Maydica

Review Open Access

Molecular Mechanisms and Biotechnological Strategies for Abiotic Stress Tolerance in Maize (*Zea mays* L.)

Mir Muhammad Adil [1]*, Mahira Shaikh[2]

1 Department of Biosciences, Shaheed Zulfikar Ali Bhutto Institute of Science and Technology (SZABIST), 90 and 100 Clifton, Karachi, Sindh 75600, Pakistan.

2 Department of Biomedical Engineering, Ziauddin University, Karachi, Sindh, Pakistan

*Corresponding author: E-mail: *mirmdadil@gmail.com*

Keywords: *maize, drought stress, heat stress, CRISPR-Cas9, molecular breeding*

Abstract

Maize (*Zea mays* L.) productivity is increasingly threatened by drought and heat, particularly when the stresses coincide around flowering and disturb water relations, photosynthesis, reproductive development and kernel set. This article reviews the molecular architecture through which maize senses stress, integrates abscisic acid, calcium, reactive oxygen species and kinase signals, and activates transcriptional programs that protect cellular function while preserving growth. Emphasis is placed on evidence-rich maize regulatory modules that illustrate why regulatory dosage, tissue specificity and developmental timing are as important as gene identity. The review then evaluates transgenic approaches, genome editing, natural-allele deployment and multi-omics discovery in a common translation framework. A central conclusion is that a useful stress-tolerance target must connect a defined mechanism to repeatable physiological and grain-yield benefits across relevant drought and heat environments. Integrating molecular breeding with high-quality phenotyping, water-conserving agronomy and locally appropriate seed delivery offers a practical route to more resilient maize production.

Abbreviations

ABA : abscisic acid
ABF : ABRE-binding factor
bZIP : basic leucine zipper
CRISPR-Cas : clustered regularly interspaced short palindromic repeats/CRISPR-associated protein
DNA : deoxyribonucleic acid
DREB : dehydration-responsive element-binding protein
ERF : ethylene-responsive factor
GWAS : genome-wide association study
HSF : heat shock factor
HSP : heat shock protein
MITE : miniature inverted-repeat transposable element
MYB : MYB transcription factor
NAC : NAM, ATAF1/2 and CUC2 transcription factor
NF-Y : nuclear factor Y
PYR/PYL/RCAR : pyrabactin resistance/PYR1-like/regulatory component of ABA receptor
QTL : quantitative trait locus
RNA : ribonucleic acid
RNA-seq : RNA sequencing
ROS : reactive oxygen species
SnRK2 : SNF1-related protein kinase 2
VPD : vapor-pressure deficit
WRKY : WRKY transcription factor

Introduction

Maize is a high-value cereal because its grain, stover and industrial products underpin food, feed and bio-based value chains. Its productivity, however, is highly exposed to water deficit and high temperature. Long-term global analyses have already linked warming trends with losses in major crop production (Lobell *et al.*, 2011). For maize, the agronomic danger is not merely a lower daily photosynthetic rate. Drought can delay silk emergence, restrict assimilate supply, and reduce kernel set, while heat can destabilize membranes, impair pollen function, and accelerate developmental transitions. When both stresses occur around flowering stage, the phenotype can be qualitatively different from either stress alone (Sato *et al.*, 2024).

A useful review on stress tolerance must therefore move beyond a list of stress-induced genes. Drought toleran-

ce in maize is polygenic, stage-dependent and strongly conditioned by soil depth, vapor-pressure deficit (VPD), temperature trajectory and recovery conditions. Recent maize-focused syntheses highlight the importance of the reproductive phase, although its molecular regulation remains incompletely understood (Singh *et al.*, 2023). Reproductive stages are particularly vulnerable to heat, as reproductive tissues may become non-functional before leaves show signs of injury (Zenda *et al.*, 2022). In production environments, drought and heat may co-occur and interact with variation in radiation, nutrient availability and atmospheric demand, making single-trait selection unreliable (Fahad *et al.*, 2017).

The biological question is therefore not whether a genotype survives a treatment applied, but whether it has a useful grain yield and recovers after a stress event, while still performing well when conditions are favorable or treatment is not applied. Molecular mechanisms are important, because they reveal potential points of intervention, such as selection of a promoter with a naturally higher expression level, editing of a promoter or a regulatory sequence, or development of a tissue-specific expression strategy that avoids growth penalties generally associated with a constitutive stress response (Shi *et al.*, 2017; Chen *et al.*, 2019).

This review first links drought and heat injury to measurable maize traits. It then examines signaling pathways and transcription factor networks controlling adaptive responses, causal genes supported by functional evidence, biotechnology and omics-based breeding. The final sections translate these tools into sustainable production systems in which molecular gain is tested against the actual environments in which farmers require reliable maize performance.

Literature Search and Review Approach

This review was structured around peer-reviewed literature on drought and heat tolerance in maize, with emphasis on molecular signaling, transcriptional regulation, causal loci, biotechnology, multi-omics and field translation. Studies were prioritized when they provided maize-specific genetic, functional, physiological or grain-yield evidence, while heterologous findings were distinguished from in-maize and field-level validation. Recent research was considered alongside established foundational studies to connect molecular mechanisms with applied maize breeding and production outcomes. The review is narrative rather than systematic; therefore, no formal meta-analysis or quantitative risk-of-bias assessment was performed.

Stress injury, developmental context and trait architecture

Why drought and heat are not interchangeable

Drought can be considered as a hydraulic and osmotic disturbance. As soil water availability decreases, the water potential of leaves decreases, cell expansion is reduced and the benefits of stomatal control increase. The thermal load that is the cause of heat is a change in membrane behavior, elevation of respiratory requirement, and an increase in protein misfolding. Both stresses can increase reactive oxygen species (ROS), decrease photosynthetic carbon assimilation and disrupt the hormone balance, but with different immediate demands. Drought-induced decreases in stomatal conductance can help to maintain water status. The same closure can reduce transpirational cooling and exacerbate tissue temperature under severe heat. The optimal response is therefore context-dependent rather than maximal (Fahad *et al.*, 2017; Sato *et al.*, 2024).

This separation is the reason why combined stress may not easily be extrapolated from drought and heat experiments conducted separately. A tolerant line should balance water conservation with canopy energy balance, maintain integrity of photosynthetic apparatus and maintain reproductive competence. Small changes in anthesis-silking synchrony, silk growth and/or pollen viability at flowering can produce a disproportionate yield loss. The correct target should not be a static stress marker, but rather a timed response that is of magnitude, tissue, and duration appropriate for the production scenario (Araus *et al.*, 2018; Sato *et al.*, 2024).

From molecular response to a meaningful maize phenotype

A robust phenotype panel should separate stress avoidance, cellular tolerance and post-stress recovery. Flowering-stage grain yield, anthesis-silking interval, kernel number, canopy temperature, leaf water status, stomatal conductance, chlorophyll fluorescence, and speed of return to normal growth, as well as root depth and hydraulic capacity, are useful readouts. The above characteristics do not all go hand in hand. For instance, a cooler canopy may also be desired to show an effective transpirational cooling effect when water is available, but it may be undesirable if it shows excess water loss in a terminal drought. It is therefore necessary to interpret the traits with the help of environmental information and an explicit water regime hypothesis (Araus *et al.*, 2018; Nelson *et al.*, 2007).

Screening can still be used to detect genes, especially if it shows natural alleles or reproducible molecular differences. Such findings alone are insufficient as

cultivar-release criteria. The strongest translation pipeline test is one that tests a candidate in a controlled environment, field stress and multi-location trials and compares the stress gains with yield under non-stress conditions. This architecture is key to preventing one of the usual pitfalls of abiotic-stress research: selecting a molecular response that does not translate into improved grain yield (Araus *et al.*, 2018; Nelson *et al.*, 2007).

Timing, tissue specificity and recovery

Stress timing creates distinct molecular priorities. Before flowering, water deficit can constrain leaf area, root extension and the size of the source canopy. At flowering, a short stress episode can have a much larger reproductive consequence because silk growth, pollen release, pollen-tube growth and early kernel development are sensitive to hydraulic and thermal disruption. After flowering, the principal problem can shift toward maintenance of photosynthetic source strength, remobilization of assimilates and protection of developing kernels. A single gene can therefore be beneficial at one stage and neutral or costly at another if its expression is not appropriately controlled (Sato *et al.*, 2024; Zenda *et al.*, 2022).

Tissue specificity is equally important. Guard cells need rapid ion and hormone responses; roots need hydraulic, metabolic and growth adjustments; reproductive organs need protection of cell division, membrane integrity and carbohydrate supply. Whole-leaf RNA measurements are informative but may miss the decisive signal in silk, anther, root tip or vascular tissue. This supports the use of tissue-aware promoters, cell-type-sensitive phenotyping and sampling designs that reflect the target stress event rather than relying on a single bulk-tissue time point (Araus *et al.*, 2018; Yang *et al.*, 2021).

Recovery deserves the same attention as injury. Transiently reduced growth due to a genotype may be compensated for by increased photosynthesis, silk growth, and kernel filling when water and temperature return to normal. Conversely, an apparently healthy plant may have damaged chloroplasts or reproductive organs, with delayed consequences for survival or yield. Hence, time-series phenotyping should involve stress onset, stress peak, and stress recovery, and yield measured at maturity. This prevents choosing an early molecular signature which is not related to the end agricultural product (Guo *et al.*, 2021; Sato *et al.*, 2024).

Stress perception and signal integration

ABA, calcium and kinase signaling

Plants do not appear to use one universal drought or heat sensor. Rather, calcium signatures and phosphorylation cascades are used to decode the altered turgor, membrane state, cell-wall properties, redox balance and metabolism. The calcium sensors, calcium-dependent protein kinases, calcineurin B-like protein-interacting kinases and mitogen-activated protein kinase pathways can transduce transient physical perturbations into information for the regulation of genes. This distributed architecture is well suited to maize, since the intensity and timing of stress in the field are different for different tissues and different days (Aslam *et al.*, 2022; Cutler *et al.*, 2010).

A particularly important drought response hub is provided by abscisic acid (ABA). Its fundamental mechanisms include ABA perception by PYR/PYL/RCAR receptors, inhibitory regulation of clade A protein phosphatases 2C, and activation of SnRK2 kinases, which control ion transport and transcriptional programs (Cutler *et al.*, 2010). This pathway regulates stomatal movements, osmotic adjustment and stress genes. It should not be thought of as a growth turn-off valve. To prepare the appropriate response, it is necessary to tune the response to ABA, since its extended signal or ectopic signal may suppress carbon capture and developmental processes. Current evidence also suggests that ABA is not acting alone but rather in a network with jasmonate, ethylene and auxin (Aslam *et al.*, 2022).

ROS, hormone crosstalk and heat-response coupling

Many examples of ROS demonstrate the need for dosage control. ROS can be produced under drought and/or heat conditions in chloroplasts, mitochondria, peroxisomes and the apoplast. At controlled levels, ROS act as signaling molecules that influence kinases and transcriptional regulators, but when production exceeds antioxidant buffering, they cause damage to lipids, proteins and nucleic acids. A tolerance mechanism should therefore be evaluated both from the perspective of signaling competence and redox protection, such as superoxide dismutase, catalase, peroxidases, ascorbate-glutathione metabolism and membrane stability (Nadarajah, 2020).

This systems view is backed up by comparisons of maize transcriptomes. The difference between drought-tolerant and sensitive genotypes is not a single pathway, but in their induction of oxidative defense, photosynthetic electron transport, protein modification, phytohormone responses and transcription factors

Table 1 - Evidence-rich maize molecular modules for drought and heat resilience. The table distinguishes natural variation, in-maize functional evidence and heterologous evidence so that discovery status is not confused with deployment readiness.

Module	Mechanistic or functional evidence	Deployment interpretation
ZmDREB2.7	Promoter polymorphisms associated with seedling drought tolerance in a 368-line maize panel; drought-responsive expression and heterologous functional support (Liu *et al*., 2013).	Favorable regulatory allele is a selection candidate; flowering-stage and field validation remain essential.
ZmNAC111	An 82-bp promoter MITE represses expression through epigenetic mechanisms; elevated expression improved seedling drought tolerance and water-use efficiency in maize (Mao *et al*., 2015).	A natural regulatory mechanism with a clear expression direction; assess allelic effects in elite germplasm.
ZmVPP1	A 366-bp promoter insertion containing MYB cis-elements increases drought-inducible expression; enhanced expression improved roots and photosynthetic performance (Wang *et al*., 2016).	Promoter haplotypes or regulatory editing can be prioritized over coding-sequence disruption.
ZmNF-YB2	Increased expression supported leaf-water relations, photosynthesis and grain yield under water-limited conditions (Nelson *et al*., 2007).	Useful transgenic dosage example; deploy only after checking maturity, non-stress yield and background effects.
ZmWRKY106	Drought-induced WRKY gene that conferred drought and heat tolerance in transgenic plants (Wang *et al*., 2018a).	Mechanistically promising, but evidence should be treated as heterologous until maize field yield is demonstrated.
Zmhdz9-ZmWRKY120 / ZmTCP9	Zmhdz9 promotes ABA and lignin-related responses through ZmNCED1 and ZmKNOX8 regulatory routes; overexpression and editing experiments improved drought resistance (Jiao *et al*., 2024).	Network-level candidate; test pleiotropy because ABA and lignin affect development as well as stress response.
DSD1/ZmICEb	Mutations alter stomatal development, improve water-use efficiency and drought tolerance, and reduce drought-associated yield loss (Zhou *et al*., 2025).	Direct water-productivity lever; validate trade-offs in canopy cooling and carbon capture across environments.
ZmHsf28 / ZmHSFA2d	ZmHsf28 integrates ABA and jasmonate signaling with stomatal and ROS responses; ZmHSFA2d supports heat and drought tolerance and photosystem protein maintenance (Cao et al., 2025; Liu *et al*., 2025).	Attractive multi-stress modules; use tissue-, stage- and dosage-aware designs to avoid broad stress-response costs.

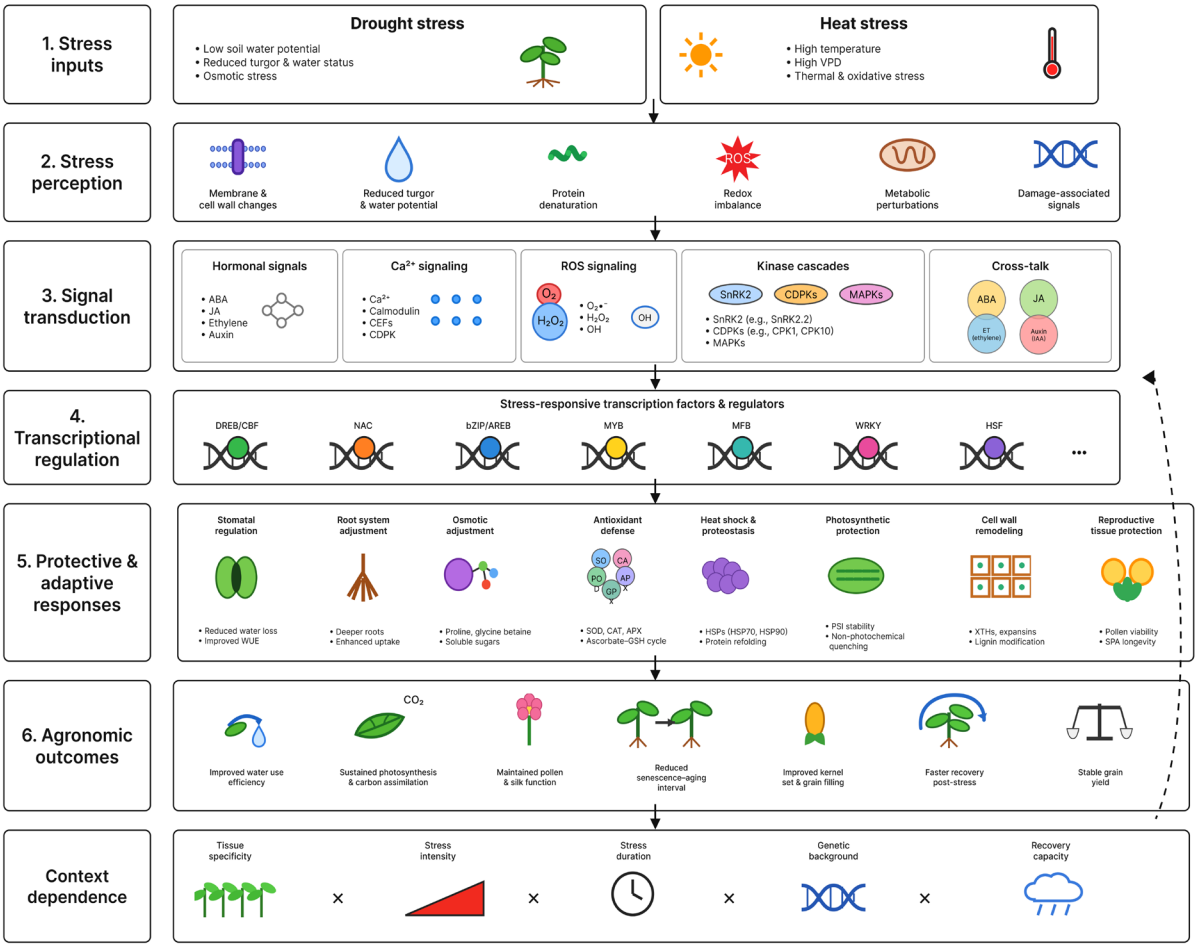


Fig. 1 - Integrated molecular architecture of drought and heat tolerance in maize. Hydraulic, thermal, membrane and redox disturbances activate ABA, calcium, ROS and kinase signaling pathways. These pathways interact with hormone networks and stress-responsive transcription factors to regulate stomatal behavior, root function, antioxidant defense, proteostasis, photosynthetic protection and reproductive resilience.

(Wang *et al.*, 2022). A recently published maize module makes this hormonal connection more obvious: ZmHsf28 interacts with ZmSnRK2.2 and is inhibited by ZmJAZ14/17, connecting ABA and jasmonate biosynthesis with stomatal closure and ROS control during drought (Liu *et al.*, 2025). Heat shock factors (HSFs) and heat shock proteins (HSPs) also provide a proteostasis layer. Maize contains 25 annotated HSF and 22 HSP70 genes, and members of both families respond to heat treatment (Jiang *et al.*, 2021).

Figure 1 summarizes this integrated architecture. The diagram deliberately includes a context filter between signaling and output. The same upstream signal can produce a different agronomic result according to developmental stage, tissue, genetic background and stress duration. This is why causal mechanisms must be paired with field-aware phenotyping rather than inferred from transcript induction alone.

Reversible signaling and stress memory

Signal integration must also be reversible. A productive maize plant cannot maintain a full stress program after the environment improves because long-term stomatal restriction, defense metabolism and growth suppression consume carbon that should support biomass and grain filling. The useful molecular property is therefore not simply strong induction; it is an appropriate rise, persistence and decline in activity. Kinase phosphorylation, feedback from ROS-scavenging systems, hormone turnover and transcriptional repressors all contribute to this temporal control (Sato *et al.*, 2024; Yoon *et al.*, 2020).

Stress memory is a related but distinct idea. Prior exposure can leave molecular or metabolic states that improve a later response, but such states must be tested for duration, cost and transferability across genotypes. In a breeding context, a memory-associated

Table 2 - Biotechnology and breeding options for abiotic-stress resilience in maize, with the validation requirement that prevents a molecular result from being mistaken for a field-ready trait.

Approach	Biological opportunity	Critical validation and risk control
Natural-allele deployment	Use favorable promoter or haplotype variation, such as ZmDREB2.7, ZmNAC111 and ZmVPP1, through marker-assisted introgression or haplotype selection (Liu *et al.*, 2013; Mao *et al.*, 2015; Wang *et al.*, 2016).	Confirm direction of effect in elite backgrounds and at flowering; avoid assuming that a seedling allele predicts grain yield.
Transgenic dosage control	Increase or localize expression of a validated regulator, as illustrated by ZmNF-YB2 (Nelson *et al.*, 2007).	Use tissue- and stage-appropriate promoters; quantify non-stress yield, developmental effects and stability.
CRISPR regulatory editing	Modify promoter activity or 5-prime regulatory regions while preserving coding sequence. ARGOS8 shows field-level proof of concept (Shi et al., 2017).	Sequence-confirm the edit, test off-target risks, and measure yield under both managed stress and well-watered conditions.
Targeted knockout or multiplex editing	Remove a defined negative regulator or combine independently validated modules; modern editing platforms broaden the design space (Zhang *et al.*, 2018; Chen *et al.*, 2019).	Screen pleiotropy, compensation and epistasis before stacking; do not combine modules solely because each works separately.
Multi-omics prioritization	Integrate genomics, transcriptomics, proteomics, metabolomics and physiology to identify causal candidates and pathway state (Guo *et al.*, 2021; Yang *et al.*, 2021).	Require temporal and tissue-specific sampling; correlation alone does not establish a causal breeding target.
Genomic prediction and high-throughput phenotyping	Predict polygenic resilience using markers, environmental covariates, canopy temperature, imaging and growth trajectories (Crossa *et al.*, 2017; Araus *et al.*, 2018).	Train and test models across the intended target population of environments; report prediction accuracy and genotype-by-environment behavior.

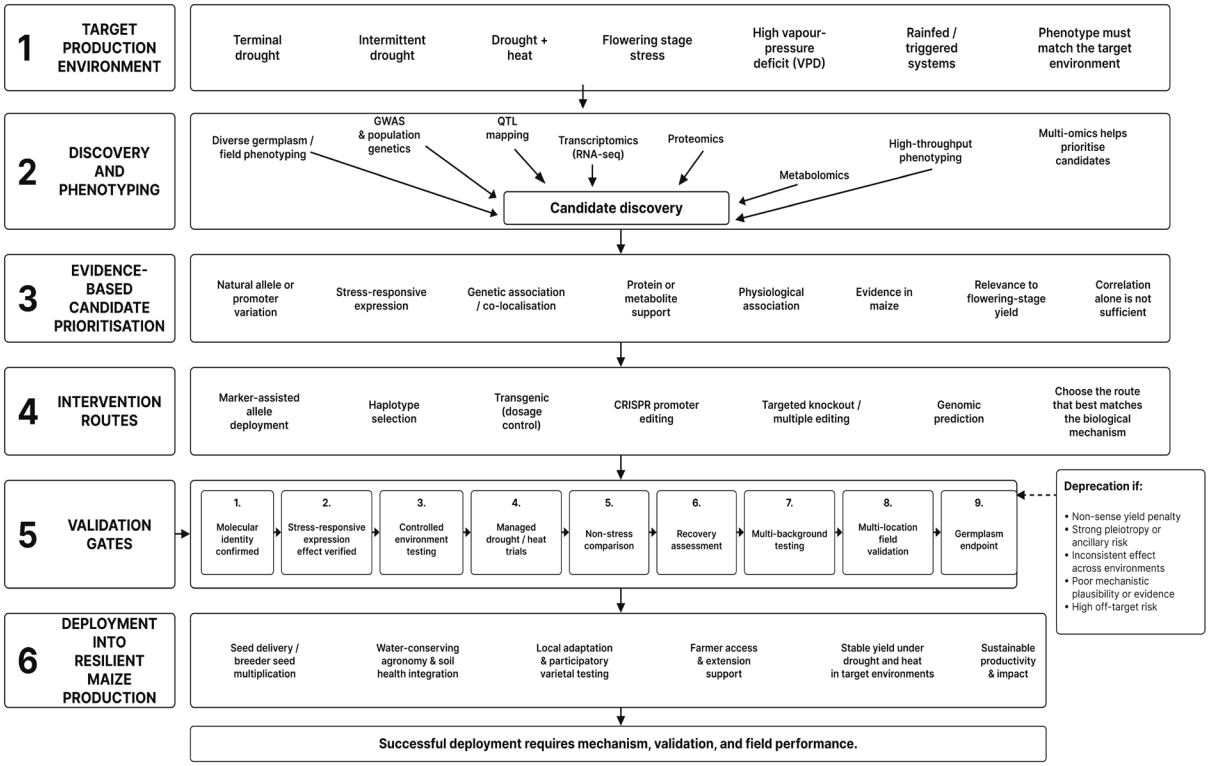


Fig. 2 - Evidence-gated pipeline for translating abiotic-stress mechanisms into resilient maize cultivars. Candidate discovery through genetics, multi-omics and phenomics is followed by evidence-based prioritization, selection of an appropriate breeding or biotechnology intervention, molecular and physiological confirmation, managed-stress testing and multi-environment yield validation.

signature becomes useful only if it improves recovery or yield under a realistic recurrent-stress scenario. This requirement helps separate an attractive mechanistic observation from a trait worth carrying into a breeding program (Guo *et al.*, 2021; Sato *et al.*, 2024).

Transcriptional networks and protective effectors

DREB/ERF, NAC, bZIP/ABF and NF-Y modules

Stress-responsive transcription factors convert signal integration into coordinated changes in metabolism, transport, cell-wall remodeling and development. DREB/ERF, NAC, bZIP/ABF, NF-Y, MYB, WRKY and HSF families recur across abiotic-stress studies because each family can regulate multiple downstream effectors. Their value for breeding is not equivalent: some act as broad hubs with a high risk of pleiotropy, whereas others offer more tractable regulatory alleles or tissue-specific targets (Yoon *et al.*, 2020).

The DREB family has especially strong relevance to dehydration signaling. In maize, a genome-wide analysis of 18 ZmDREB genes associated promoter-region variation in ZmDREB2.7 with natural variation in seedling drought tolerance. The favorable allele was linked to drought-responsive expression, and ZmDREB2.7 overexpression improved drought tolerance in *Arabidopsis* (Liu *et al.*, 2013). This evidence illustrates a recurring opportunity: a regulatory allele can be more useful than a coding-sequence change when it changes the amplitude or timing of an otherwise functional protein.

NAC and NF-Y factors provide complementary examples. An 82-bp miniature inverted-repeat transposable element in the ZmNAC111 promoter is associated with drought sensitivity because it represses expression through RNA-directed DNA methylation and H3K9 dimethylation; raising ZmNAC111 expression increased water-use efficiency and drought-responsive gene expression in transgenic maize (Mao *et al.*, 2015). Earlier field-oriented work showed that enhanced ZmNF-YB2 expression supported photosynthesis, leaf-water relations and grain yield under water-limited conditions (Nelson *et al.*, 2007). These studies justify work on transcriptional regulators, but they also warn against treating a promoter or overexpression result as a universal cultivar solution.

MYB, WRKY and HSF networks

MYB factors often connect stress response with secondary metabolism, cell-wall properties and promoter-level control. Their relevance is visible in a natural drought-tolerance allele of ZmVPP1: the favorable 366-bp promoter insertion contains three MYB cis-elements, increases drought-inducible expression and is associa-

ted with stronger root development and photosynthetic performance (Wang *et al.*, 2016). Thus, MYB-related regulation can be exploited through cis-regulatory sequence rather than by indiscriminate activation of a whole transcription-factor family.

WRKY proteins provide another layer of signal-to-gene coupling. ZmWRKY40 improved drought resistance in transgenic *Arabidopsis* (Wang *et al.*, 2018b), while ZmWRKY106 conferred both drought and heat tolerance in transgenic plants (Wang *et al.*, 2018a). These findings are valuable for hypothesis generation but should be classified as heterologous evidence until a maize field phenotype is established. In contrast, a recent maize study placed Zmhdz9 within a more resolved network: it activates ZmWRKY120 and ZmTCP9, thereby promoting the ABA-biosynthetic gene ZmNCED1 and a lignin-associated pathway through ZmKNOX8 (Jiao *et al.*, 2024).

While the most useful maize modules incorporate drought signals, the HSFs play a key role in the heat acclimation process by inducing chaperone expression and repair of the proteome. HSF regulatory logic includes transcriptional activation, phosphorylation, hormone crosstalk and HSP feedback (Guo *et al.*, 2016). ZmNF-YA1 is involved in maize thermotolerance, controlling heat-shock responses (Yang *et al.*, 2024). ZmHSFA2d provides direct multi-stress evidence: its overexpression resulted in heat tolerance, enhanced antioxidant activity and preservation of photosystem proteins, while its CRISPR/Cas9 knockout led to a decrease in heat and drought tolerance (Cao *et al.*, 2025). These examples provide the field with a step beyond mere heat-shock-protein catalogs to testable regulatory modules.

Effectors that preserve function under stress

Transcriptional regulation only becomes agronomically useful through downstream effectors. Those effectors under water deficit are ion transport, osmotic adjustment, root growth, cell-wall modification, antioxidant systems and protection of photosynthetic electron transport. Under heat, protein folding capacity, membrane composition, ribosome function and photosystem repair become particularly important. Proteomic comparisons of contrasting maize varieties reveal coordinated changes in protein abundance associated with physiological differences, rather than a single tolerance marker (Li *et al.*, 2021). From a practical standpoint, it means that the process of monitoring an edited or selected regulator should be done for the physiological output(s) that it actually regulates, and not for the transcript level alone (Li *et al.*, 2021; Yoon *et al.*, 2020).

Chromatin, promoter architecture and post-transcriptional control

Promoter architecture is a major source of useful variation because it can alter dose without necessarily changing protein function. As illustrated in the examples of ZmDREB2.7 and ZmVPP1, promoter polymorphisms can alter drought-responsive expression and as in the example of ZmNAC111, a transposable element can recruit epigenetic repression and create a population-level difference in drought tolerance (Liu *et al.*, 2013; Mao *et al.*, 2015; Wang *et al.*, 2016). These cases make a case for sequencing regulatory regions as well as coding regions in drought and heat diversity panels.

Post-transcriptional and post-translational layers add another level of control. Without a big steady-state transcript difference, output can change due to alternative splicing, mRNA stability, small RNAs, protein phosphorylation, protein turnover or subcellular localization. This means that RNA-seq data should be correlated with protein, metabolite and physiological data, where possible. Therefore, editing a promoter or binding motif may be preferable to knocking out a transcription factor with multiple developmental functions (Chen *et al.*, 2019; Yang *et al.*, 2021).

Causal maize loci and evidence tiers

Stronger evidence comes from convergent genetics and function

Not all candidate genes deserve the same deployment priority. A valuable evidence ladder starts with a transcript that is responsive to stress, or a protein that is no longer present or is increased in abundance, moves on to the association of a locus or haplotype with a phenotype and is most convincing when altered gene dosage or sequence changes the phenotype in maize and the effect persists in field trials. Natural variation in regulation can be illustrated in the case of ZmVPP1, ZmNAC111 and ZmDREB2.7. Targeted expression changes can impact yield under water-limiting conditions, as is seen in the case of ZmNF-YB2 and ARGOS8. All these examples illustrate why cis-regulatory logic is appealing: it can modify when (and where) a response is switched on without actually removing a basic function (Cao *et al.*, 2025; Nelson *et al.*, 2007; Zhou *et al.*, 2025).

Stomatal development is an example of another intervention class. The dsd1 mutant reveals that DSD1/ZmICEb is a basic helix-loop-helix factor that is necessary for proper stomatal development. Mutations led to decreased stomatal density, increased water use efficiency and increased drought tolerance with decreased yield loss under drought (Zhou *et al.*, 2025). This water-productivity pathway is a promising one, but can-

not be assessed in isolation; a reduced stomatal density could have different impacts on cooling capacity versus carbon gain versus disease interactions, depending on location (humid, arid, heat-prone). Table 1 distinguishes these evidence levels and identifies the accompanying deployment question for each molecular module.

What the evidence says about trait stacking

The loci in Table 1 should not be interpreted as a shopping list for unrestricted stacking. Combining modules that all reduce stomatal opening, for example, may improve water conservation under terminal drought but impose excessive carbon or cooling costs under intermittent drought and heat. Likewise, constitutive activation of broad transcriptional hubs can alter development. A better stacking logic is functional complementarity: one module may preserve root hydraulics, another may stabilize proteostasis, and a third may improve reproductive resilience. Their combined value must be estimated in the same genetic background and target environment rather than inferred from separate publications (Chen *et al.*, 2019; Liu *et al.*, 2013; Wang *et al.*, 2016).

A causal locus also needs a clear intervention route. Natural promoter alleles can enter breeding through marker-assisted introgression or haplotype selection. A known regulatory sequence can be recreated through precision editing. A module whose evidence rests on heterologous transgenics may need in-maize functional validation before either route is justified. This distinction protects breeding programs from spending resources on candidates whose direction of effect is still uncertain in elite maize (Liu *et al.*, 2013; Mao *et al.*, 2015; Wang *et al.*, 2016).

Evidence thresholds for cultivated germplasm

A discovery population can expose a causal allele that is absent, rare or poorly expressed in elite material. Before investment in a breeding route, the candidate should be surveyed across the intended heterotic groups and tested for linkage drag, allele frequency, promoter context and interaction with local disease and maturity constraints. A strong association in a diverse panel is not a guarantee of utility in a commercial hybrid. Conversely, a rare favorable allele can motivate a targeted edit if it cannot be efficiently introduced through conventional crossing (Araus *et al.*, 2018; Crossa *et al.*, 2017).

This cultivated-germplasm filter is a scientific as well as a practical necessity. It tests whether a mechanism remains directional when combined with the many other loci that shape maize yield. It also helps distinguish a pathway marker from a deployable allele. The decision is strengthened when molecular, physiological and yield evidence all point to the same response and when the effect is reproducible in more than one elite background (Araus *et al.*, 2018; Crossa *et al.*, 2017).

Biotechnological strategies for resilient maize

Transgenic expression and dosage control

Transgenic approaches remain informative when a trait requires increased dosage, a novel allele or a tissue-specific response that does not exist yet in the breeding pool. ZmNF-YB2 is an early example of a transcriptional regulator that enhanced field performance under water limitation (Nelson *et al.*, 2007). The strongest contemporary design principle is to avoid blanket overexpression whenever possible. Promoters should be selected for the relevant tissue and developmental window, and the construct should be tested for non-stress yield penalties, altered maturity, root-shoot trade-offs and stability across genetic backgrounds. A transgene that improves a seedling assay but reduces grain yield under favorable conditions is not a resilience trait.

CRISPR-Cas editing and regulatory engineering

CRISPR-Cas systems expand the options because they can create loss-of-function alleles, modify promoters, change cis-regulatory architecture or assemble multiple edits. The most persuasive maize example is ARGOS8. Rather than introducing a random change, CRISPR-Cas9 was used to insert or replace the native promoter with the moderately constitutive GOS2 promoter. The resulting ARGOS8 variants increased transcript abundance and raised yield by five bushels per acre under flowering stress without a yield loss under well-watered field conditions (Shi *et al.*, 2017). This is a model for regulatory engineering: a precise molecular change, verified sequence identity and an agronomically relevant field endpoint.

Genome editing should still be matched to biological mechanism. If a favorable promoter allele already exists, allele replacement or targeted promoter editing may preserve useful native regulation. If a negative regulator has a defined, limited function, a knockout can be considered, but pleiotropy and compensatory networks must be assessed. Base editors and prime-editing approaches offer additional precision for selected nucleotide changes, whereas multiplex editing may be appropriate only after each component has been independently validated. General crop-editing reviews emphasize that technical precision does not remove the need for trait-level validation (Zhang *et al.*, 2018; Chen *et al.*, 2019).

Omics, phenomics and predictive breeding

Multi-omics does not replace experimentation; it helps prioritize experiments. Genomics identifies haplotypes and structural variation, transcriptomics reveals stress-responsive programs, proteomics shows whether transcription reaches the protein level, and metabolomics captures biochemical state. Integration is most useful when these data are collected from the relevant tissue, growth stage and stress trajectory, then connected to genetic mapping and physiology (Yang *et al.*, 2021). The resulting evidence can distinguish a correlated transcript from a locus that plausibly causes a trait.

Genomic selection can use dense markers and environmental covariates to predict complex, polygenic performance, while high-throughput phenotyping can measure canopy temperature, spectral indices, growth dynamics and water-use proxies at scale (Crossa *et al.*, 2017; Araus *et al.*, 2018). These approaches are particularly valuable where many small-effect loci contribute to yield stability. They should not, however, conceal uncertainty. A study of combined drought and cold stress in maize demonstrated that metabolite, ABA and transcriptional responses can vary over time and between stress and recovery (Guo *et al.*, 2021). Figure 2 therefore frames omics as one step in a gated pipeline, not as an endpoint. Table 2 translates that pipeline into design choices and quality controls.

Data integration should produce a ranked intervention, not an undirected list

A practical multi-omics workflow starts with a clearly defined production scenario, samples the relevant tissue before irreversible injury, and asks whether independent data layers converge. For example, a high-priority target might show a stress-responsive promoter allele, co-localize with a QTL, change transcript and protein abundance in a tolerant genotype, and associate with a physiological trait such as canopy cooling or kernel set. By contrast, a gene that appears only in a broad differential-expression list should be treated as a hypothesis, not a breeding target. This ranking discipline makes gene editing and transformation experiments more efficient and more interpretable (Araus *et al.*, 2018; Yang *et al.*, 2021). Data integration also benefits from negative information. A candidate should be deprioritized if it produces an undesirable maturity shift, loses benefit under non-stress conditions, has an inconsistent direction of effect across environments or cannot be distinguished from a tightly linked causal locus. Reporting these outcomes improves collective breeding knowledge and reduces repeated investment in pathways that are biologically responsive but agronomically unsuitable (Chen *et al.*, 2019; Crossa *et al.*, 2017).

Integration into sustainable maize production systems

Breeding resilient maize for target production environments

Molecular tools are most useful when they are integrated with an explicit production objective. The target may be late-season water deficit, intermittent drought with heat spikes, shallow soils, a rainfed flowering window or a high-VPD environment. Each objective implies a different combination of traits, phenotyping schedule and validation sites. A variety designed for water savings under terminal drought may need different stomatal and root behavior from a variety designed to maintain cooling during episodic heat. Recent reviews of maize stress molecular biology underline the value of combining transcriptomics, GWAS, QTL analysis and gene editing, but the methods only converge when the phenotype is defined with agronomic precision (Meng *et al.*, 2024).

The sustainable production frame also prevents molecular solutions from being oversold. Stress-tolerant genetics should complement water-conserving soil management, residue retention where appropriate, timely planting, nutrient management, climate information and seed-system access. The desired outcome is not maximal expression of a stress gene. It is stable grain output per unit of water and land, with a cultivar that farmers can access and manage under local conditions. Molecular breeding has its greatest impact when it shortens the route from field problem to validated cultivar rather than when it substitutes for agronomy (Fahad *et al.*, 2017; Meng *et al.*, 2024).

A practical validation standard

For candidate loci and edited events, a minimum standard should include confirmation of molecular identity, expression or protein effect, relevant physiological readouts, replicated field grain-yield data, comparison with non-stress controls and evaluation in more than one genetic background. Environmental data should report temperature, rainfall or irrigation, soil conditions and stress timing. If an event is intended for deployment, off-target assessment, inheritance, seed quality, regulatory requirements and stewardship should be addressed in parallel with efficacy. This standard is demanding, but it converts a promising mechanism into a credible contribution to food security (Chen *et al.*, 2019; Crossa *et al.*, 2017; Shi *et al.*, 2017).

Responsible innovation and access

Deployment also has a governance dimension. The molecular characterization and transparency of performan-

ce data help breeders, regulators and growers distinguish a precisely characterized regulatory edit from an unverified stress-tolerance claim. Access is as important as molecular novelty: the value of a drought- or heat-resilient event is limited if it is confined to a background, a seed channel or management system that does not reach the exposed production areas. Public germplasm, local testing, seed multiplication and farmer feedback can contribute to equitable and lasting resilience if integrated in partnerships (Chen *et al.*, 2019; Meng *et al.*, 2024).

Conclusion

Drought and heat tolerance in maize emerge from coordinated regulation rather than a single master gene. ABA, calcium, ROS and kinase networks integrate changing environmental information, while DREB/ERF, NAC, NF-Y, MYB, WRKY and HSF modules organize downstream responses. The most useful genetic resources are those with evidence across scales: a defined allele or edit, a mechanistic effect, a measurable physiological response and repeatable yield performance in relevant field environments. ZmVPP1, ZmNAC111, ZmDREB2.7, ARGOS8, DSD1/ZmICEb and emerging HSF-linked modules demonstrate the promise of this approach.

Future progress will depend on coupling causal discovery with stage-specific phenotyping, multi-environment validation and careful control of regulatory dosage. CRISPR-Cas systems, transgenic expression, multi-omics and genomic prediction are complementary tools when used within this evidence framework. Their shared goal should be to deliver maize cultivars that sustain productive physiology and kernel set under realistic drought and heat scenarios while fitting sustainable, locally adaptive production systems.

Acknowledgments

The authors acknowledge their respective institutions supporting the scholarly work that informed this review.

Conflict of Interest

The authors declare no conflict of interest.

References

Araus JL, Kefauver SC, Zaman-Allah M, Olsen MS, Cairns JE, 2018. Translating high-throughput phenotyping into genetic gain. Trends Plant Sci 23:451-466. *https://doi.org/10.1016/j.tplants.2018.02.001*

Aslam MM, Waseem M, Jakada BH, Okal EJ, Lei Z, Saqib HSA, Yuan W, Xu W, Zhang Q, 2022. Mechanisms of abscisic acid-mediated drought stress responses in plants. Int J Mol Sci 23:1084. *https://doi.org/10.3390/ijms23031084*

Cao Y, Zhang C, Chen X, Zeng T, Wang H, Du X, Wu X, Zhu B, Gu L, 2025. ZmHSFA2d positively regulates maize seedling heat and drought tolerance by modulating photosystem protein synthesis. Theor Appl Genet 138:266. *https://doi.org/10.1007/s00122-025-05052-1*

Chen K, Wang Y, Zhang R, Zhang H, Gao C, 2019. CRISPR/Cas genome editing and precision plant breeding in agriculture. Annu Rev Plant Biol 70:667-697. *https://doi.org/10.1146/annurev-arplant-050718-100049*

Crossa J, Pérez-Rodríguez P, Cuevas J, Montesinos-López OA, Jarquín D, de los Campos G, Burgueño J, González-Camacho JM, Pérez-Elizalde S, Beyene Y, Dreisigacker S, Singh R, Zhang X, Gowda M, Roorkiwal M, Rutkoski J, Varshney RK, 2017. Genomic selection in plant breeding: methods, models, and perspectives. Trends Plant Sci 22:961-975. *https://doi.org/10.1016/j.tplants.2017.08.011*

Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR, 2010. Abscisic acid: emergence of a core signaling network. Annu Rev Plant Biol 61:651-679. *https://doi.org/10.1146/annurev-arplant-042809-112122*

Fahad S, Bajwa AA, Nazir U, Anjum SA, Farooq A, Zohaib A, Sadia S, Nasim W, Adkins S, Saud S, Ihsan MZ, Alharby H, Wu C, Wang D, Huang J, 2017. Crop production under drought and heat stress: plant responses and management options. Front Plant Sci 8:1147. *https://doi.org/10.3389/fpls.2017.01147*

Guo M, Liu J, Ma X, Luo DX, Gong ZH, Lu M, 2016. The plant heat stress transcription factors (HSFs): structure, regulation, and function in response to abiotic stresses. Front Plant Sci 7:114. *https://doi.org/10.3389/fpls.2016.00114*

Guo Q, Li X, Li N, Jameson PE, Zhou W, 2021. Transcription-associated metabolomic adjustments in maize occur during combined drought and cold stress. Plant Physiol 186:677-695. *https://doi.org/10.1093/plphys/kiab050*

Jiang L, Hu W, Qian Y, Ren Q, Zhang J, 2021. Genome-wide identification, classification and expression analysis of the Hsf and Hsp70 gene families in maize. Gene 770:145348. *https://doi.org/10.1016/j.gene.2020.145348*

Jiao P, Jiang Z, Miao M, Wei X, Wang C, Liu S, Guan S, Ma Y, 2024. Zmhdz9, an HD-Zip transcription factor, promotes drought stress resistance in maize by modulating ABA and lignin accumulation. Int J Biol Macromol 258:128849. *https://doi.org/10.1016/j.ijbiomac.2023.128849*

Li H, Yang M, Zhao C, Wang Y, Zhang R, 2021. Physiological and proteomic analyses revealed the response mechanisms of two different drought-resistant maize varieties. BMC Plant Biol 21:513. *https://doi.org/10.1186/s12870-021-03295-w*

Liu L, Tang C, Zhang Y, Sha X, Tian S, Luo Z, Wei G, Zhu L, Li Y, Fu J, Luo P, Wang Q, 2025. The SnRK2.2-ZmHsf28-JAZ14/17 module regulates drought tolerance in maize. New Phytol 245:1985-2003. *https://doi.org/10.1111/nph.20355*

Liu S, Wang X, Wang H, Xin H, Yang X, Yan J, Li J, Phan Tran LS, Shinozaki K, Yamaguchi-Shinozaki K, Qin F, 2013. Genome-wide analysis of ZmDREB genes and their association with natural variation in drought tolerance at the seedling stage of Zea mays L. PLoS Genet 9:e1003790. *https://doi.org/10.1371/journal.pgen.1003790*

Lobell DB, Schlenker W, Costa-Roberts J, 2011. Climate trends and global crop production since 1980. Science 333:616-620. *https://doi.org/10.1126/science.1204531*

Mao H, Wang H, Liu S, Li Z, Yang X, Yan J, Li J, Phan Tran LS, Qin F, 2015. A transposable element in a NAC gene is associated with drought tolerance in maize seedlings. Nat Commun 6:8326. *https://doi.org/10.1038/ncomms9326*

Meng L, Zhang J, Clarke N, 2024. A critical review of recent advances in maize stress molecular biology. Int J Mol Sci 25:12383. *https://doi.org/10.3390/ijms252212383*

Nadarajah K, 2020. ROS homeostasis in abiotic stress tolerance in plants. Int J Mol Sci 21:5208. *https://doi.org/10.3390/ijms21155208*

Nelson DE, Repetti PP, Adams TR, Creelman RA, Wu J, Warner DC, Anstrom DC, Bensen RJ, Castiglioni P, Donnarummo MG, Hinchey BS, Kumimoto RW, Maszle DR, Canales RD, Krolikowski KA, Dotson SB, Gutterson N, Ratcliffe OJ, Heard JE, 2007. Plant nuclear factor Y (NF-Y) B subunits confer drought tolerance and lead to improved corn yields on water-limited acres. Proc Natl Acad Sci USA 104:16450-16455. *https://doi.org/10.1073/pnas.0707193104*

Sato H, Mizoi J, Shinozaki K, Yamaguchi-Shinozaki K, 2024. Complex plant responses to drought and heat stress under climate change. Plant J 117:1873-1892. *https://doi.org/10.1111/tpj.16612*

Shi J, Gao H, Wang H, Lafitte HR, Archibald RL, Yang M, Hakimi SM, Mo H, Habben JE, 2017. ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. Plant Biotechnol J 15:207-216. *https://doi.org/10.1111/pbi.12603*

Singh A, Pandey H, Pandey S, Lal D, Chauhan D, Aparna, Antre SH, Santhosh B, Kumar A, 2023. Drought stress in maize: stress perception to molecular response and strategies for its improvement. Funct Integr Genomics 23:296. *https://doi.org/10.1007/s10142-023-01226-6*

Wang CT, Ru JN, Liu YW, Li M, Zhao D, Yang JF, Fu JD, Xu ZS, 2018a. Maize WRKY transcription factor ZmWRKY106 confers drought and heat tolerance in transgenic plants. Int J Mol Sci 19:3046. *https://doi.org/10.3390/ijms19103046*

Wang CT, Ru JN, Liu YW, Yang JF, Li M, Xu ZS, Fu JD, 2018b. The maize WRKY transcription factor ZmWRKY40 confers drought resistance in transgenic Arabidopsis. Int J Mol Sci 19:2580. *https://doi.org/10.3390/ijms19092580*

Wang X, Wang H, Liu S, Ferjani A, Li J, Yan J, Yang X, Qin F, 2016. Genetic variation in ZmVPP1 contributes to drought tolerance in maize seedlings. Nat Genet 48:1233-1241. *https://doi.org/10.1038/ng.3636*

Wang Y, Guo H, Wu X, Wang J, Li H, Zhang R, 2022. Transcriptomic and physiological responses of contrasting maize genotypes to drought stress. Front Plant Sci 13:928897. *https://doi.org/10.3389/fpls.2022.928897*

Yang Y, Li Z, Zhang J, 2024. ZmNF-YA1 contributes to maize thermotolerance by regulating heat shock response. Int J Mol Sci 25:6275. *https://doi.org/10.3390/ijms25116275*

Yang Y, Saand MA, Huang L, Abdelaal WB, Zhang J, Wu Y, Li J, Sirohi MH, Wang F, 2021. Applications of multi-omics technologies for crop improvement. Front Plant Sci 12:563953. *https://doi.org/10.3389/fpls.2021.563953*

Yoon Y, Seo DH, Shin H, Kim HJ, Kim CM, Jang G, 2020. The role of stress-responsive transcription factors in modulating abiotic stress tolerance in plants. Agronomy 10:788. *https://doi.org/10.3390/agronomy10060788*

Zenda T, Wang N, Dong A, Zhou Y, Duan H, 2022. Reproductive-stage heat stress in cereals: impact, plant responses and strategies for tolerance improvement. Int J Mol Sci 23:6929. *https://doi.org/10.3390/ijms23136929*

Zhang Y, Massel K, Godwin ID, Gao C, 2018. Applications and potential of genome editing in crop improvement. Genome Biol 19:210. *https://doi.org/10.1186/s13059-018-1586-y*

Zhou W, Yin J, Zhou Y, Li Y, He H, Yang Y, Wang X, Lian X, Dong X, Ma Z, Chen L, Hou S, 2025. DSD1/ZmICEb regulates stomatal development and drought tolerance in maize. J Integr Plant Biol 67:1487-1500. *https://doi.org/10.1111/jipb.13890*



Maydica's Editor
Dr.ssa Carlotta BALCONI
CREA - Research Centre for Cereal and Industrial Crops
Via Stezzano, 24 24126 Bergamo - ITALY
carlotta.balconi@crea.gov.it



Maydica's Technical Editor
Jacopo Barone
j.barone@masaf.gov.it

crea
Consiglio per la ricerca in agricoltura
e l'analisi dell'economia agraria