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GENOME EDITING FOR ENHANCING ABIOTIC STRESS TOLERANCE IN MAIZE: MOLECULAR MECHANISMS, PHYSIOLOGICAL RESPONSES AND FUTURE PROSPECTS

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ABSTRACT
Maize (Zea mays L.) is one of the major crops that contribute to food security worldwide and is increasingly exposed to abiotic stresses like drought, heat, and salinity stress, worsened by climate change. The polygenetic nature of stress tolerance and long breeding cycles are the limitations of conventional breeding approaches. This review provides a summary of recent advances, molecular and physiological mechanisms and perspectives of precision genome editing such as CRISPR-Cas9 systems and TALENs to improve abiotic stress resistance in maize. We identify important molecular networks that underpin responses to stress including, among others, DREB/NAC transcription factor networks for drought, HSFs/HSPs networks for heat and HKT/NHX networks for salinity. Promoter changes were successfully made in ARGOS8, conferring a 5 bushels per acre (around 8-10% yield improvement) advantage under drought conditions, and the knock-in of the teosinte-derived ZmHKT1;2 allele, which lowered shoot Na^+ by 80% in saline conditions. While there are concerns about the possible off-target effects, delivery efficiency and regulations, genome editing has the potential to revolutionize maize breeding for climate resilience. The translational capacity of editing technologies is highlighted in this review for the purpose of protecting global food systems from a changing climate.

KEYWORDS: Abiotic stress, CRISPR-Cas9, drought tolerance, genome editing, heat tolerance, maize, salinity tolerance

1. INTRODUCTION

Maize (Zea mays L.) plays an important role in global food security as a primary source of calories, animal feed and bioenergy. Maize, which is produced at an annual level of more than 1.2 billion metric tons, plays a key role in the livelihoods of millions and is the backbone of agricultural economies in the world (FAO, 2024). However, the productivity of maize is becoming increasingly threatened by the increasing frequency and severity of abiotic stresses, particularly drought, heat stress and salinity, which are all made worse by climate change. The Intergovernmental Panel on Climate Change (IPCC) projects a 10–25% reduction in maize yield by 2050 due to moderate emission scenarios, potentially falling above 80% in climate change susceptible areas such as sub-Saharan Africa and South Asia (IPCC, 2024). These stresses disrupt vital physiological functions, which include uptake of water, pollination, and ion balance, posing major problems for maize production around the world. Abiotic stress tolerance in maize is a complex trait governed by interconnected physiological and molecular mechanisms that regulate water relations, photosynthesis, ion homeostasis, antioxidant defense, and reproductive development. Understanding these mechanisms is essential for developing resilient cultivars under changing climatic conditions.

In the past, the most common way to increase crop yields has been through conventional breeding. A significant success story is the Green Revolution that doubled maize yields from the 1960s onwards. But there are serious challenges to this approach in the case of polygenic traits, such as drought, heat or salinity tolerance. The breeding process can be time consuming (8–12 years for each variety) and can suffer from the presence of undesirable alleles with the target quantitative trait loci (QTLs) through linkage drag. In addition, high levels of inbreeding in elite germplasm can reduce genetic diversity (Varshney et al., 2021). Vavilov's concept of using this genetic variation, first proposed in 1922, is highly applicable to the present breeding for stress resistance. Recent developments in pan-genome analyses have expanded upon this concept, demonstrating that wild relatives and landraces have variants that were not selected for domestication, but can now be precisely reintroduced by genome editing (Raza et al., 2023). Besides yield losses, abiotic stresses put a significant strain on smallholder farmers in developing countries who have limited resources available for irrigation and better cultivars. Genome editing offers a rapid and precise alternative for improving complex stress-responsive traits while minimiznig linkage drag and accelerating cultivar development (Chen et al 2023).

Abiotic stress responses involve coordinated signaling networks mediated by phytohormones, calcium signaling, reactive oxygen species (ROS), mitogen-activated protein kinase (MAPK) cascades, and stress-responsive transcription factors such as DREB, NAC, WRKY, HSF, and bZIP. These regulatory pathways have emerged as major targets for precision genome editing aimed at improving stress resilience in maize. The engineering of drought-inducible ARGOS8 promoters to increase water-use efficiency and crop yield during drought and the precise knock-in of ancestral ZmHKT1;2 alleles to significantly reduce sodium buildup in shoots during salinity stress (Shi et al., 2017; Zhang et al., 2023). Recent Advances in CRISPR-based genome editing have enabled precise manipulation of stress – responsive genes governing physiological adaptation to drought, heat, and salinity.
This review integrates current knowledge of the physiological and molecular mechanisms underlying drought, heat, and salinity tolerance in maize with recent advances in genome-editing applications targeting these pathways. Rather than providing a detailed description of genome-editing technologies, the review emphasizes how precise modification of stress-responsive genes has enhanced physiological adaptation and stress resilience. In addition, recent developments in multiplex genome editing, multi-omics integration, phenomics-assisted target discovery, and future breeding strategies for developing climate-resilient maize cultivars are discussed.

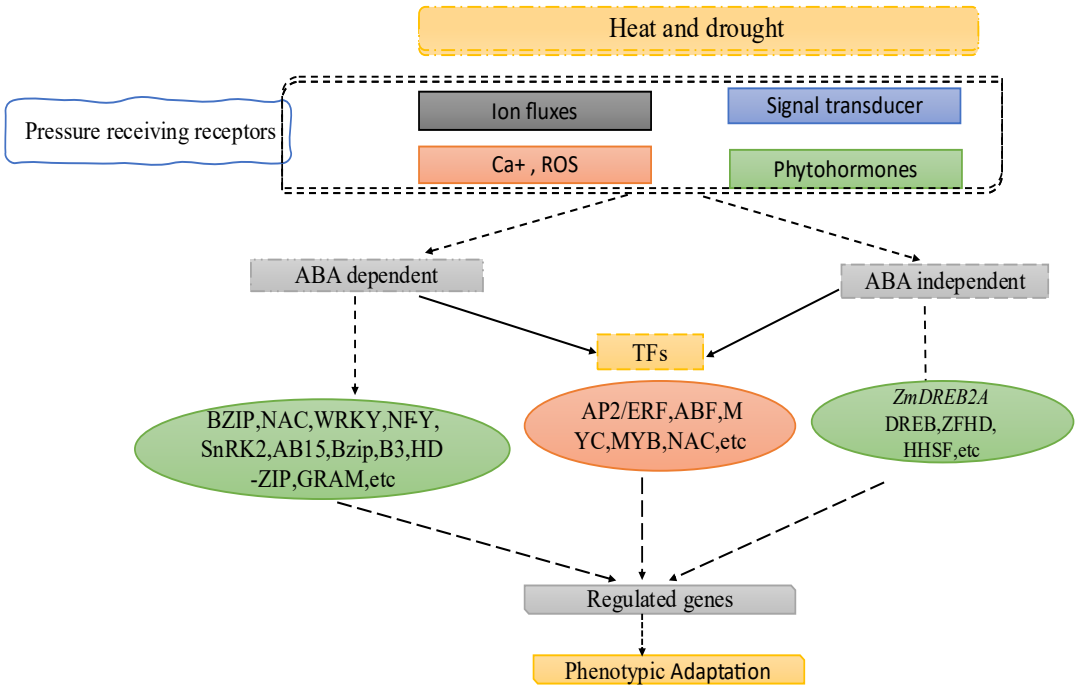

Figure 1: Heat and drought stress activate receptors, ion fluxes (Ca^{2+}, ROS), and phytohormone signaling, leading to ABA-dependent (AP2/ERF, ABF, MYC, MYB, NAC) and ABA-independent (ZmDREB2A, DREB, ZFHD, HHSC) transcription factors that regulate stress-responsive genes and drive phenotypic adaptation.

2. MOLECULAR BASIS OF ABIOTIC STRESS TOLERANCE IN MAIZE

Maize is highly responsive to abiotic stresses via a complex network of abiotic stress sensors which are located at the cell membrane, such as mechanosensitive calcium channels (e.g., ZmOSCA1.1), receptors with kinase activity (e.g., ZmRLK7), and histidine kinases (e.g., ZmHK3). These sensors trigger fast signaling cascades such as bursts of cytosolic Ca^{2+}, bursts of reactive oxygen species (ROS) and fluxes of phytohormones (ABA, ethylene, JA, SA)

(Tong et al.,2021). These primary signals converge on a group of stress responsive transcription factors (TFs) that are responsible for the transcriptional reprogramming of more than 10% of the maize genome within hours. These early signalling events coordinate the physiological and molecular responses that enable maize to adapt to drought, heat, and salinity and also provide important targets for genome-editing strategies. Table 1 shows some of the major morphological and physiological responses of maize to drought, heat and salinity stress.

Table 1: Morphological and physiological adaptations to abiotic stress in maize.

Feature	Stress Type	Key Adaptive Traits	Reference
Morphological Features	Drought	35% increased deeper root system; root wall remodeling via lignin biosynthesis	Wang et al., 2024; Xia et al., 2023
	Heat	Maintained pollen viability; reduced silk desiccation; delayed flowering (via ZmELF3 knockout)	Liu et al., 2023
	Salinity	Reduced shoot Na^+ accumulation (lowers by 90%); vacuolar Na^+ sequestration	Zhang et al., 2023
Physiological Features	Drought	Accumulation of proline (15–25 mg/g DW); high water-use efficiency (WUE) by 30%; ROS scavenging (reduced 50% oxidative damage)	Bai et al., 2022
	Heat	Induction of HSP70/90/101; protection of PSII efficiency (Fv/Fm +0.2); activation of antioxidant enzymes (ZmSODCp, ZmCAT2)	Liu et al., 2023; Pessa et al., 2024
	Salinity	Ion homeostasis (K^+/Na^+ ratio increases 2x); osmotic adjustment; H_2O_2 detoxification via ZmAPX2 and ZmCAT2	Li et al., 2023
	Cross-Stress (ROS)	Enhanced activity of ZmSOD3a, ZmAPX2, ZmCAT2, ZmGR1; regulation by ZmNAC111, ZmDREB2A, ZmWRKY71	Mittler et al., 2022

Drought Tolerance

Drought stress causes a succession of physiological issues which results in fewer germinating seeds, smaller leaf area, and a substantial decrease in photosynthetic capacity and kernel set. A long anthesis-silking interval -silking interval (ASI) may worsen this situation, impacting yield directly (Seleiman et al., 2021; Khan et al., 2022). At the cellular level, stomatal closure is the first defense, which is triggered by K^+ efflux which is mediated by stomatal ABA receptors such as ZmSLAC1. This will save water, but at the cost of CO_2 fixation which may reduce up to 60% of the photosynthesis (Silva et al., 2022). Plants respond to these challenges by triggering highly coordinated networks of transcription factors (TFs) that regulate complex molecular networks. This response is controlled by an ABA-dependent and an ABA-independent pathway. The concept of the role of phytohormones as internal messengers, proposed by Chailakhyan (1968), is an important starting point for the understanding of stress responses that are mediated by ABA. ZmDREB2A is a member of the AP2/ERF family and is an important member of the ABA-independent pathway. There is, however, also the cross-talk between ABA signalling via protein-protein interaction with AREB/ABF transcription factors, which forms a regulatory hub that links both pathways (Hu et al., 2022). This TF is known to be a master regulator that binds to the promoters of >100 downstream genes containing DRE/CRT cis-elements (ACCGAC). It activates the synthesis of osmoprotectants such as the amino acid proline and sugars, which are like molecular sponges that hold water in cells. Accumulation of osmoprotectants such as proline is one of the major biochemical responses to water deficit, the biological function and regulation of which has been extensively discussed by Kuznetsov and Shevyakova (1999). The critical role of ZmDREB2A has been verified using CRISPR/Cas9 knockout technique where loss of ZmDREB2A led to increased dehydration stress sensitivity in plants due to the repression of the key downstream effectors such as LEA and P5CS (Shi et al., 2017).

Also, the NAC TF family is able to integrate various stress signals in both ABA dependent and independent pathways. One of the integrators is ZmNAC111. In addition to the transcriptional regulators, genes related to ABA biosynthesis are also important editing targets, such as the ZmABA2 gene that was recently identified to directly regulate the drought-stress responses in maize (Li et al.,2025).Natural variation in its promoter, in particular, a MITE transposon insertion detected in drought-tolerant lines through genome-wide association study (GWAS), is a useful breeding target that is associated with higher expression of this gene (Engelhorn et al., 2025). ZmNAC111 directly stimulates the expression of lignin biosynthetic genes, such as ZmCAD2 and ZmCCR1, which allows remodeling of the root wall, thereby allowing a 25-35% deeper root system and enhanced water foraging ability (Wang et al., 2024; Xia et al., 2023). Moreover, it triggers the expression of ROS scavenging enzymes, such as ZmSOD3a and ZmAPX2, which can decrease ROS-induced cellular damage up to 50% in direct binding evidence obtained from ChIP-qPCR assays (Engelhorn et al., 2025). Other NAC TFs such as ZmNAC4/JUB1/NAP are finely co-expressed with ZmNAC111 ($r > 0.85$), and proper regulation of both is crucial for providing stress tolerance without compromising agronomic yield, as described by Luo et al., 2022. Supplementary Table 1 summarizes key transcription factors that are responsive to drought stress, their binding

motifs, validated targets, and their effects on the phenotype. In Figure 2, a transcriptional regulatory network involved in drought tolerance in maize is shown, where the ABA-dependent and ABA-independent pathways are integrated.

When these programmes of transcription are being biochemically executed, they involve the accumulation of compatible solutes to aid in osmotic adjustments. Overexpression of the gene for proline biosynthesis ZmP5CS1 leads to a 30% higher water-use efficiency (WUE) with proline accumulation of 15–25 mg/g dry weight. Likewise, the PSII efficiency (Fv/Fm) increased by 0.15 for plants that had not been transgenically engineered with ZmBADH1, demonstrating the crucial role of this compound in stress-induced PSII stability. Collectively ZmDREB2A, ZmNAC111, and ZmABA2 represent major regulatory nodes governing drought adaptation. Their central roles in ABA signalling, osmotic adjustment, root development, and ROS detoxification make them attractive genome-editing targets for improving tolerance in maize.

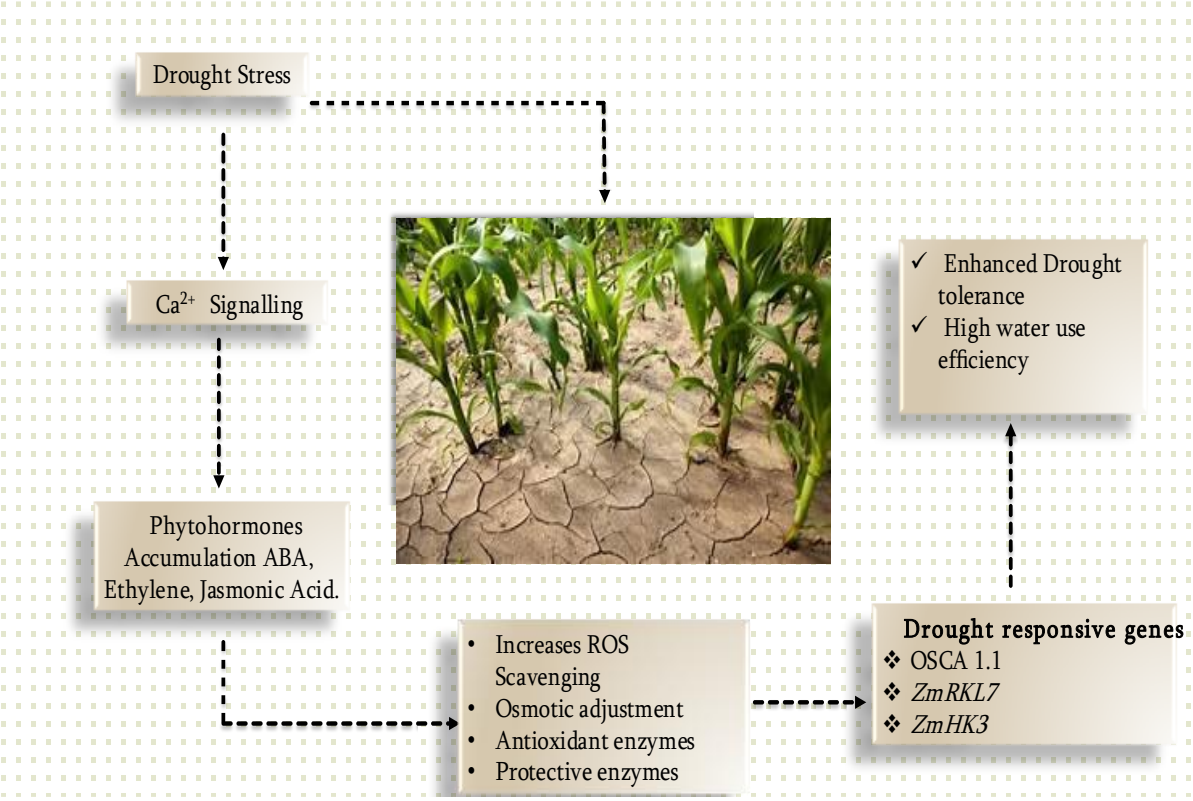

Figure 2 Transcriptional regulatory network governing drought tolerance in maize.

Drought stress signals are perceived by membrane sensors, activating ABA-dependent and ABA-independent signaling pathways. Master transcription factors including ZmDREB2A (ABA-independent) and ZmNAC111 (ABA-dependent/independent) regulate downstream effector genes involved in osmotic adjustment (P5CS1, BADH1), ROS scavenging (SOD3a, APX2), and root architecture remodeling (CAD2, CCR1).

Heat Tolerance

Maize may be quite sensitive to heat stress, particularly during the developing and grain-filling stages. The high temperature may disrupt the function of the tapetum and the dehydration of silk may reduce the pollen viability and consequently, interfere in the fertilization process. In addition to this, high temperatures cause significant disruption to important enzymatic activities that are needed for starch synthesis and storage, leading to poor grain filling and yield losses sometimes over 50% (Hatfield and Prueger, 2015).

Proteotoxic stress is sensed by Heat Shock Factors (HSFs). They trimerize and bind to Heat Shock Elements (HSEs) in promoters of Heat Shock Protein (HSP) genes, launching the main molecular line of defence. Specific HSPs serve as important molecular chaperones such as HSP70 and the plastidic HSP101 (Fujimoto et al., 2023). These proteins regulate the cellular balance, avoiding the aggregation of denatured proteins when the temperature rises, helping them to refold, and protecting the photosynthetic apparatus and metabolic enzymes from denaturation. For example, the expression level and transactivation activity of ZmHsf28 have been linked to thermotolerance in maize, and increasing the expression of ZmHsf28 can greatly improve maize seedling survival under severe heat stress (Liu et al., 2023). This points to the importance of the specific role played by the HSFs. Chronic heat conditions (high daytime and night time temperatures, >35°C) or sudden heat events (high temperatures for 2-4 hours, >40°C) can severely affect reproduction, with pollen viability dropping by 90% after only 3 hours at 38°C as a result of programmed cell death in the tapetum and loss of starch and sugars in the

locules. Silks get dried, which causes a greater anthesis-silking interval (ASI), and the filling of kernel stops while starch synthase (SSIIa, SSIII) denatures, and its Km increases 3-fold.

Healthiness of plants could be assessed by the canopy temperature depression (CTD) which was less than 2°C, as a measure of heat tolerance (Rose et al., 2023). ZmHsf28 is a nuclear and cytosolic protein which forms trimers in response to heat stress and binds to Heat Shock Elements (HSEs; consensus 5' nGAAn-3') in the promoters of Heat Shock Protein (HSP) genes. More than 150 direct targets have been identified by ChIP-seq analysis, such as the cytosolic HSP70/90, which is involved in the refolding of 60% of denatured Rubisco, and plastid HSP101 involved in PSII turnover (Lang et al., 2021). ZmHsf28 is a fast-response molecular switch to heat stress at a molecular level. This protein produces protective heat-shock proteins 12 hours earlier than normal when the protein is increased in its expression. This results in enhanced pollen germination by 45% under high heat stress and enhances grain yield by 18% in the field, without compromising normal plant growth. It is further enhanced by being used in combination with another regulator (ZmHSFA6b), whose natural variant is also closely linked to heat-tolerant tassels. Furthermore, plants can avoid heat stress through a delay in flowering; for example, zmelef3 gene knockouts can delay flowering 8-12 days and thus escape the heat stress. For the identification of naturally heat-resistant pollen, pollen cells which are stable and contain certain heat-resistant elements, such as ZmAMS and ZmTDF1, are targeted. Heat stress may cause the buildup of toxic ROS in mitochondria but ZmHsf28 works by inducing genes associated with antioxidant enzymes (ZmSODCp and ZmCAT2) to reduce the damage caused by heat stress. This action helps to maintain the energy balance in the cell and avoid damaging the cell membrane, decreasing by 40% one of the cell injury markers in tolerant plants (Pessa et al., 2024). The successful manipulation of ZmHsf28 and ZmELF3 demonstrates how genome editing can improve both heat tolerance and reproductive stability under elevated temperatures. If you wish to read more about the sequential molecular reaction to heat stress, from perception to recovery, read on in Supplementary Table 2. The molecular pathways involved in the mechanisms of heat stress response and thermotolerance upon HSF activation are shown in the Figure 3.

Table 2: Key stress-responsive genes edited in maize for abiotic stress tolerance.

Stress Type	Gene Edited	Editing Strategy	Molecular Function	Phenotypic Outcome	Reference
Drought	ARGOS8	Promoter modification (deletion)	Negative regulator of ethylene response	↑ Yield by 5 bu/acre under drought; ↑ WUE; delayed senescence	Shi et al. (2017)
	ZmPL1	Knockout (KO)	RINGv E3 ubiquitin ligase (ABA signaling modulator)	↑ Root length by 30%; ↑ germination & survival under osmotic stress	Khan et al. (2022)
	ZmNAC111	Overexpression (OE) via promoter	NAC TF; regulates root lignin & ROS scavenging	↑ Root depth by 35%; ↑ survival under 15% PEG stress	Bai et al. (2022)
Heat	ZmHsf28	Transcriptional activation	Master heat shock transcription factor	↑ Pollen viability by 45% at 40°C; ↑ yield by 18% under heat stress	Liu et al. (2023)
	ZmELF3	Knockout (KO)	Flowering time regulator (evening complex)	Delays flowering by 10 days, enabling escape from peak heat periods	Liu et al., 2023
Salinity	ZmHKT1;2	Knock-in (SNP947-G allele)	Xylem parenchyma Na^+ uniporter	↓ Shoot Na^+ by 90%; ↑ biomass by 30% under saline conditions	Zhang et al. (2023)
	ZmNHX1	Overexpression (OE) / RNP editing	Vacuolar Na^+/H^+ antiporter	↑ Vacuolar Na^+ storage; ↑ PSII efficiency (Fv/Fm +0.23); ↑ cellular detoxification	Li et al., 2023
Cold	ZmG6PDH1	Knockout (KO) for validation	Rate-limiting enzyme in oxidative pentose phosphate pathway	KO led to redox collapse (NADPH ↓50%), ↑ ROS, severe chlorosis under cold stress	Liu et al. (2023)
Cross-Stress	ZmAPX2	Overexpression (OE)	Cytosolic ascorbate peroxidase (ROS scavenging)	Enhanced H_2O_2 detoxification; improved recovery under combined drought & heat	El-Sappah et al. (2022)
	ZmCIPK15	Knockout (KO)	CBL-interacting protein kinase (ion homeostasis)	Improved K^+/Na^+ homeostasis; ↓ ionic toxicity symptoms under salinity	El-Sappah et al. (2022)

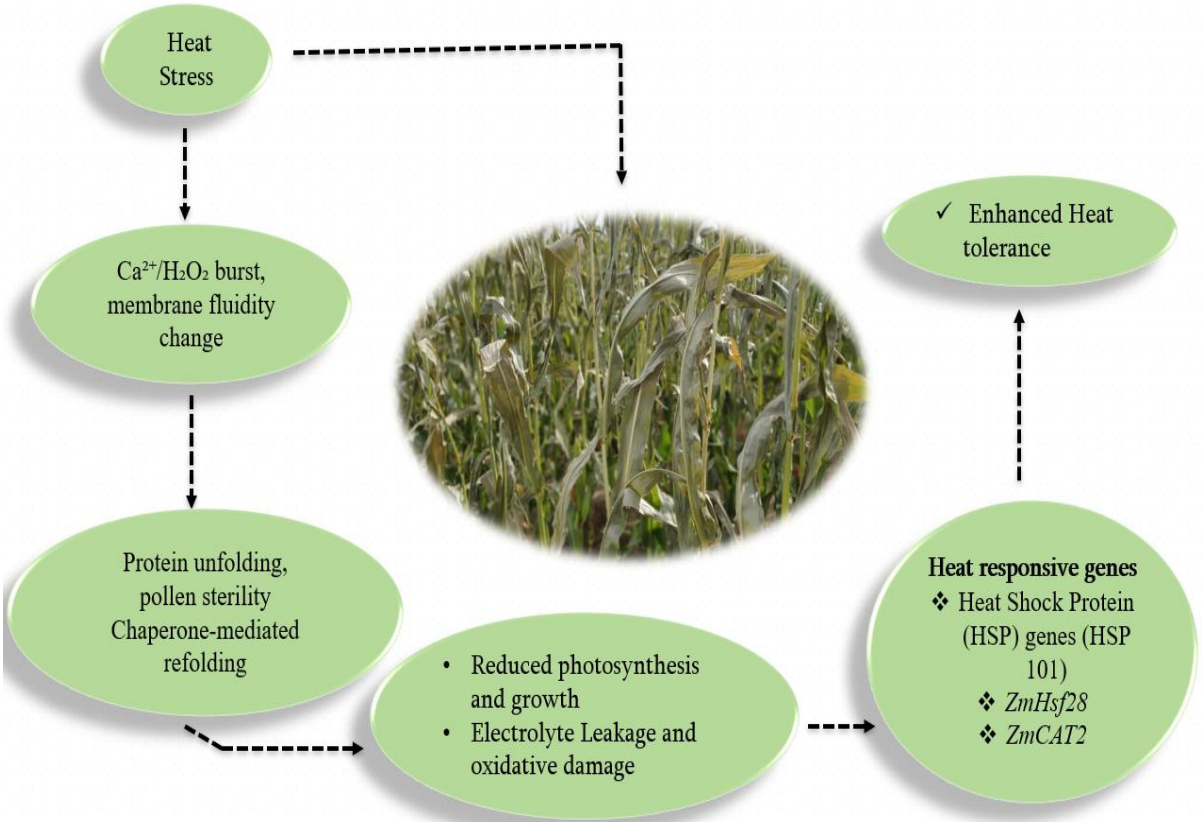

Figure 3. Molecular mechanism of heat stress response and HSF-mediated thermotolerance in maize. Heat stress induces protein unfolding, which activates heat shock factors (HSFs).

ZmHsf28 trimerizes, translocates to the nucleus, and binds heat shock elements (HSEs) in the promoters of heat shock protein (HSP) genes. HSP70/90/101 function as molecular chaperones to refold denatured proteins, maintain PSII efficiency, and protect pollen viability. ZmELF3 knockout delays flowering, enabling heat escape. Green arrows indicate activation; red arrows indicate stress effects.

Salinity Tolerance

Van Zelm et al., 2020, state that salinity stress creates two important challenges: osmotic stress which causes plants to have difficulties absorbing available water and ionic stress from the high levels of Na^+ ions that promote the blockage of enzymes and imbalance of K^+ ions. Ion transporters join forces to address these problems in maize. The Tonoplast's Na^+/H^+ Exchangers (NHX), for example, ZmNHX1, are important pumps that pump Na^+ into the vacuole. This helps to adjust the osmolarity by utilizing the collected ions and it also decreases the cytosolic Na^+ level and thus its toxicity. A comprehensive table of maize key stress-responsive genes edited, along with editing strategies, molecular function of the edited gene, and phenotypic changes, is provided in Table 2. Maize has the potential to improve its salt tolerance through increased vacuolar Na^+ sequestration (Zhang et al., 2023). Furthermore, the root xylem parenchyma cells are also known to contain High-affinity K^+ Transporters (HKTs), especially those of subfamily I, such as ZmHKT1. It is thought that these transporters remove Na^+ from xylem sap, thereby reducing the amount of Na^+ reaching the shoot which is performing photosynthesis (shoot exclusion; Zhao et al., 2017). Production of Reactive Oxygen Species (ROS) such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2) is generally increased under conditions of drought, heat and salinity. ROS can trigger lipid, protein and DNA oxidation at high concentrations (Mittler 2022). The maize is able to resist this by having a well developed antioxidant system with multiple layers. First line of defence: enzymes such as Superoxide Dismutase (SOD) which converts O_2^- into H_2O_2. Following that, Catalase (CAT) and a set of ascorbate-glutathione cycle enzymes, such as Ascorbate Peroxidase (APX), quickly mop up H_2O_2, turning it into oxygen and water (Mittler, 2022).

The same transcription factors responsible for the expression of other stress response genes often also regulate these ROS-scavenging genes. DREB and NAC TFs may interact directly with the promoters of SOD and APX genes, establishing an integrated defense network that is simultaneously able to cope with oxidative, ionic and osmotic damage.

ZmHKT1;2, a member of class I HKT family, is localized in the plasma membranes of root xylem parenchyma cells and acts as a selective Na^+ uniporter (ZmNC3) (Ser-67 is Na^+ selective compared to K^+) (Zhang et al.,2023). Corn plants have sophisticated defences to survive in saline soils, in simple terms. The principle is to prevent the presence of toxic sodium (Na^+) ions in the sensitive working regions of the cell. One key player is ZmNHX1, a protein that functions as a pump on the membrane of a large storage compartment that is located inside the cell,

known as the vacuole. It actively pumps sodium from the interior of the cell into this vacuole using energy obtained from another pump to achieve this. Not only does it help the cell to flush toxins from its system, but it can also help the cell hold on to water. Other related pumps, such as ZmNHX2/4, are activated by a kinase protein (ZmCIPK15) which function as a switch and up-regulates their activity under salt stress. At the edge of the root, however, there is a strong partnership between two parts to determine what enters the cell (Behzadi et al., 2021). ZmSOS1 protein acts as a sodium ejector which actively pushes the salt out into the soil. In close cooperation, the ZmHAK4 protein is a precise potassium (K+) collector, which will uptake K+ even if sodium (Na+) is available in excess. The plant can thus keep a good homeo-stasis, through these safe operations of fixing sodium, expelling it and selectively absorbing potassium. This coordinated defense enables it to maintain its core activity, photosynthesis, which is considerably more efficient in the case of plants that are salt tolerant (Malakar and Chattopadhyay, 2021). Key ion transporters involved in salinity tolerance and their localization, function and phenotypic gains on editing are summarized in Supplementary Table 3. Figure 4, shows the coordinated ion transport mechanisms which control salinity tolerance in maize and includes xylem Na^+ retrieval, vacuolar sequestration, and root exclusion. These ion transporters constitute the principal genome-editing targets for improving Na^+ exclusion, vacuolar sequestration, and K^+ homeostasis in maize cultivated under saline conditions.

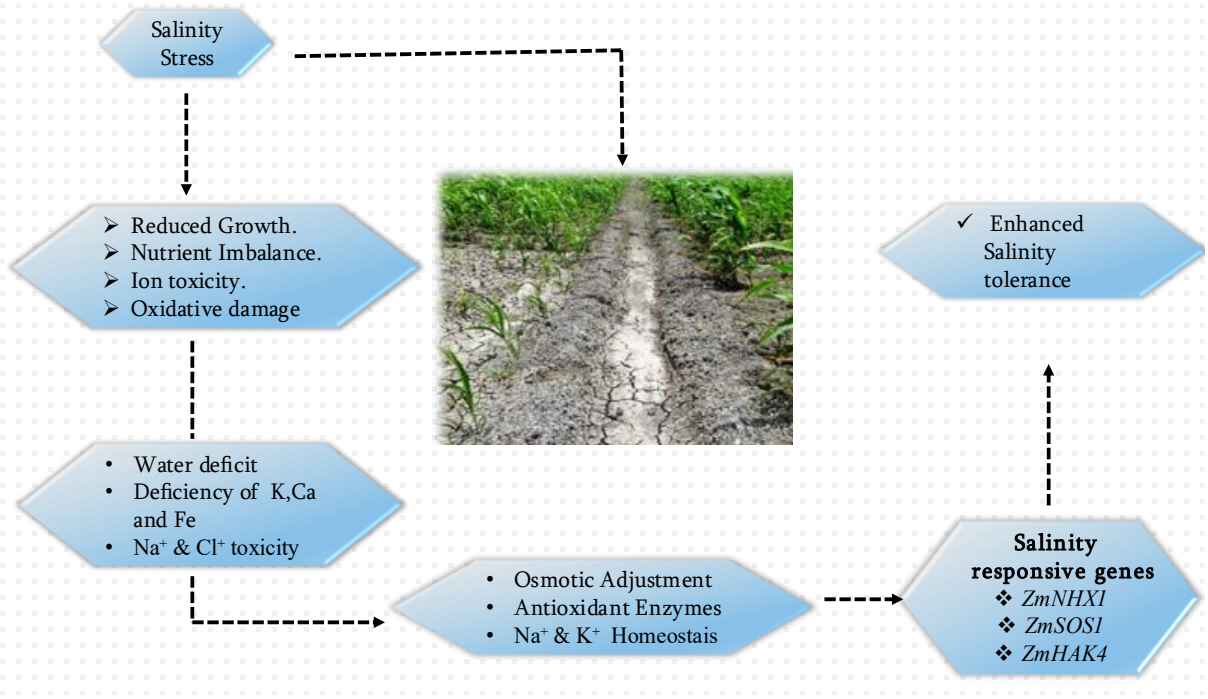

Figure 4. Ion transport mechanisms governing salinity tolerance in maize.

(1) ZmHKT1;2 in root xylem parenchyma retrieves Na^+ from xylem sap, reducing shoot Na^+ accumulation; (2) ZmNHX1 sequesters Na^+ into vacuoles via tonoplast Na^+/H^+ exchange; (3) ZmSOS1 mediates Na^+ efflux from root epidermal/cortical cells into apoplast; (4) ZmHAK4 enables high-affinity K^+ uptake under Na^+-dominant conditions. PM, plasma membrane; Ton, tonoplast; Xy, xylem.

Cross-Talk: ROS Scavenging and Common Integrative Pathway

All abiotic stresses are associated with an increased generation of reactive oxygen species (ROS): superoxide (O_2^-) through the Mehler reaction, hydrogen peroxide (H_2O_2) in the photorespiration process, and singlet oxygen (O_2) from the triplet chlorophyll reaction. A sophisticated antioxidant defence system was built up in maize, which is tightly regulated by various transcription factors. Information on the core ROS scavenging enzymes, their cellular localization and transcription factors is provided in Supplementary Table 4.

These are called Enzymatic Antioxidant Fortress: ZmSOD3a (Cu/Zn-SOD) present in cytosol, and ZmSODCp (Fe-SOD) present in the chloroplast. High levels of H_2O_2 are regulated by catalase (ZmCAT2, peroxisome), ascorbate peroxidase (ZmAPX2, cytosol) and glutathione peroxidase (ZmGPX, mitochondria) in the ascorbate-glutathione (AsA-GSH) cycle (Mittler, 2022). Redox Regeneration: GSH is converted to GSSG by the enzyme glutathione reductase (ZmGR1) using NADPH that is acted on primarily by the oxidative pentose phosphate pathway enzyme ZmG6PDH1 (Mittler, 2022).

Transcription factors such as ZmDREB2A, ZmNAC111 and ZmWRKY71 bind to ROS-Responsive Elements (DRE and ABRE) located in the promoters of antioxidant genes and create stress-dependent feed-forward loops. ABA/JA: These hormones activate MAPK cascades (ZmMAPK3→MPK4) that activate NADPH oxidases

(ZmRBOHs) that cause a burst of ROS and then stimulates the expression of antioxidant genes, such as ZmAPX2 (Mittler, 2022).

Ethylene: It regulates the activity of E3 ligases, such as ZmPL1, that regulate NAC transcription factor turnover (Mittler, 2022). Because ROS signaling integrates drought, heat, and salinity responses, antioxidant regulatory genes represent promising candidates for multiplex genome-editing strategies aimed at improving tolerance to multiple abiotic stresses simultaneously.

3. PHYSIOLOGICAL BASIS OF ABIOTIC STRESS TOLERANCE AND ITS ENHANCEMENT VIA GENOME EDITING

The shift from proof-of-concept towards actual implementation is a major step forward in crop biotechnology. Genome editing has proved to be a powerful tool to improve the abiotic stress tolerance of maize, allowing targeted manipulation of the important genetic pathways involved in abiotic stress tolerance. Using the novel genome editing tools CRISPR-Cas9, TALENs and new editing platforms, scientists have been able to generate maize lines with enhanced drought, heat, salinity, and cold tolerance, with minimal loss in optimum growing conditions. This section provides examples of key applications, organized by type of stress, that demonstrate the benefits of specific genetic interventions in terms of measurable phenotypic improvement in controlled settings and field experiments.

Enhancing Drought Tolerance

Maize production remains as most serious constraint of drought. Addressing this problem, two approaches have been taken: modification of the promoters of the positive regulators, and mutations of the negative regulators. They directly target molecular networks underlying drought tolerance, including the DREB/NAC transcription network and osmotic adjustment. One of the most innovative drought-resilience promoter editing examples is the editing of ARGOS8, a negative regulator of ethylene signalling. Shi et al. (2017) used the CRISPR-Cas9 method to target the 5' untranslated region (UTR) of the native ARGOS8 gene, creating a 16-base pair deletion and the inclusion of a drought-responsive element from the GOS2 gene. This modified allele leads to increased expression of ARGOS8, particularly under water-limited conditions. The field testing of these edited lines had resulted in less senescence, increased water-use efficiency, and increased yield (up to 5 bushels per acre) under drought conditions, without reduction in yield in well-watered situations. This research exemplifies a brilliant breeder's method of creating alleles that are responsive to stress, yet have a positive physiological effect. Knockout strategies of negative regulators have also been promising. Khan et al. 2022 used CRISPR-Cas9 to edit ZmPL1, a gene encoding a RING-type E3 ubiquitin ligase that plays a role in ABA signalling and root development. Root lengths and biomass of the zmpl1 knockout mutants were 30% longer and 25% greater, respectively, under osmotic stress (15% PEG6000). These enhancements in root architecture led to improved water-uptake from the soil and increased survivability over time and less wilting when drought-stressed, demonstrating that genetic suppressors can be removed to unleash the plant's natural tolerance to stress. These studies demonstrate that editing genes regulating ethylene signalling, ABA responses, and root architecture can substantially improve drought adaptation while maintaining yield under favourable environments.

Enhancing Heat Tolerance

Heat stress, especially during flowering, can have a devastating effect on the viability of pollen and the number of set kernels. The editing applications have thus been directed toward improving this protective system by modulation of the transcription factors.

Liu et al. (2023) showed that the master regulator ZmHsf28 can be transcriptionally activated in maize using CRISPR which resulted in an accelerated and stronger induction of downstream heat shock proteins (HSP70, HSP101). When exposed to acute heat stress (45% greater viability of pollen when exposed to 40°C for 3h) edited lines preserved pollen viability 45% better than wild-type controls. kernel set significantly improved in field trials which simulated a heatwave at anthesis with 18% greater yield than unedited isolines. This method goes beyond the constitutive overexpression, utilizing and amplifying the plant's stress-response circuit, thus introducing minimal fitness costs. Other approaches involve the engineering of flowering time genes for example, ZmELF3, to shift the time of anthesis to avoid the peak heat stress period, which has been successfully shown to maintain yield under terminal heat stress (Hill et al., 2022).

Enhancing Salinity Tolerance

The transport mechanisms for Na^+ exclusion and sequestration are the main regulators of salinity tolerance in maize. Conventional backcrossing is a long and complex process compared to introgression by editing, which is used to incorporate desirable alleles that have been lost during domestication without linkage drag.

One example is the accurate editing of ZmHKT1;2, a xylem parenchyma Na^+ transporter. Zhang et al. (2023) found one SNP allele, SNP947-G/Y316C, in teosinte that was superior in Na+ retrieval from the xylem stream. They transferred this allele in the elite, salt-sensitive B73 background by the introduction of the allele using a homology-directed repair (HDR) strategy based on CRISPR-Cas9. The shoot Na^+ loading and shoot biomass for the edited near-isogenic lines were considerably lower than that of control plants, with a 90% decrease and a 30% increase, respectively, when cultured at EC=12 dS/m in saline field conditions. This is a high-quality example of rewilding crops by editing, bringing to the table much-needed historical genetic diversity in a smart and efficient

way to face the problems of modern agriculture. Complementary approaches have focused on vacuolar sequestration through the overexpression of the ZmNHX1, which increases the compartmentalisation of toxic ions and increases osmotic adjustment under salt stress. This study illustrates how precise introgression of beneficial alleles from wild relatives through genome editing can accelerate salinity improvement while avoiding linkage drag.

4. MULTIPLEXING AND TRANSGENE-FREE DELIVERY FOR ADVANCED EDITING STRATEGIES

We need to look at more than single gene editing, as abiotic stress tolerance is a very complex trait. With multiplex editing, we can simultaneously edit multiple genetic regions. This is achieved by the systems that can express multiple sgRNAs from a single polycistronic transcript, either via tRNA or Csy4-processing. This will enable us to transform multiple alleles, such as ZmNAC111 with deeper rooting, ZmHKT1;2 with sodium exclusion, and ZmHsf28 with better heat tolerance in a single transformation event. This really accelerates the development of plant varieties that are able to stand multiple stresses (Sarfraz et al., 2025). At the same time, it's essential to produce edited plants that are free from transgenes to make regulatory approval smoother and to ease public concerns. Delivering pre-assembled CRISPR-Cas9 ribonucleoproteins (RNPs) into protoplasts or embryos has shown to be very effective for this. Liu et al (2023) for instance were able to edit ZmNHX1 for salinity tolerance using RNPs, generating plants of the T_0 generation that contained no foreign DNA whatsoever. The approach is a transient delivery that considerably reduces the breeding window, avoids transgene integration and streamlines the regulatory process for the deployment of the edited varieties.

5. FUTURE PROSPECTS

Expansive genome-wide association studies (GWAS) are bringing the study of novel editing targets to a revolutionized state by combining with predictive computational biology. High-resolution pan-genome GWAS have been conducted on more than 30,000 maize accessions and have unearthed hundreds of quantitative trait loci (QTLs) such as promoter elements including insertion of a MITE in the ZmNAC111 gene (Wang et al., 2023). Top candidate genes, like ZmBADH1 involved in glycine betaine accumulation, can be validated in just six months with the help of rapid CRISPR-Cas. Structural prediction tools, such as AlphaFold3, are used to model transcription factor-DNA complexes (e.g., DREB2A) and subsequently create precise base edits, which then lead to improved DNA binding affinity and/or modulation of the regulatory activity in a non-perturbing way, hence shifting the paradigm from disruption to functional modulation (Abramson et al., 2024).

Epigenome editing, vast genomics and quick breeding are rapidly combining to be a game-changer in fundamental research into climate-resilient crops that can be grown in the field. A significant field of study involves epigenome engineering, where a disabled Cas9 (dCas9) is fused to an effector domain, such as VPR transcriptional activator, KRAB repressor or TET1 demethylase. It can be used for more accurate and heritable changes in the expression of a gene by altering chromatin marks, such as the methylation of a promoter, that do not affect the DNA sequence (Gallego-Bartolomé, 2020).

Multiplex stacking is particularly important for dealing with polygenic traits. In this way, multiple genes, e.g., NAC111, HKT1;2, Hsf28, and APX2, which all play a role in abiotic stress resistance, are targeted by polycistronic tRNA-gRNA or Csy4-processed sgRNA arrays, typically around 5 to 10 genes. The delivery of pre-assembled ribonucleoprotein (RNP) complexes through lipid nanoparticles (LNPs) has achieved impressive editing efficiencies of about 70% in T_0 plants. However, the AI-designed sgRNA is predicting biallelic rates over 95%, accelerating the development of non-transgenic stacked varieties (Abramson et al., 2024).

It is possible to generate homozygous, trait-stacked lines with doubled-haploid (DH) pipelines and multiplex editing in just three years. In advanced trials in India, a combined stress of drought and salinity has resulted in a 15% yield improvement in hybrids combining edits in ARGOS8 and HKT1;1 (ICAR-CRIDA, 2025). This progress is supported by speed breeding protocols that involve controlled LED lighting and night-day temperature cycles, as well as high-throughput drone-based phenomics for fast assessment of plant traits such as the Normalized Difference Vegetation Index (NDVI) and Anthesis-Silking Interval (ASI) for plant selection in early generations. Large scale public-private partnerships, like the ones between CGIAR institutes and Corteva Agriscience, are now increasing production to eventually make use of climate resilient hybrids in 50 million hectares by 2030, with a particular focus on developing early maturing, stress-tolerant hybrids for Sub-Saharan Africa (CGIAR, 2023).

6. CONCLUSIONS

Conventional breeding approaches have been replaced by precise molecular manipulation of important targets such as ZmDREB2A, ZmNAC111, ZmHsf28, ZmHKT1;2, and ZmNHX1 in the study of maize's responses to abiotic stress. New, cutting-edge tools such as CRISPR-Cas9, and newer tools like Cas12a and base editors are taking us to an unprecedented degree of specificity. For example, the ARGOS8 promoter variants have achieved remarkable field gains of +5 bushels per acre during drought and ZmHKT1;2 SNP knock-ins have been able to lower Na^+ levels in the shoot by a whopping 90%. Plus, activating ZmHsf28 has improved pollen viability by 45% at 40°C, all without needing any agronomic practices in stress-free environments. The results emphasize the superiority of editing over conventional approaches, without linkage drag and accelerate polygenic stacking with

multiplex RNPs and transgene-free recovery. Maize is now under attack with maternal haploid inducers and AI-optimized sgRNA arrays an impressive 80% of doubled-haploids were achieved. This puts our varieties in a strong position to be quickly deregulated under favourable conditions, such as the US/EU SDN-1 exemptions. We are developing a comprehensive map of ~ 500 loci to be edited that regulate osmotic adjustment, ROS quench, ion exclusion and chaperone cascades, and we are incorporating multi-omics analysis of GWAS-discovered QTLs, ChIP-seq regulons, and scRNA-seq hotspots.

In the future, these technologies of epigenome modification (dCas9-TET1 to activate H3K27ac), prime editing to seamlessly swap alleles, as well as the speed of breeding, have the potential of creating next-generation varieties which are drought-, heat- and salinity-tolerant.

Public-private partnerships (PPP) require attention with respect to hybrid development (eg : CGIAR, large seed companies). These coordinated actions can provide 20 to 30 percent yield protection by directly maintaining the root structure, photosynthetic systems and heat-tolerant reproductive stage for an expected temperature increase of 2 to 3°C, and with a target of introducing edited maize to more than 50 million hectares by 2030, impact on food security could be significant.

Future integration of genome editing with genomics, phenomics, artificial intelligence, and speed breeding is expected to accelerate the development of climate-resilient maize cultivars. Continued improvements in transformation efficiency, regulatory harmonization, and field validation will facilitate the deployment of genome-edited varieties for sustainable crop production under changing climatic conditions.

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SUPPLEMENTARY DATA

Supplementary Table: 1 Key drought-responsive transcription factors and their functions in maize.

Regulator	Binding Motif	Validated Target Genes	Primary Phenotypic Impact	Editing Strategy Demonstrated	References
ZmDREB2A	DRE/CRT (ACCGAC)	LEA3, P5CS1, ZmPIP1;5	↓65% survival in KO mutants under severe drought	CRISPR-Cas9 Knockout (KO)	Zhang et al.,2022
ZmNAC111	Secondary cell wall motif	CAD2, SOD3a, APX2	↑35% root depth; ↑ ROS scavenging	Promoter MITE editing;	Jin et al., 2023

				Overexpression (OE)	
ZmAREB1	ABRE (ACGTG)	Stomatal closure genes	↑ Water-Use Efficiency (WUE) by 25%	CRISPR-mediated activation	Ding et al., 2023
ZmJUB1	NACBS (CACGCG)	Antioxidant module genes	Delayed leaf senescence; context-dependent yield effects	Knockout (KO)	Farooqi et al., 2022

Supplementary Table 2: Phased molecular response to heat stress in maize.

Phase	Cellular Impact	Key Regulator	Downstream Effector	Physiological Outcome	References
Perception (0-1h)	Ca^{2+}/H_2O_2 burst, membrane fluidity change	Sensor kinases (e.g., ZmHKs)	ZmHsf28 trimerization & activation	Rapid signal transduction	Ahmed et al ., 2022
Acclimation (1-6h)	Protein unfolding, aggregation	ZmHsf28, ZmHSFA6b	HSP70/90/101 induction	PSII repair (Fv/Fm +0.2), proteostasis	Khan et al., 2022
Reproductive Damage (24-72h)	Tapetal PCD, pollen sterility	ZmAMS, ZmTDF1	Carbohydrate metabolism genes	↓90% pollen viability, kernel abortion	Yang et al., 2025
Recovery	Chaperone-mediated refolding	Sustained HSP expression	Protein re-solubilization	Kernel set recovery, reduced yield loss	Khan et al ., 2022

Supplementary Table 3: Key ion transporters governing salinity tolerance in maize.

Component	Transporter/ Gene	Localization	Primary Function	Phenotypic Gain from Editing	References
Xylem Na^+ Retrieval	ZmHKT1;2	Root xylem parenchyma (PM)	Na^+ uniport from xylem sap	↓90% shoot Na^+, ↑30% biomass under salinity	Zhang et al., 2023
Vacuolar Sequestration	ZmNHX1	Tonoplast	Na^+/H^+ exchange (vacuolar loading)	Fv/Fm ↑0.23, ↑ osmotic adjustment	Yang et al ., 2025
Root Exclusion	ZmSOS1	Root epidermis/cortex (PM)	Na^+ efflux to apoplast	K^+/Na^+ ratio ↑2x, reduced ion toxicity	Faizan et al ., 2021
K^+ Homeostasis	ZmHAK4	Root cortex (PM)	High-affinity K^+ uptake	Enzyme protection,	Li et al., 2023

Supplementary Table 4: Core ROS scavenging enzymes and their regulation in maize.

Enzyme	Subcellular Location	Reaction Catalyzed	Primary TF Regulator	Stress Induction
ZmSOD3a	Cytosol	$2\ O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$	ZmNAC111, ZmWRKY71	4x (Drought/Heat)
ZmCAT2	Peroxisome	$2\ H_2O_2 \rightarrow O_2 + 2\ H_2O$	ZmDREB2A	Constitutive, non-saturable
ZmAPX2	Cytosol	H_2O_2 + AsA → DHA + $2\ H_2O$	ZmAREB1 (ABA-dependent)	3x (Drought, Salt)
ZmGPX1	Mitochondria	$H_2O_2 + 2$ GSH → GSSG + $2\ H_2O$	ZmHSF28	Heat-specific upregulation
ZmGR1	Cytosol/Chloroplast	GSSG + NADPH → 2 GSH + $NADP^+$	ZmJUB1	Essential for cycle recycling