimulants and intelligent irrigation management, promoting the transformation of the corn production system towards "high density and stable yield, stress resistance and water conservation, high efficiency and green".

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Conflict of Interest Disclosure

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

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