

COLD STRESS TOLERANCE IN ZEA MAYS: REGULATORY MECHANISMS

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Abstract Maize (*Zea mays* L.) is one of the most important C4 cereal crops grown globally. Originating from the tropical and subtropical regions of Mesoamerica, maize is sensitive to temperatures between 0 and 15°C. As maize is cultivated beyond these temperature thresholds, spring frosts can cause severe physiological damage to the plant. However, in recent years, advances in the field of maize genomics have enabled scientists to better understand how the maize genome regulates cold and freezing tolerance within the plant. This review will examine how recent studies on maize have shed light on the physiological impacts of cold stress on maize plants, the role of the ICE1-CBF-COR regulative pathway, as well as other regulative pathways that control freezing tolerance in maize plants. Also, this review will discuss the various breeding strategies that have been employed to create maize varieties with increased resistance to cold stress.

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Introduction

not have the same level of cold tolerance as other cereals like wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) (Kosová et al., 2015). The lack of such adaptations to cold temperatures makes maize more susceptible to disorders in the plant that are initiated by low temperatures (Miedema, 1982). The initial primary sensing event of low temperature happens at the plasma membrane. Cold stress induces a conformational shift in the lipid bilayers of the plasma membrane from a flexible, liquid-crystalline state to a rigidified gel phase (Mendoza, 2014). This phase transition compromises the membrane's fluidity, leading to the formation of physical micro-fractures within the membrane and the leakage of electrolytes from the cell. Photoinhibition in C4 plants, such as maize, happens due to the inhibition of PSII by cold temperatures (Long and Spence, 2013). This inhibition can lead to a reduction in the maximum quantum efficiency of PSII (Fv/Fm). Also, maize's C4 metabolism is dependent upon several enzymes that are sensitive to low temperatures, such as PPDK and PEPC. The inhibition of these enzymes can lead to the development of chlorosis in the leaves of maize.

The impairment of the electron transport chain during photosynthesis results in an accumulation of reactive

oxygen species (ROS) within the plant cells, such as superoxide anions (O₂•⁻), hydrogen peroxide (H₂O₂), and hydroxyl radicals (•OH) (Alam et al., 2025). These species are harmful to plants, leading to lipid peroxidation, protein oxidation and even programmed cell death (Ali et al., 2019). Cold stress can have devastating effects upon the physiological functions of plants. Cold stress can lead to the destabilization of the plant's plasma membrane, the suppression of enzymes necessary for the process of photosynthesis in the C4 pathway, and even the bursting of cellular components due to the formation of reactive oxygen species (Wang et al., 2024). However, some maize species exhibit protective mechanisms against cold stress that allow them to remain healthy and functioning despite the cold temperatures. These protective mechanisms include keeping the photosystem II reaction center within chloroplasts and maintaining the fluidic nature of the plasma membrane (Yamamoto, 2016).

Core Molecular & Regulation

The ICE1-CBF-COR Transcriptional Cascade

Cellular defense against cold stress is coordinated through interconnected signaling and gene-regulatory networks that begin with temperature-associated changes at the plasma membrane, including altered

membrane fluidity and Ca^{2+} influx, and culminate in transcriptional and metabolic reprogramming. The ICE-CBF-COR pathway is a major component of this response, although its relative contribution varies among plant species and genotypes (Jin et al., 2018).

ZmICE1: ICE1 proteins are MYC-like basic helix-loop-helix transcription factors that regulate cold-responsive transcription (Jiang et al., 2022). In the canonical model established primarily in *Arabidopsis*, ICE1 is constitutively expressed and binds MYC-recognition elements in CBF promoters (Table 1). Its activity and stability are controlled by post-translational mechanisms, including phosphorylation, ubiquitination, and SUMOylation (Chen et al., 2020). Homologous ICE1-centered regulation has been reported in maize, but individual ZmICE1-promoter interactions and phosphorylation events should be supported with maize-specific experimental evidence rather than inferred directly from *Arabidopsis*.

ZmCBF/DREB1 family: CBF/DREB1 proteins belong to the AP2/ERF transcription-factor family. Following cold induction, they recognize C-repeat/dehydration-responsive elements, generally represented by the core sequence CCGAC, in promoters of responsive genes (Akhtar et al., 2012). The broader CRT/DRE consensus is often written as A/GCCGAC. Because CBF nomenclature and function are not always directly equivalent among species, maize genes should be identified using validated locus names rather than assuming that ZmCBF1, ZmCBF2, and ZmCBF3 correspond functionally to the three principal *Arabidopsis* CBFs (Caccialupi et al., 2023).

COR genes and cellular protection: The CBF regulon includes genes involved in membrane stabilization, osmotic adjustment, reactive-oxygen-species detoxification, and protection of proteins and cellular structures (Guo et al., 2025). These may encode dehydrins, late embryogenesis abundant proteins, antioxidant components, and enzymes involved in compatible-solute metabolism. Accumulation of sugars and other osmoprotectants can contribute to freezing or chilling tolerance, but not every gene involved in proline, raffinose, or trehalose metabolism is necessarily a direct CBF target (Roychowdhury et al., 2025). Moreover, some CBF-regulated genes lack recognizable CRT/DRE motifs and may therefore be controlled indirectly through secondary transcription factors.

CBF-Independent Networks and Hormonal Cross-Talk

Cold acclimation is not governed exclusively by the CBF regulon. MYB, NAC, WRKY, CAMTA, bZIP, and other transcription-factor families participate in CBF-dependent and CBF-independent responses. These networks interact with Ca^{2+} signaling, mitogen-activated protein kinase cascades, circadian

regulation, chloroplast-derived signals, redox status, and phytohormones.

Absciscic acid: ABA contributes to cold and dehydration responses through PYR/PYL-PP2C-SnRK2 signaling, activation of ABA-responsive transcription factors, regulation of stomatal aperture, and induction of protective genes. Some ABA-responsive genes operate independently of CBFs, but substantial cross-talk exists, so the ABA and CBF pathways should not be described as completely separate (Ali et al., 2026).

Jasmonates: Cold stress can stimulate jasmonate biosynthesis. In the best-characterized model, jasmonate signaling promotes degradation of JAZ repressors, relieving their inhibition of ICE1-related transcriptional activity and thereby supporting CBF expression. Writing that JA "frees ZmICE1" is too maize-specific unless direct interaction between particular maize JAZ and ZmICE1 proteins has been demonstrated.

Salicylic acid: SA may enhance cold tolerance by influencing antioxidant defenses, redox balance, and stress-responsive gene expression (Song et al., 2023). Increases in superoxide dismutase, catalase, and peroxidase activities have been observed in some experimental systems, but the direction and magnitude of SA effects depend on species, tissue, concentration, developmental stage, and stress conditions (Moravcová et al., 2018). It is therefore safer to state that SA can modulate antioxidant capacity rather than that it universally increases SOD, CAT, and POD activity.

Epigenetic Architecture & Post-Translational Controls

In maize, cold responsiveness is precisely regulated thru dynamic chromatin remodeling and post-translational mechanisms:

Post-Translational Modifications (PTMs): In order to avoid growth penalties, ZmICE1 is targeted for proteasomal destruction by RING-type E3 ubiquitin ligases. On the other hand, ZmICE1 is shielded from destruction during early cold onset by SIZ1-mediated sumoylation (Li et al., 2025).

Epigenetic Reconfiguration & Small RNAs: Rapid histone changes brought on by cold stress, such as the enrichment of H3K9ac and H3K4me3, open chromatin architecture at ZmCBF loci. In order to preserve metabolic energy, microRNAs like miR166, miR319, and miR396 simultaneously regulate developmental phase transitions.

Genomic Architecture: GWAS, QTL, and Candidate Genes

High-throughput genotyping of diverse maize germplasm—including inbred lines, landraces, and the Nested Association Mapping (NAM) population—has unraveled the highly polygenic genetic architecture underlying low-temperature tolerance.

Table 1. Gene loci and their functions

Candidate Locus	Molecular Class	Functional Role in Cold Resilience
ZmCBF1 / ZmDREB1A	AP2/ERF Transcription Factor	Directs downstream COR expression; primary driver of freeze acclimation.
ZmICE1	bHLH Transcription Factor	Upstream activator of CBF genes; regulated via sumoylation and phosphorylation.
ZmPPDK	C4 Metabolic Enzyme	Key rate-limiting photosynthetic enzyme; cold-tolerant variants sustain C4 carbon fixation.
ZmSnRK2.x	Serine/Threonine Kinase	Central kinase node in ABA signal transduction during osmotic and cold stress.
ZmGRAS20	GRAS Family Protein	Regulates root tissue elongation and cellular survival under chilling temperatures.
ZmSOD1 / ZmCAT1	Antioxidant Enzymes	Scavenges toxic reactive oxygen species (H ₂ O ₂ , O ₂ •-) during the cold rewarming phase.

Molecular Breeding & Biotechnological Perspectives

To accelerate the development of cold- and freezing-tolerant maize commercial hybrids, molecular insights must be integrated into modern breeding workflows:

High-Throughput Digital Phenomics: Utilizing unmanned aerial vehicles (UAVs) equipped with thermal infrared sensors, high-resolution RGB, and multispectral cameras enables large-scale, non-destructive screening of quantum yield (Fv/Fm), canopy temperature, and early vigor across breeding field trials (Niu et al., 2025).

Genomic Selection (GS) & Marker-Assisted Breeding: Because cold tolerance is governed by hundreds of small-effect QTLs, Genomic Selection models combining high-density SNP arrays with phenomics yield significantly higher prediction accuracy than traditional Marker-Assisted Selection (MAS) (Mallikarjuna et al., 2022).

CRISPR/Cas Promoter Engineering: Targeted edit of cis-regulatory promoter elements in ZmCBF or ZmCOR loci can enhance cold-induced expression without incurring yield drag under optimal thermal conditions. Knockout of negative regulators (e.g., specific JAZ or E3 ligase genes) offers another powerful strategy.

Introgression of Highland Landraces & Teosinte Alleles: High-altitude Mexican landraces (e.g., Palomero Toluqueño) and wild relatives (*Zea mays* ssp. *huehuetenangensis*) harbor valuable, naturally selected alleles for cold-hardiness and photosynthetic efficiency at low temperatures that were lost during modern industrial breeding.

Conclusion

In light of shifting climatic paradigms, expanding the northern and high-altitude limits of maize agriculture is essential to ensuring global food production. A complex network including the ICE1–CBF–COR regulatory axis, parallel signal cascades, hormone cross-talk, and structural chromatin alterations has been revealed by deciphering the genomic architecture of cold and freezing tolerance. To create high-yielding, cold-resilient maize cultivars for

tomorrow's agriculture, future efforts must focus on multi-omics integration (transcriptomics, metabolomics, and epigenomics) in conjunction with multiplexed CRISPR genome editing and high-altitude landrace allele mining.

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Statements and Declarations

Data Availability statement

All relevant data are within the manuscript file.

Author's Contribution Statement

ZA, and SS collected data and wrote manuscript equally. SK, and ZA make final editing. SK supervised the whole research work. All authors have read the final manuscript and approve its submission.

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Declaration of Generative AI and AI-assisted Technologies in the Writing Process

During the preparation of this work, the author(s) have not used any ChatGPT (OpenAI) tool.



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