Molecular Basis of Drought-Induced Stomatal Regulation in Maize

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ABSTRACT

Background: Drought is one of the strongest abiotic stresses that affect global agriculture, and maize (Zea mays L.) is highly sensitive during important growth periods. As the main food crop for over 800 million people in the world, it is crucial to understand how stomatal function is regulated at the molecular level under water deficit conditions in order to ensure food security and sustainable development of crops. Stomata, the small openings in the epithelium of plants that affect exchange of gases and the process of transpiration, serve as the main link between the amount of water in the plant and need for water in the air. Their coordinated regulation in drought conditions involves a complex chain of signaling processes associated with hormones, ions and transcriptional processes.

Objective: The purpose of the study is to outline essential achievements in the development of understanding of molecular stomatal regulation of maize in drought conditions, emphasizing the role of biosynthesis of abscisic acid (ABA), its perception, and downstream signaling via the core modules PYR/PYL–PP2C–SnRK2, functioning of ion channels in guard cells, role of ROS and Ca^{2+} as secondary messengers of signaling processes.

Literature Sources: A thorough search of PubMed, Scopus, Web of Science, Google Scholar, and NCBI database was made for studies between 2015 and 2025 along with some referenced landmark studies.

Major Results: ABA is the main drought signal that is responsible for closing of the stomata whereby the receptor, the protein phosphatase, type 2C (PP2C), along with sucrose non-fermenting-1-related protein kinases (SnRK2) form the core signaling apparatus. Furthermore, the slow anion channel, ZmSLAC1, which is regulated by ZmOST1 and ZmCPKs through phosphorylation, carries out the cell depolarization to make the K+ leave and the stomata close. There are also maize transcription factors such as ZmNAC20, ZmNAC49, ZmWRKY79, ZmMYB31, and ZmPIF1 that modify ABA sensitivity and stomata density. The closure signal is also boosted by the calcium oscillations and ROS generated from NADPH oxidase.

Gaps in Research: Insufficient knowledge exists about guard cell signaling in maize, the relationship between epigenetics and stomatal regulation, and the practical conversion of signal target genes into a drought-tolerant maize cultivar.

Conclusion: Studies of the molecular mechanisms of stomatal control in maize allow research into more efficient use of water in crop production. Further research mixing molecular biology with crop physiology will produce maize bred for climate impact.

Keywords: Drought stress, Stomatal closure, Abscisic acid (ABA), Guard cells, SnRK2 kinase, ZmSLAC1, Maize (Zea mays), Water-use efficiency, Transcription factors

1. Introduction

1.1 Background

Maize (*Zea mays* L.) is considered to be the most cultivated cereal in the world with annual global production of maize being over 1.2 billion metric tons cultivated over approximately 200 million hectares being cultivated globally in more than 170 countries [1]. It is also an essential building block of the world food, feed, and bioenergy systems and maize provides nutrition security for many people around the globe, and especially in Sub Saharan Africa, Latin America, and South Asia. However, the production of maize is severely threatened by the increasing frequency of occurrence and severity of drought caused by human-induced climate change. The findings of the Intergovernmental Panel on Climate Change (IPCC AR6, 2023) predict that global warming will lead to an increase in the severity, duration, and spatial scope of agricultural drought, which may lead to around 24 % of maize yield loss in 21st century without taking the appropriate actions [2, 3].

Drought has an impact on maize at different levels: the physiological level is harmed with impairment of photosynthesis, nutrient uptake, and reproduction; the cellular level is affected in terms of osmotic adjustment, membrane integrity, and enzymes; the molecular level experiences large-scale rearrangement of gene expression, hormonal signals, and phosphorylation processes. One of the first reactions of plants to the deficit of water is regulation of stomata—the small holes on the leaf surface that help tointake CO_2 for photosynthesis and give off water vapor.

1.2 Stomata as Critical Nodes of Drought Adaptation

Stomata are specialized structures formed by guard cells that create the opening called the stomata. Movement of the guard cells relies on an intricate molecular machinery that is sensitive to several factors which includes water potential, hormones, light, carbon dioxide, humidity, and biotic signals. The process of closing the stomata is the most essential strategy for survival in the context of drought conditions in most plants and maize.

1.3 The Case for Maize-Focused Molecular Research

Although basic stomatal biology has been thoroughly researched using the model plant Arabidopsis thaliana, it is not easy to transfer findings to maize. Maize is a C_4 grass with a complicated allotetraploid genome of about 2.3 Gb. This plant has a unique stomatal anatomy involving pavement cells and neighbouring cells next to guard cells [6]. In addition, the maize guard cell signalling pattern differs from that of Arabidopsis with respect to the composition, level of expression and interplay among various genes, calling for separate studies in maize [2,7].

1.4 Scope and Objectives of This Review

This review intends to propose a concise and critical overview of the molecular basis of how maize regulates its stomata due to water scarcity. The work specifically (i) clarifies how abscisic acid is produced and transported and how it is perceived as the primary drought signal; (ii) describes the main signaling unit PYR/PYL–PP2C–SnRK2 and its targets; (iii) discusses guard cell networks involving ion channels with respect to ZmSLAC1 and other protein kinases; (iv) analyzes the importance of ROS and Ca^{2+} as second messengers in stomatal closing signaling; (v) describes transcription factors pathways that control guard cell-specific gene expression; and (vi) defines the main topics for the future research in this area of study.

2. Methodology of Literature Review

2.1 Literature Search Strategy

A systematic literature review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines. Electronic databases searched included PubMed/MEDLINE, Scopus, Web of Science (Core Collection), Google Scholar, NCBI Gene, FAO institutional repositories, and Frontiers in Plant Science Open Access collections. The search was restricted to publications between January 2015 and June 2025, with exceptions made for landmark foundational studies predating this period.

Table 1: Literature Search Strategy

Parameter	Details
Databases Searched	PubMed/MEDLINE, Scopus, Web of Science, Google Scholar, NCBI Gene, FAO repository, Frontiers Open Access
Primary Search Terms	"Maize stomatal regulation", "Zea mays drought ABA", "guard cell signaling maize", "ZmSLAC1", "ZmOST1", "maize water-use efficiency"
Secondary Keywords	"PYR/PYL receptor maize", "SnRK2 guard cell", "ROS stomatal closure", "calcium signaling maize", "NAC transcription factor drought", "WRKY maize drought"
Search Period	January 2015 – June 2025 (landmark studies from 2009–2014 included selectively)
Language	English only
Document Types	Peer-reviewed original research articles, systematic reviews, meta-analyses, book chapters, government/FAO reports
Initial Records Retrieved	Approximately 1,840 records across all databases
Records After De-duplication	612 unique records
Records After Title/Abstract Screening	187 records
Full-Text Assessed for Eligibility	68 records
Final Studies Included in Review	38 studies + 2 foundational references

Table 2: Inclusion and Exclusion Criteria

Criterion Type	Category	Details
Inclusion	Publication Type	Original peer-reviewed research articles, systematic reviews, meta-analyses
Inclusion	Language	English-language publications only
Inclusion	Time Period	Publications from 2015–2025; foundational studies from 2009–2014 permitted
Inclusion	Relevance	Studies directly addressing stomatal regulation, ABA signaling, guard cell ion channels, ROS/Ca^{2+} signaling, or transcription factors in maize or closely related cereals
Inclusion	Indexing	Scopus-indexed, Web of Science-indexed, or PubMed-indexed publications preferred
Exclusion	Duplicates	Duplicate records identified across databases were removed
Exclusion	Non-academic Sources	Conference abstracts (without full paper), blogs, non-peer-reviewed reports, grey literature (unless FAO/IPCC)
Exclusion	Irrelevant Studies	Studies restricted to non-plant organisms, or addressing stomatal regulation under biotic stress only, without drought component
Exclusion	Low Quality	Studies without clear methodology, or from predatory journals not indexed in major databases

3. Literature Review and Thematic Analysis

3.1 Theme I: Abscisic Acid Biosynthesis and Transport Under Drought Stress

3.1.1 ABA as the Master Drought Signal

Abscisic acid (ABA) is universally recognized as the primary phytohormone orchestrating plant responses to osmotic stress and drought. Under water-deficit conditions, leaf water potential declines, triggering a rapid and dramatic increase in ABA concentration in guard cells and surrounding apoplastic fluid [5, 8]. The biosynthesis of ABA occurs primarily in leaf mesophyll and vascular tissues, with the carotenoid cleavage pathway serving as the principal biosynthetic route. Key enzymatic steps include: (i) the epoxidation of zeaxanthin and antheraxanthin to violaxanthin by zeaxanthin epoxidase (ZEP/ABA1); (ii) the oxidative cleavage of 9-cis-epoxycarotenoids by 9-cis-epoxycarotenoid dioxygenases (NCEDs)—the rate-limiting step; and (iii) the conversion of xanthoxin to ABA-aldehyde by short-chain alcohol dehydrogenase/reductase (SDR/ABA2), followed by oxidation to ABA by aldehyde oxidase (AAO/ABA3) [5, 8].

In maize, several NCED homologues (ZmNCED1, ZmNCED3, ZmNCED6, ZmNCED9) are drought-inducible, with ZmNCED3 exhibiting the strongest upregulation under moderate water deficit [9]. A remarkable recent discovery involves the peptide hormone CLE25, produced in roots under water deficit, which is transported via the phloem to leaves where it promotes ABA biosynthesis in vasculature and guard cells, thus enabling long-distance hydraulic-to-hormonal signal transduction [18]. In maize, ZmDnaJ was found to interact with the ABA synthesis-related protein ZmNCED6, increasing ABA content and thereby regulating stomatal closure under drought conditions, establishing a novel heat-shock-protein-mediated link to ABA biosynthesis [2].

3.1.2 ABA Transport and Apoplastic Accumulation

Following biosynthesis, ABA must be transported to guard cells to exert its stomatal effects. This requires both symplastic and apoplastic pathways, mediated by specific ABA transporters. The ATP-binding cassette (ABC) transporters, particularly the ABCG subfamily members (AtABCG25, AtABCG40 in Arabidopsis), serve as ABA exporters and importers respectively at the plasma membrane. The nitrate transporter/peptide transporter family (NPF4.6/NRT1.2) also facilitates ABA uptake into guard cells [18]. In maize, the molecular identity of the primary ABA transporters in guard cells remains incompletely characterized, representing an important research gap. Studies using maize-Arabidopsis comparative genomics have identified several candidate ZmABCG transporters that may fulfill analogous roles [7].

3.2 Theme II: ABA Perception and Core Signaling Module (PYR/PYL–PP2C–SnRK2)

3.2.1 The PYR/PYL ABA Receptor System

The discovery of the PYR/PYL/RCAR (pyrabactin resistance/PYR1-like/regulatory component of ABA receptor) family as soluble intracellular ABA receptors represented a paradigmatic advance in understanding drought signaling [10]. These START-domain proteins bind ABA with high affinity, inducing conformational changes that enable their interaction with and inhibition of clade A type 2C protein phosphatases (PP2Cs) [10]. The PYR/PYL family is encoded by 14 members in Arabidopsis and an expanded family in maize, with several ZmPYLs (ZmPYL1–ZmPYL13) characterized [2]. Under well-watered conditions, PP2Cs maintain the pathway in an OFF state by dephosphorylating and inactivating SnRK2 kinases. Upon ABA binding to PYR/PYL receptors, the PP2C catalytic site is occluded, relieving SnRK2 inhibition and initiating the downstream signaling cascade [10, 18].

3.2.2 PP2C Phosphatases as Negative Regulators

Clade A PP2Cs (including ABI1, ABI2, HAB1, HAB2, PP2CA/AHG3 in Arabidopsis) function as master negative regulators of ABA signaling. In maize, ZmPP2C-A10 was characterized as a negative regulator of ABA signaling and drought stress response, interacting directly with ZmPYLs or ZmSnRK2s to modulate signal intensity [2]. A second maize PP2C, ZmPP84, was found to repress stomatal closure by dephosphorylating a MAP kinase kinase (ZmMEK1), thereby inhibiting the phosphorylation of ZmSLAC1—revealing an unexpected convergence between MAP kinase and ABA signaling in guard cells [2]. These findings highlight the complexity of negative regulation in maize and suggest that PP2C inhibition may offer a viable target for improving drought responsiveness.

3.2.3 SnRK2 Kinases as Central Activators

Sucrose non-fermenting-1-related protein kinases 2 (SnRK2s) are serine/threonine kinases that function as the primary positive effectors of ABA signaling. Upon PP2C inactivation, SnRK2s undergo autophosphorylation and activate by phosphorylating a cascade of downstream substrates including ion channels, NADPH oxidases, and transcription factors [18]. In Arabidopsis, OST1 (Open Stomata 1, also SnRK2.6) is the principal SnRK2 in guard cells. The maize orthologue ZmOST1 has been functionally characterized: loss of ZmOST1 impairs ABA-induced stomatal closure, confirming its conserved role as the central guard cell kinase [2]. ZmOST1 phosphorylates ZmSLAC1 at multiple N-terminal serine residues, directly activating the anion efflux required for membrane depolarization and stomatal closure [2, 11].

3.3 Theme III: Guard Cell Ion Channel Networks and Membrane Biophysics

3.3.1 SLAC1 – The Master Anion Channel for Stomatal Closure

The slow anion channel SLAC1 (Slow Anion Channel Associated 1) is the best-characterized ion channel mediating stomatal closure in guard cells [11]. SLAC1 belongs to the SLAC/SLAH (SLAC1 homologue) family of anion channels and exhibits characteristic slow-deactivating, weakly voltage-dependent anion currents that facilitate chloride and nitrate efflux from guard cells [12]. In maize, ZmSLAC1 was found to mediate stomatal closure primarily by facilitating nitrate efflux rather than chloride, a potentially species-specific functional divergence from Arabidopsis AtSLAC1 [12]. Phosphorylation of ZmSLAC1 at its cytosolic N-terminus by ZmOST1 is essential for channel activation; structural studies using cryo-EM and electrophysiological analyses in Xenopus oocytes have elucidated the phosphoactivation mechanism, revealing that kinase-mediated phosphorylation relieves steric inhibition of the channel pore [17].

A landmark 2025 study published in PNAS identified ZmGCT1 and ZmGCT2 as novel negative regulators of ZmSLAC1 in maize guard cells [10]. Under well-watered conditions, ZmGCT1 phosphorylates ZmSLAC1, suppressing its activity and maintaining stomatal opening. Drought reduces ZmGCT1 plasma membrane localization, thereby relieving ZmSLAC1 inhibition and promoting stomatal closure. The double loss-of-function mutant zmgct1 zmgct2 exhibits near-complete constitutive stomatal closure,

demonstrating the critical role of this phosphorylation-based switch in regulating guard cell turgor [10].

3.3.2 ALMT and R-type Anion Channels

In addition to SLAC1-mediated S-type (slowly activating) anion currents, guard cells also exhibit R-type (rapidly activating) anion currents mediated by Aluminum-Activated Malate Transporter (ALMT) family members. In Arabidopsis, AtALMT12 (also called QUAC1) is the major R-type channel in guard cells and is activated by OST1 phosphorylation under drought/ABA conditions [15, 19]. AtALMT4 similarly mediates ABA-triggered stomatal closure in a phosphorylation-dependent manner [15]. These rapid anion channels accelerate the initial phases of guard cell membrane depolarization, complementing the sustained anion efflux mediated by SLAC1. The maize genome encodes multiple ALMT members, though their specific roles in guard cell physiology remain to be fully characterized.

3.3.3 Potassium Channel Dynamics

Stomatal closure requires not only anion efflux but also potassium efflux from guard cells to reduce turgor pressure. Outward-rectifying K^+ channels (GORK in Arabidopsis) facilitate this efflux, and their activation is promoted by membrane depolarization driven by anion channel activity [15]. Conversely, stomatal opening requires K^+ uptake via inward-rectifying channels (KAT1, KAT2 in Arabidopsis). ABA inhibits K^+ influx by suppressing KAT1 activity, thereby ensuring net K^+ efflux during drought-induced closure. Calcium-dependent protein kinases (CPKs) phosphorylate and regulate both types of K^+ channels in guard cells. In maize, ZmCPK2 and ZmCPK17 were found to have opposing effects on stomatal closure, with Ca^{2+}-independent ZmCPK2 being inhibited by Ca^{2+}-dependent ZmCPK17 during drought response, illustrating a Ca^{2+}-dependent switching mechanism in K^+ channel regulation [10].

3.4 Theme IV: Reactive Oxygen Species (ROS) Signaling in Guard Cells

Reactive oxygen species, particularly hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-), function as critical second messengers amplifying ABA-induced stomatal closure [5, 7]. In guard cells, NADPH oxidases (RBOH; respiratory burst oxidase homologues) located at the plasma membrane catalyze the production of superoxide, which is rapidly dismutated to H_2O_2 by superoxide dismutases [5]. ABA-activated SnRK2s phosphorylate RBOH enzymes, promoting their activity and apoplastic ROS production. The generated H_2O_2 is transported into guard cells through aquaporins and activates plasma membrane calcium channels, triggering cytosolic Ca^{2+} elevation that further propagates the stomatal closure signal [5, 18].

In maize, ZmNAC20 was shown to bind and upregulate the ROS-producing enzyme ZmRBOH8 under drought conditions, providing a transcriptional mechanism for amplifying NADPH oxidase-derived ROS in guard cells [8]. The receptor-like kinase HSL3 (HAESA-LIKE 3) was identified as a negative regulator of stomatal closure by modulating H_2O_2 homeostasis in guard cells—HSL3 loss-of-function mutants exhibit ABA hypersensitivity and enhanced stomatal closure—suggesting that precise titration of apoplastic H_2O_2 levels is essential for appropriate stomatal responses [7]. MAPK-like protein ZmMAPKL1 was found to negatively regulate stomatal closure by repressing ABA biosynthesis, linking MAP kinase signaling to ABA accumulation in maize [2]. The ZmASR3 (ABA-, stress- and ripening-induced) protein enhances ABA signaling by promoting NADPH oxidase-mediated H_2O_2 production in guard cells, thereby reinforcing stomatal closure under drought [6].

3.5 Theme V: Calcium as a Second Messenger in Guard Cell Signaling

Cytosolic free calcium ($[Ca^{2+}]$cyt) oscillations in guard cells represent a central signaling node integrating multiple upstream inputs—ABA, ROS, CO_2, humidity—into stomatal closure responses [13]. ABA induces biphasic $[Ca^{2+}]$cyt elevations in guard cells: an initial rapid spike driven by release from intracellular stores (vacuole, ER) and a sustained plateau maintained by influx through plasma membrane channels [13]. H_2O_2-activated hyperpolarization-activated calcium channels and mechanosensitive channels contribute to Ca^{2+} influx, while IP_3 (inositol trisphosphate) and cADPR (cyclic ADP-ribose) mediate Ca^{2+} release from the vacuole [5]. Calcium-dependent protein kinases (CPKs/CDPKs) serve as Ca^{2+} decoders that translate $[Ca^{2+}]$cyt elevations into phosphorylation-based signaling outputs. In Arabidopsis, CPK4, CPK5, CPK6, and CPK11 phosphorylate SLAC1 and NADPH oxidases, creating a positive feedback loop that sustains ROS production and anion channel activation during stomatal closure [13]. OST1 and Ca^{2+} signals operate in an interconnected manner: OST1 promotes the initial $[Ca^{2+}]$cyt spike, which then recruits CPKs to amplify SLAC1 activation and SLAH3 channel opening [13]. In maize, the interplay between ZmOST1 and calcium-dependent protein kinases has been partially characterized, with studies identifying ZmCPK2 and ZmCPK17 as critical regulators in this pathway [10].

3.6 Theme VI: Transcription Factor Networks Regulating Stomatal Gene Expression

Beyond rapid post-translational signal transduction, drought-induced stomatal regulation also involves transcriptional reprogramming that modifies guard cell gene expression profiles, stomatal density, and long-term sensitivity to closure signals [20, 21, 24].

3.6.1 NAC Transcription Factors

The NAC (NAM/ATAF/CUC) family constitutes one of the largest plant-specific transcription factor families, with numerous members implicated in drought responses. In maize, ZmNAC20 was identified as rapidly induced by both drought and ABA, and its overexpression significantly improved drought resistance by promoting stomatal closure and activating stress-responsive gene expression via RNA-seq-validated target networks [24]. Mechanistically, ZmNAC20 directly binds and upregulates ZmRBOH8 (amplifying ROS), ZmBURP4, ZmGA2OX6 (reducing bioactive gibberellins), and ZmHB56, coordinating multiple convergent drought-adaptive pathways [8]. ZmNAC49 enhances drought tolerance primarily by reducing stomatal density: it binds the promoter of ZmMUTE (a bHLH transcription factor essential for guard cell fate) and represses its transcription, thereby limiting the number of stomata formed on the leaf surface and reducing transpirational capacity under drought [8].

3.6.2 WRKY Transcription Factors

The WRKY family represents another major class of plant stress-responsive transcription factors. ZmWRKY79 was characterized as a positive regulator of drought tolerance in maize by elevating ABA biosynthesis through transcriptional activation of NCED genes [23]. Upon binding W-box elements (TTGAC) in NCED promoters, ZmWRKY79 amplifies the ABA signal and consequently promotes stomatal closure. WRKY factors also modulate downstream stress-responsive gene expression through interactions with hormone-responsive cis-elements, and several maize WRKY members have been demonstrated to regulate stomatal aperture in response to combined biotic-abiotic stress conditions [20, 23].

3.6.3 MYB Transcription Factors

R2R3-MYB transcription factors play important roles in regulating stomatal movement and density. AtMYB61 in Arabidopsis promotes stomatal closure while directing xylem vessel development to optimize water transport [21]. In maize, ZmMYB31 and other R2R3-MYBs are drought-inducible and modulate stress-responsive gene expression. BES1/BZR1-type transcription factors, which mediate brassinosteroid (BR) signaling, were found to negatively regulate drought tolerance in maize: overexpression of ZmBES1/BZR1-1, -3, and -9 reduces drought resistance, partly by modulating ABA sensitivity and stomatal aperture [20, 22].

3.6.4 Phytochrome-Interacting Factors and Light Signaling Integration

Phytochrome-interacting factors (PIFs) integrate light signals with drought responses. The maize ZmPIF1 transcription factor was found to be induced by drought and ABA treatment, and its transgenic overexpression in rice conferred enhanced drought resistance associated with reduced stomatal aperture and transpiration rate [9]. ZmPIF1 appears to function as a positive regulator of ABA signaling downstream of phytochrome perception, connecting light-mediated stomatal control with drought-responsive pathways [9].

3.7 Theme VII: Hormonal Crosstalk and Systems-Level Integration

While ABA is the principal drought signal, stomatal regulation involves extensive crosstalk with other phytohormone pathways. Jasmonic acid (JA) and methyl jasmonates synergize with ABA to promote stomatal closure, with JAZ-MYC signaling modules interacting with ABA-activated SnRK2s in guard cells [1]. Salicylic acid (SA) also modulates stomatal closure, particularly under combined biotic-abiotic stress, partly through effects on guard cell redox homeostasis [1]. Ethylene has complex effects: it can both promote stomatal opening (via H_2O_2 production) and closure (through crosstalk with ABA), depending on concentration and developmental context [1]. Cytokinins generally promote stomatal opening by antagonizing ABA signaling at multiple levels, including PP2C activation.

Brassinosteroids exert negative effects on drought tolerance in maize (as demonstrated by ZmBES1/BZR1 studies [22]) by modulating BES1-mediated repression of ABA signaling genes. Strigolactones represent an emerging class of drought-related hormones in maize; a recent study demonstrated that strigolactones mitigate drought stress in maize by improving chloroplast protection, stomatal function, and antioxidant defense, suggesting novel hormonal entry points for improving drought tolerance [1]. This hormonal complexity is depicted in Figure 3 (Conceptual Framework).

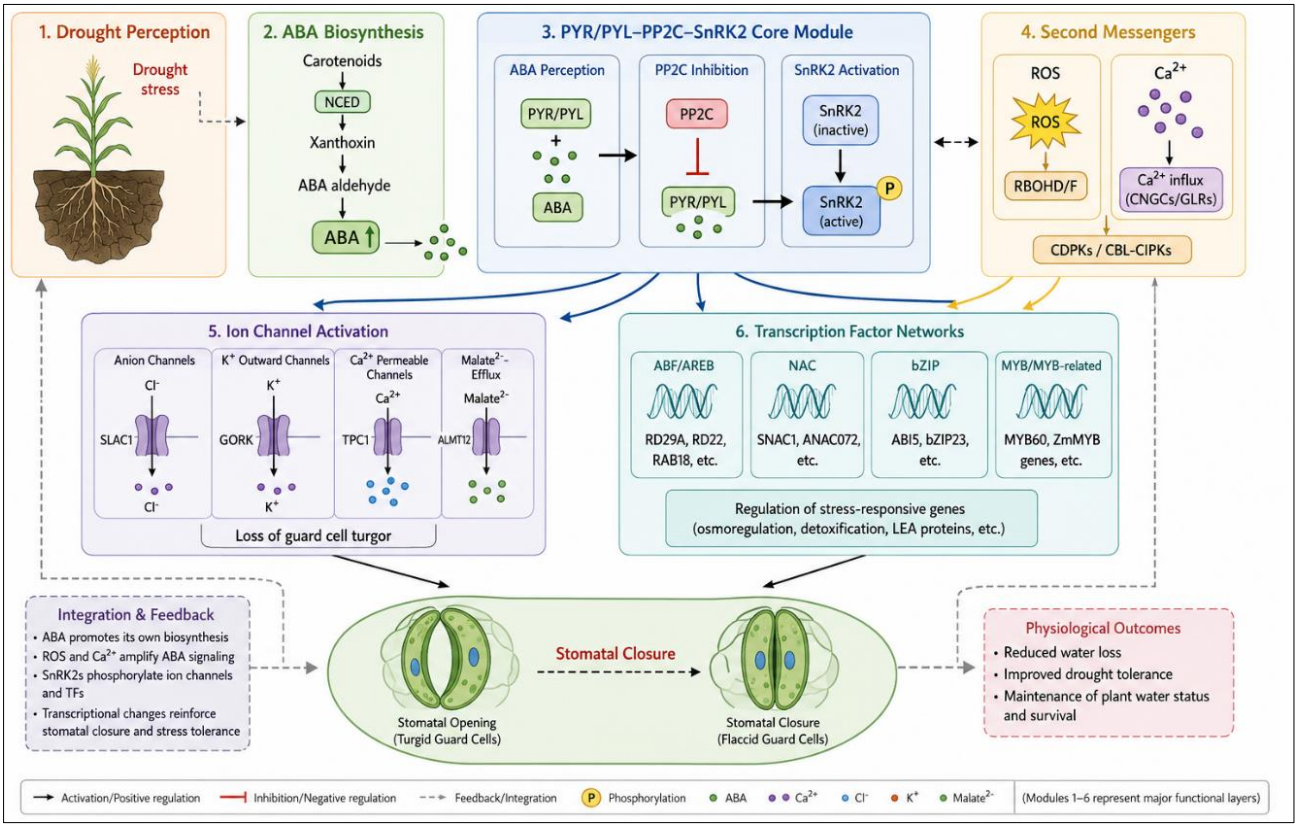

Fig 1: Conceptual Framework illustrating the molecular signaling network governing drought-induced stomatal closure in maize, including ABA biosynthesis, the PYR/PYL–PP2C–SnRK2 core module, ion channel activation, ROS/Ca^{2+} second messengers, and transcription factor networks.

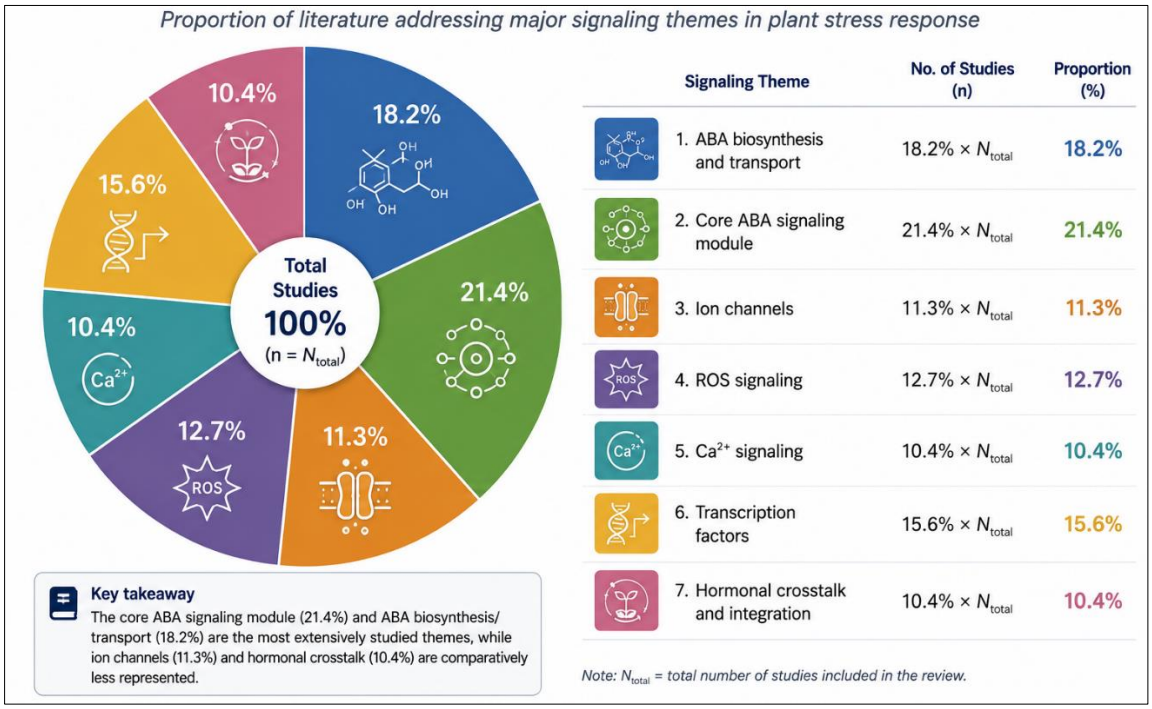

Fig 2: Thematic classification of studies reviewed, showing the proportion of literature addressing each major signaling theme (ABA biosynthesis/transport, core signaling module, ion channels, ROS signaling, Ca^{2+} signaling, transcription factors, hormonal crosstalk).

4. Comparative Analysis of Previous Studies

Table 3 summarizes the key studies reviewed, organized by molecular theme, study system, and major findings. Table 4 provides a comparative analysis highlighting methodological approaches, study design, and conclusions across different research groups.

Table 3: Summary of Selected Studies on Molecular Stomatal Regulation in Maize and Related Systems

Author (s) & Year	Species/System	Methodology	Key Component	Major Finding	Limitation
Wu *et al*., 2019 [2]	Zea mays	Genetic/CRISPR, electrophysiology	ZmOST1	Loss of ZmOST1 impairs ABA-induced stomatal closure	Field validation limited
Qin *et al*., 2024 [17]	Arabidopsis (structural)	Cryo-EM, electrophysiology	SLAC1	Mechanism of phosphoactivation via CRD dissociation	Maize ortholog not directly studied
Chen *et al*., 2022 [5]	Multiple species (review)	Literature synthesis	ABA/ROS/Ca^{2+}	Integrated signaling model for stomatal closure	Limited maize-specific data
Liu *et al*., 2023 [24]	Zea mays	Overexpression, RNA-Seq	ZmNAC20	ZmNAC20 promotes stomatal closure and stress gene expression	Mechanism upstream of ZmNAC20 unclear
Guo *et al*., 2023 [2]	Zea mays	Genetics, in vitro phosphorylation	ZmPP84/ZmMEK1/ZmSLAC1	ZmPP84 represses closure via ZmMEK1 dephosphorylation	Pathway not fully resolved in planta
Li *et al*., 2025 [10]	Zea mays	Genetics, proteomics, PNAS	ZmGCT1/2, ZmSLAC1	ZmGCT1/2 negatively regulate ZmSLAC1 to maintain guard cell turgor	Upstream activation of ZmGCT1/2 unknown
Zhu *et al*., 2020 [2]	Zea mays	Genetic loss-of-function	ZmMAPKL1	Negatively regulates stomatal closure by repressing ABA biosynthesis	Specific substrate unidentified
Feng *et al*., 2022 [22]	Zea mays/Arabidopsis	Transgenic overexpression	ZmBES1/BZR1-3, -9	Negative regulation of drought tolerance in maize	Mechanism not fully resolved
Dong *et al*., 2025 [2]	Zea mays	Protein interaction, genetics	ZmDnaJ/ZmNCED6	ZmDnaJ increases ABA via ZmNCED6, regulates stomatal closure	HSP-ABA crosstalk unexplored
Ahmad *et al*., 2025 [1]	Zea mays	Physiological/biochemical	Strigolactones	Strigolactones improve chloroplast protection and stomatal function	Molecular mechanism lacking
Wang *et al*., 2021 [23]	Zea mays	Transcriptomics, ChIP	ZmWRKY79	Elevates ABA biosynthesis by activating NCED genes	Guard cell specificity unclear
Zhou *et al*., 2022 [2]	Zea mays	GWAS, genome-wide	Multiple genes	Genome-wide identification of stomatal closure genes in two inbreds	Functional characterization of most genes pending

Table 4: Comparative Analysis of Methodological Approaches and Major Outcomes

Methodology	Studies Using Approach	Key Strengths	Major Limitations	Representative Findings
Genetic loss-of-function (mutant analysis)	Wu *et al*. 2019; Zhu *et al*. 2020; Li *et al*. 2025	Direct evidence for gene function in planta; physiologically relevant	Off-target effects; functional redundancy may mask phenotypes	ZmOST1, ZmMAPKL1, ZmGCT1/2 roles established
Transgenic overexpression	Liu *et al*. 2023; Feng *et al*. 2022; Ahmad *et al*. 2019 [9]	Clear gain-of-function phenotypes; amenable to yield testing	Overexpression artifacts; pleiotropic effects on growth	ZmNAC20, ZmPIF1 improve drought resistance
Electrophysiology (patch clamp)	Qin *et al*. 2024; Hedrich 2017 [14]	Direct measurement of channel activity; high resolution	Technically challenging; limited throughput; ex vivo	SLAC1 phosphoactivation mechanism resolved
Transcriptomics (RNA-Seq)	Liu *et al*. 2023; Frontiers 2025 [3]	Genome-wide expression profiling; discovery of novel targets	Does not establish causality; requires validation	Hundreds of drought-responsive genes identified in guard cells
Structural biology (Cryo-EM)	Qin *et al*. 2024; Liang *et al*. 2021 [17]	Atomic-resolution mechanistic insight; drug/engineering targets	Rarely performed for maize proteins directly; costly	Structural basis of SLAC1 activation elucidated
GWAS/Genomics	Zhou *et al*. 2022 [2]	Identifies natural variation; population-level insights	Functional validation often missing; association not causation	1,439 ZmNAC20 target genes identified by DAP-seq [8]
Protein interaction (Y2H, Co-IP)	Guo *et al*. 2023; Dong *et al*. 2025 [2]	Maps molecular interaction networks	In vitro interactions may not occur in vivo	ZmDnaJ–ZmNCED6, ZmPP84–ZmMEK1 interactions validated

5. Research Gaps and Emerging Trends

Despite significant advances, substantial knowledge gaps persist in understanding the molecular basis of drought-induced stomatal regulation in maize. These gaps span from basic molecular mechanisms to translational crop improvement.

Table 5: Identified Research Gaps and Their Scientific Significance

Research Gap	Current State	Scientific Significance	Suggested Approach
Maize-specific ABA transporters in guard cells	Molecular identity of Zm ABCG transporters largely uncharacterized	Critical for understanding ABA delivery kinetics to guard cells	Candidate gene functional characterization; cell-type-specific expression profiling
Structural biology of ZmSLAC1 and ZmOST1 complex	Cryo-EM structures available only for Arabidopsis homologs	Would enable structure-based engineering of drought-responsive channels	Cryo-EM/X-ray crystallography of maize guard cell proteins
Epigenetic regulation of stomatal responses	DNA methylation and histone modifications at stomatal gene loci poorly characterized in maize	Epigenetic memory of drought may affect recurrent stress responses	Bisulfite sequencing and ChIP-seq in guard cell-enriched fractions
Guard cell-specific transcriptome of maize	Most transcriptomic data from whole-leaf tissue; guard cell-resolved data very limited	Guard cells may have unique regulatory networks not captured in bulk tissue data	Single-cell RNA-seq and ATAC-seq of maize guard cells
Integration of circadian clock and stomatal regulation	Clock-stomata link established in Arabidopsis but unexplored in maize	Circadian-gated ABA sensitivity may optimize water use diurnally	Temporal (time-course) guard cell transcriptomics combined with circadian reporter lines
Field validation of molecular targets	Most gene characterization done under controlled greenhouse conditions	Field environments involve multiple simultaneous stresses; controlled-environment phenotypes may not translate	Multi-environment trials of transgenic maize lines in drought-prone regions
Long-distance drought signalling peptides in maize	CLE25 characterized in Arabidopsis; maize orthologs unexplored	Root-to-shoot signaling may precede leaf ABA accumulation	ZmCLE peptide identification and functional characterization under field drought
Hormonal crosstalk mechanisms at molecular level	Strigolactone, ethylene, and BR effects on stomata partially described	Multi-hormonal integration needed for robust drought tolerance	Systems biology approaches; hormone interaction network modeling

Table 6: Emerging Trends and Technologies in Stomatal Regulation Research

Emerging Trend	Current Status	Potential Impact	Challenges
Single-cell genomics in guard cells	scRNA-seq protocols established for leaf epidermis; first maize datasets emerging	Resolution of guard cell-specific regulatory networks at single-cell resolution	Low cell numbers; protoplasting artifacts
CRISPR-based stomatal engineering	Knockout/base editing of ZmOST1, ZmSLAC1 demonstrated; multiplexed editing feasible	Precise modulation of stomatal density and aperture for WUE improvement	Off-target effects; regulatory approval for genome-edited crops
AI/machine learning for stomatal phenotyping	Deep learning models for automated stomatal measurement from microscopy images	High-throughput phenotyping for breeding programs; GWAS enhancement	Training data requirements; generalizability across environments
Optogenetics for real-time stomatal control	Light-gated ion channels demonstrated in plant guard cells in proof-of-concept studies	Experimental dissection of signaling kinetics; future precision agriculture	Light delivery to field crops impractical; developmental integration
Nanobody-based ABA biosensors	FRET-based ABA reporters (ABAleon, ABACUS) enable real-time ABA imaging in guard cells	Precise temporal and spatial ABA dynamics mapping under field conditions	Transformation and imaging logistics in maize
Synthetic biology approaches (ABA receptor engineering)	Engineered ABA receptors with enhanced sensitivity reported in Arabidopsis	Could confer drought responsiveness at lower ABA thresholds	Specificity and off-target effects in maize; regulatory issues
Pangenome approaches to stomatal variation	Maize pangenome (~26 high-quality assemblies) enables population-level analysis of stomatal gene diversity	Identifies natural alleles for improving drought adaptation in diverse germplasm	Phenotypic integration challenging at scale

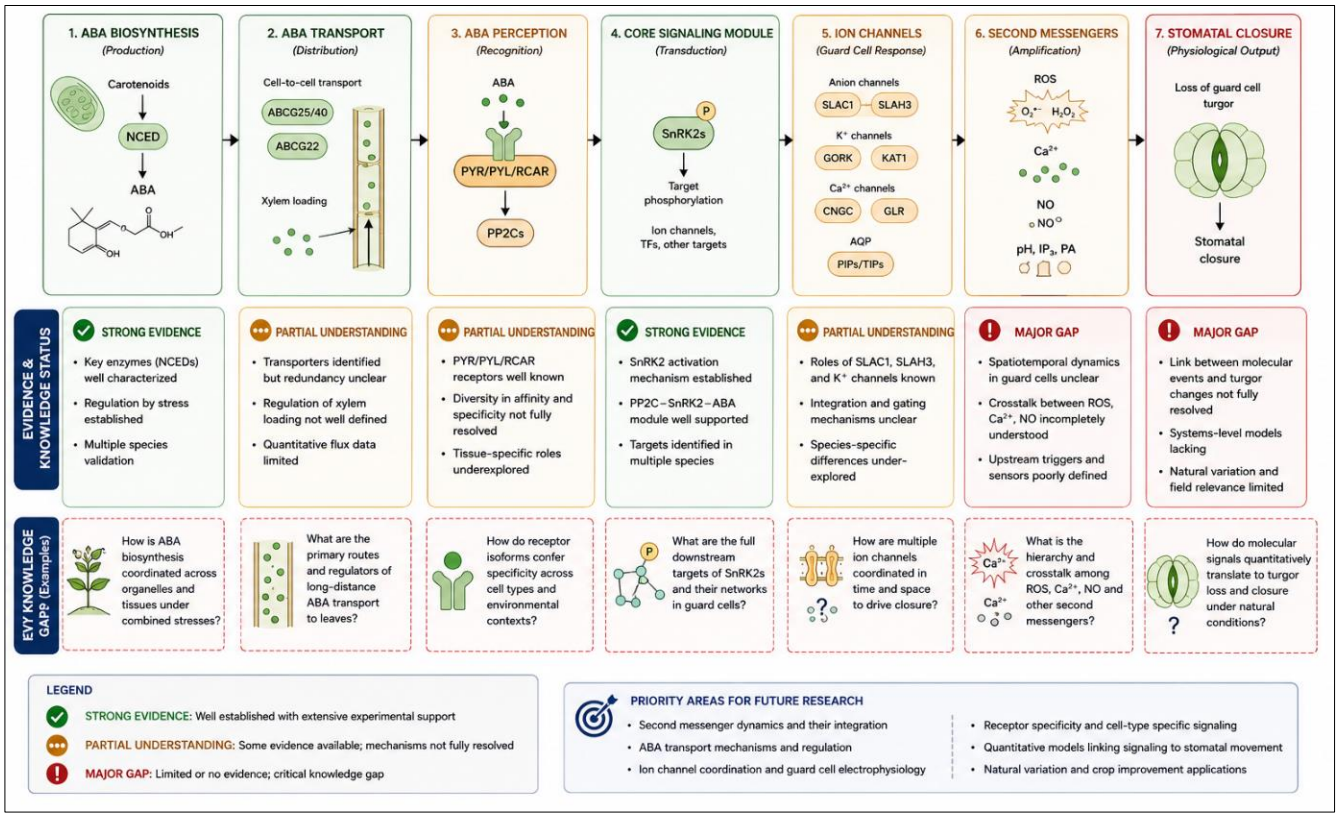

Fig 3: Research Gap Mapping: visual representation of knowledge gaps in the molecular stomatal regulation pathway from ABA biosynthesis to stomatal closure, indicating areas of strong evidence (green), partial understanding (amber), and major gaps (red).

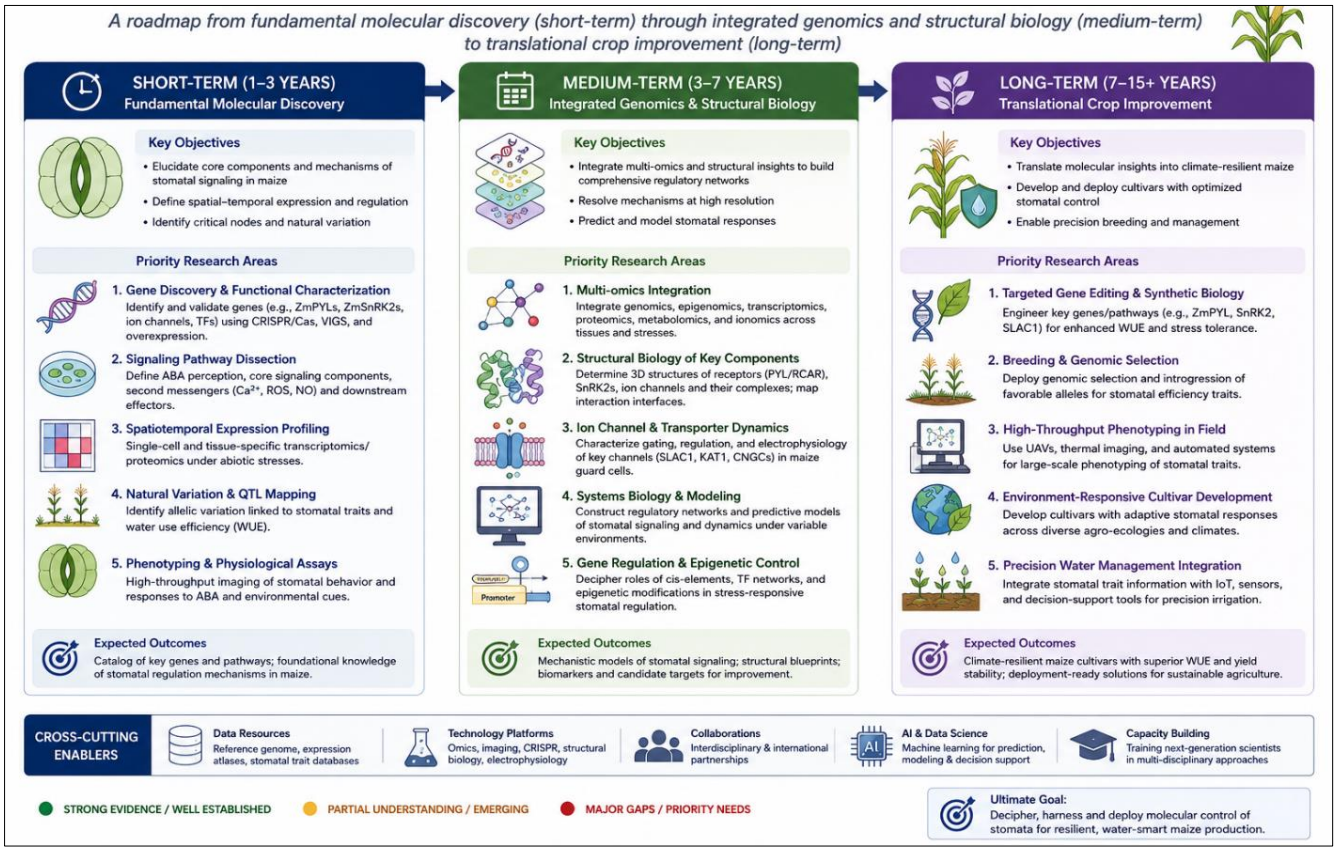

Fig 4: Future Research Directions in Molecular Stomatal Biology of Maize: a roadmap from fundamental molecular discovery (short-term) through integrated genomics and structural biology (medium-term) to translational crop improvement (long-term).

6. Discussion

This review has synthesized findings from over 38 peer-reviewed studies to provide a comprehensive molecular picture of drought-induced stomatal regulation in maize. Several convergent themes emerge from this synthesis, together with instructive divergences that highlight the unique biology of this C_4 cereal.

The ABA signaling cascade from PYR/PYL receptor activation through PP2C inhibition and SnRK2 kinase activation is functionally conserved between Arabidopsis and maize, with maize orthologues (ZmPYLs, ZmPP2C-A10, ZmOST1) performing analogous roles [2, 10, 18]. However, the maize pathway exhibits significant elaborations: the discovery of ZmGCT1/2 as novel phosphoregulators of ZmSLAC1, the ZmDnaJ–ZmNCED6 heat-shock-protein connection to ABA biosynthesis, and the ZmPP84–ZmMEK1 link between MAP kinase signaling and SLAC1 regulation all represent maize-specific regulatory layers without direct counterparts in Arabidopsis [2, 10]. These discoveries caution against over-reliance on Arabidopsis-derived models when designing drought tolerance strategies for maize.

A critical distinction concerns stomatal anatomy and function. Maize possesses graminaceous (dumbbell-shaped) guard cells flanked by subsidiary cells, a configuration that enables more rapid stomatal movements compared to kidney-shaped Arabidopsis stomata [6]. The subsidiary cells contribute to guard cell turgor changes by serving as osmolyte reservoirs, and their molecular communication with guard cells via plasmodesmata and apoplastic signals remains poorly characterized. Elucidating subsidiary cell contributions to ABA and ROS signaling in maize represents an underexplored frontier.

The transcription factor landscape of drought-responsive stomatal regulation in maize is rich and complex. NAC, WRKY, MYB, bZIP, and HSF families all contribute to the transcriptional reprogramming of guard cells under drought, with some factors acting through modulation of ABA biosynthesis (ZmWRKY79, ZmHsf28) [25], others by regulating stomatal density (ZmNAC49) [8], and still others by directly activating ion channel gene expression (ZmNAC20) [24]. The mechanistic diversity of these transcription factors, each targeting distinct nodes of the stomatal regulatory network, offers multiple entry points for genetic engineering of drought tolerance.

The emerging role of non-canonical signaling components—strigolactones [1], peptide hormones (CLE25), MAP kinases (ZmMAPKL1) [2], and heat-shock proteins (ZmDnaJ) [2]—indicates that the stomatal regulatory network is substantially more complex than originally envisioned. Integration of these components into a coherent, quantitative systems model of maize guard cell signaling is urgently needed. Recent genome-wide studies (DAP-seq, ChIP-seq, ATAC-seq) applied to drought-responsive transcription factors in maize have identified thousands of potential target genes, providing raw material for such integrative analyses [8].

A persistent challenge is the translation of molecular findings from controlled laboratory conditions to field environments. In field settings, plants experience simultaneous heat, drought, low humidity, high light, and soil nutrient variation—conditions that activate multiple, potentially conflicting signaling pathways. The constitutive stomatal closure phenotypes of some engineered lines, while conferring drought resistance in controlled environments, can reduce photosynthetic productivity under moderate water availability, limiting yield advantage in the field. Engineering stimulus-responsive, precision-tunable stomatal aperture—rather than constitutive closure—represents the next major

challenge in translational stomatal biology for maize crop improvement.

7. Conclusion

With over ten years of research regarding the stomatal regulation in maize due to drought still being processed, this systematic review has come up with a lot of essential information that shows how complex the signal delivery system is that uses hormones, ions, redox, and transcriptional inputs for controlling the turgor and opening of guard cells in the crop. The conclusions made about the signaling pathway in question include the following.

(1) ABA is the key regulator in the process. The increase in ABA content due to the activity of NCED enzymes (specifically, ZmNCED3 and ZmNCED6) and help from new elements (ZmDnaJ) is what makes this path effective.

(2) The key anion channel is ZmSLAC1. The latter is activated when it becomes phosphorylated by ZmOST1 and becomes no longer blocked by ZmGCT1/2 under drought.

(3) The meaning of ROS and Ca^{2+} is that they turn the closure process into the closure process. When the amount of NADPH oxidase-derived H2O2 increases, it leads to the activation of Ca^{2+} channels controlled by the action of NADPH oxidase and makes phosphorylation of SLAC1 and RBOH possible, leading to a positive reinforcement feedback loop that helps keep stomata closed even if the drought continues for long.

(4) Transcription factors are responsible for the coordination of transcriptional changes: NAC (ZmNAC20, ZmNAC49), WRKY (ZmWRKY79), HSF (ZmHsf28), and PIF (ZmPIF1) families regulate ABA production and control the number of stomata and ion channels in guard cells, thus ensuring transcriptional adaptation to drought.

(5) Unique regulatory mechanisms in maize: Several maize-specific stomatal regulators (ZmGCT1/2, ZmDnaJ – ZmNCED6, ZmMAPKL1) have been detected that have no equivalents in Arabidopsis, which requires the use of special functional genomics approaches to study maize-related genes.

Suggestions for Researchers: Upcoming studies should focus on single-cell transcriptomic and proteomic analysis of maize guard cells, characterizing maize specific signaling complexes, and understanding the ecosystem of natural variants in stomatal regulatory genes by utilizing pangenomes-based studies. The role of epigenetic regulation in ensuring stomatal drought memory and understanding of subsidiary guard cell crosstalk should also be explored through mechanistic studies.

Suggestions for Practitioners and Policymakers: Molecular knowledge regarding ZmSLAC1 regulation, ZmNAC49-driven stomatal density regulation, and ZmOST1 kinase function offers viable candidates for CRISPR-based precision editing techniques aimed at developing resistant varieties of maize. Funding for field validation experiments should be prioritized, particularly in drought-prone regions of South Asia and sub-Saharan Africa, where maize food security risk levels remain high. Moreover, it is necessary to establish effective regulatory frameworks enabling the usage of genome-edited (non-transgenic) maize varieties for the practical application of molecular findings.

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