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STOMATAL MECHANISMS UNDERLYING DROUGHT TOLERANCE IN RICE (*ORYZA SATIVA* L.): INTEGRATING PHYSIOLOGICAL, MOLECULAR REGULATION, AND BREEDING STRATEGIES

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SUMMARY

Drought reduces rice (*Oryza sativa* L.) productivity by impairing leaf water status, activating abscisic acid (ABA)-mediated stomatal closure, and disrupting carbon-water trade-offs. Rice adapts to drought through rapid stomatal signaling, developmental modifications, and whole-plant integration of hydraulic regulation, mesophyll conductance, and canopy architecture. Growing evidence indicates that moderate reductions in stomatal density (SD) can enhance water-use efficiency (WUE) and drought tolerance with minimal yield penalties when coordinated with non-stomatal traits. However, extreme reductions in SD may restrict carbon assimilation and impair thermoregulation. Recent studies have identified SD-related quantitative trait loci (QTLs) and candidate genes, alongside successful genome editing of EPF/EPFL family members, providing valuable targets for drought-resilient rice breeding. Rice deploys morphological, physiological, and molecular strategies to maximize WUE while sustaining photosynthesis under drought, including SD and aperture modulation, ABA and reactive oxygen species (ROS)-mediated signaling, and transcriptional regulation of drought-responsive genes and transcription factors. Despite these advances, key challenges remain in integrating stomatal traits with whole-plant hydraulics, root architecture, reproductive resilience, and genotype-by-environment interactions under field conditions. Future breeding strategies should combine physiological phenotyping with genomics, genome editing, and digital agriculture tools to develop climate-resilient rice cultivars capable of sustaining productivity under drought.

Keywords: Rice (*O. sativa* L.), stomata, drought stress, ABA-signaling, guard cells, drought tolerance

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Key findings: Drought reduces rice (*O. sativa* L.) productivity by lowering leaf water status and inducing ABA-mediated stomatal closure, disrupting carbon-water balance. Moderate reductions in stomatal density enhance water-use efficiency and drought tolerance with minimal yield penalty, whereas excessive reduction limits carbon assimilation.

INTRODUCTION

Drought remains one of the most severe abiotic stresses limiting rice (*Oryza sativa* L.) productivity globally. Approximately 45% of the global rice-growing area is rainfed, where erratic rainfall causes yield losses of 17%–40% in drought years, exceeding 50%–60% during sensitive growth stages (Jarin *et al.*, 2024). Rice is particularly vulnerable to drought stress during the seedling and reproductive stages (Sadhukhan *et al.*, 2024), while increasing drought frequency under climate change continues to exacerbate yield instability across upland and lowland ecosystems (Hu *et al.*, 2025).

Drought tolerance in rice involves a complex interplay of morphological, physiological, and molecular adaptive traits. Among these, stomatal regulation is central, directly linking environmental sensing with gas exchange, transpiration control, and photosynthetic efficiency. Reduced stomatal density in rice showed an association with increased WUE and enhanced drought tolerance (Caine *et al.*, 2023). Stomatal closure is one of the earliest drought responses, limiting transpirational water loss and preventing hydraulic failure (Hassan *et al.*, 2023), but simultaneously restricting CO₂ uptake and impairing photosynthesis (Phunthong *et al.*, 2024). Precise stomatal aperture control is therefore a critical adaptive trait governing the carbon-water trade-off in rice.

Physiologically, drought triggers ABA-mediated ion channel activation and guard cell shrinkage. At the molecular level, *OsPYL* receptors, *OsP2C* phosphatases, SnRK2 kinases, and the transcription factors *OsNAC6*, *OsbZIP23*, and *OsWRKY45* regulate stomatal movement through ABA-dependent cascades, with ROS and Ca²⁺ acting as secondary messengers that amplify drought signaling (Yan *et al.*, 2023). Recent QTL mapping studies

have revealed the genetic basis of stomatal plasticity, while high-throughput phenotyping and CRISPR/Cas9 genome editing have enabled precise targeting of stomatal regulators.

Although drought-responsive ABA signaling and rice stress physiology have had extensive reviews, existing syntheses rarely integrate anatomical, physiological, and molecular dimensions of stomatal adaptation in rice nor systematically link omics-driven discoveries with applied breeding and genome-editing efforts. Emerging evidence on stomatal plasticity, high-throughput phenotyping, and CRISPR/Cas-based manipulation remains fragmented, while conflicting views persist regarding the yield penalty of reduced stomatal density and field-level translation of gene-editing successes.

This review, therefore, consolidates recent advances in rice stomatal anatomy, physiology, and ABA-centered signaling; integrates multi-omics and QTL insights with functional genetics; and critically evaluates how these findings are being translated into practical breeding strategies by explicitly examining a) trade-offs between stomatal anatomy and carbon assimilation across rice ecotypes; b) key genetic regulators suitable for marker-assisted selection (MAS) and genomic selection (GS); and c) the integration of emerging high-throughput digital phenotyping technologies into crop improvement pipelines.

MATERIALS AND METHODS

This review synthesized existing literature on stomatal mechanisms governing drought tolerance in rice (*O. sativa* L.), covering physiological, molecular, and breeding perspectives. The retrieval of relevant studies came from Web of Science, PubMed, Scopus, Crossref, and Google Scholar using targeted search terms, including “stomatal density and

drought tolerance in rice," "ABA-mediated stomatal closure," "guard cell signaling under water deficit," "stomatal conductance and water-use efficiency," "EPF/EPFL stomatal regulators," and "CRISPR genome editing drought-resilient rice." Priority studies were those published between 2020 and 2026, while including seminal earlier studies when they provided foundational knowledge. The inclusion criteria comprised peer-reviewed articles addressing stomatal physiology, molecular regulation, or breeding applications in rice under drought conditions. Figure 1 presents the PRISMA-inspired workflow used for systematic identification, screening, and selection of studies included in this review.

Search strategy

The search strategy combined keywords and Boolean operators to maximize the retrieval of relevant literature. Representative search strings included "rice AND drought AND stomata"; "stomatal density AND rice"; "guard cell signaling AND ABA"; "*OsEPF1* OR *EPFL9* OR *STOMAGEN*"; "rice drought tolerance AND QTL"; "rice GWAS stomatal traits"; "CRISPR rice drought tolerance"; "high-throughput phenotyping rice"; "artificial intelligence stomata"; and "water-use efficiency in rice." Identification of additional publications proceeded through backward and forward citation tracking of highly cited studies and primary research papers to ensure comprehensive topical coverage.

Eligibility and exclusion criteria

Studies were eligible if they satisfied one or more of the following criteria: investigated stomatal development, morphology, density, aperture, conductance, or plasticity in rice; examined physiological or molecular mechanisms of drought tolerance involving stomatal regulation; reported QTL, genome-wide association study (GWAS), transcriptomic, proteomic, metabolomic, or functional genomic analyses related to stomatal traits; evaluated breeding approaches including marker-assisted

selection (MAS), genomic selection (GS), or genome editing targeting stomatal regulation; described high-throughput phenotyping, digital imaging, artificial intelligence (AI), or machine-learning applications for stomatal characterization; or were peer-reviewed reviews providing substantial synthesis of stomatal biology and drought adaptation in rice. Evidence from other species, including wheat, maize, sorghum, soybean, and *Arabidopsis thaliana*, was included when offering mechanistic insights directly applicable to rice stomatal biology or breeding, and such studies were clearly identified as comparative evidence.

The exclusion of the following publications comprised conference abstracts without accompanying full-text manuscripts; editorials, commentaries, and opinion articles lacking primary evidence or comprehensive synthesis; studies unrelated to drought or stomatal regulation; publications focused exclusively on species without clear relevance to cereal crops or transferable stomatal mechanisms; and duplicate records retrieved from multiple databases.

Study screening and selection

Retrieved publications underwent screening in three sequential stages. First, removing duplicate records identified across multiple databases ensued (Figure 1). Second, evaluating titles and abstracts looked for relevance to stomatal biology, drought stress, and rice improvement. Third, assessing full-text articles considered scientific quality, methodological relevance, and contribution to the objectives of this review. Preference focused on studies published in high-impact, peer-reviewed journals that reported physiological experiments, molecular characterization, quantitative genetic analyses, functional validation, or breeding applications.

Data extraction

Information extracted from each eligible publication included the following: publication year; crop species; experimental conditions;

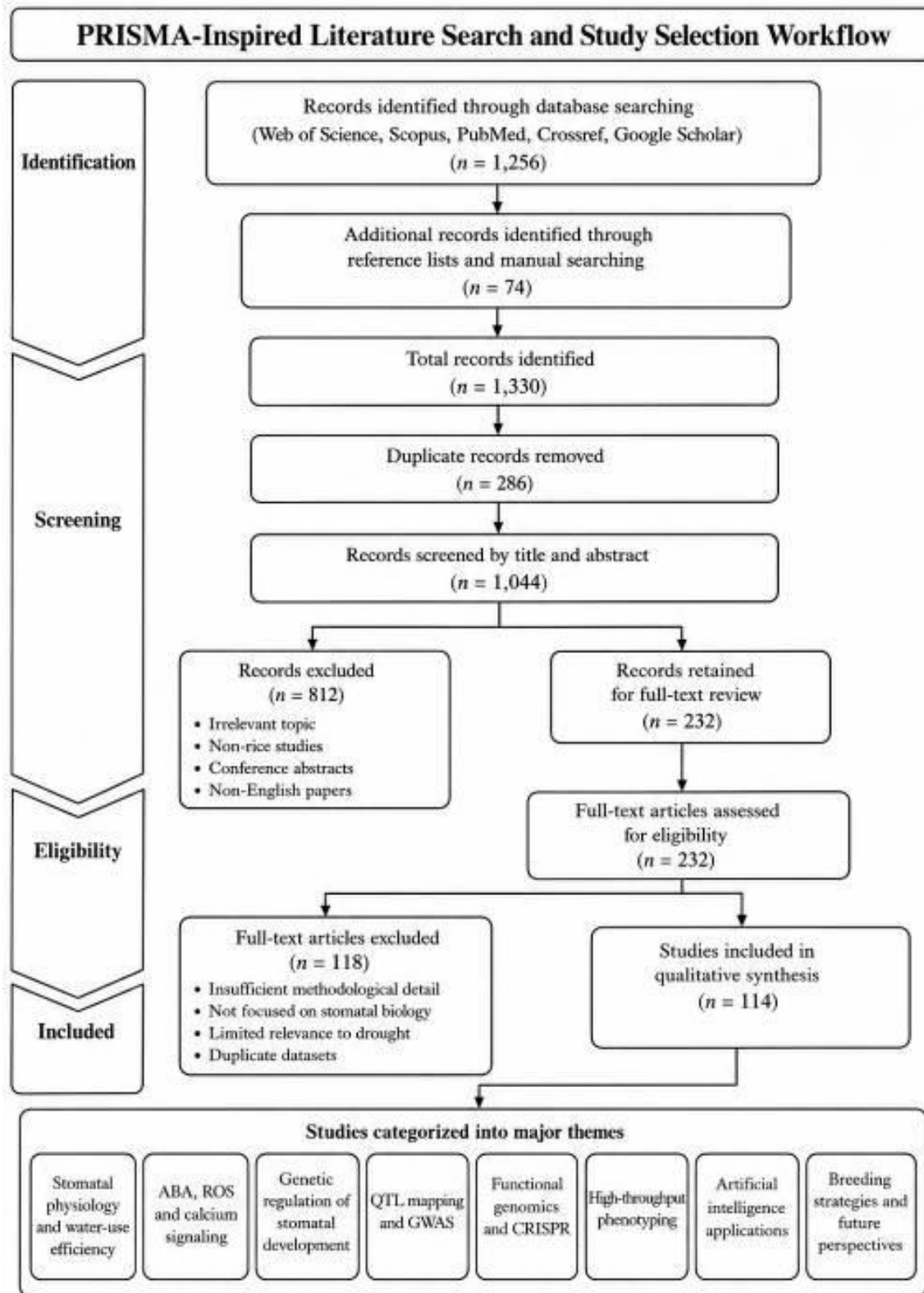


Figure 1. This PRISMA-inspired diagram illustrates the literature retrieval and study selection process used in the review to ensure a transparent, systematic, and reproducible search strategy.

stomatal traits investigated; physiological responses documented; genes, transcription factors, and signaling pathways identified; QTL and GWAS results; genome editing targets; phenotyping approaches employed; and breeding implications reported.

Literature synthesis

The selected literature's synthesis thematically sought to integrate evidence across multiple biological scales rather than summarizing individual studies sequentially. Publication grouping comprised the following major themes: functional roles of stomata in drought adaptation; physiological regulation of stomatal movement; ABA, ROS, calcium, and hydraulic signaling; genetic regulation of stomatal development; QTLs, GWAS, and candidate genes; high-throughput phenotyping and digital image analysis; artificial intelligence and machine learning applications; genome editing and functional genomics; marker-assisted and genomic selection; and future breeding strategies for climate-resilient rice. Within each theme, findings from independent studies underwent critical comparison to identify areas of consensus, conflicting evidence, methodological limitations, and emerging research priorities.

Quality assurance

Ensuring reliability and recency had the review emphasis placed on peer-reviewed publications from 2020 to 2026, while retaining seminal studies on rice stomatal development and drought physiology where appropriate. Information corroborated by multiple independent studies received preferential synthesis to minimize reliance on isolated observations and provide a balanced, evidence-based assessment of current knowledge.

Key findings span the physiological, molecular, and genetic dimensions of stomatal regulation under drought, encompassing gas exchange, high-throughput phenotyping, transcriptomics, and genome editing. The analysis revealed critical knowledge gaps, including insufficient integration of stomatal

traits with whole-plant hydraulics, limited field validation of laboratory-identified regulators, and the lack of unified frameworks linking stomatal phenotyping with genomic selection. This underscores the need for integrated breeding strategies combining stomatal optimization with yield stability.

RESULTS AND DISCUSSION

Stomata and drought tolerance

Drought triggers stomatal regulation in rice (*O. sativa* L.), directly affecting photosynthesis, transpiration, and WUE. As shown in Figure 2, stomatal closure obtains modulation from ABA-, Ca^{2+} -, and ROS-mediated signaling networks, which intersect with developmental pathways to alter stomatal patterning and function under water-deficit conditions (Matkowski and Daszkowska-Golec, 2023). Under optimal conditions, stomata remain open to facilitate CO_2 uptake for photosynthesis and transpiration-induced cooling. However, as soil water potential declines, loss of leaf turgor and hydraulic signaling trigger rapid stomatal closure (Figure 2). Based on findings, prolonged stomatal closure reduces the internal CO_2 concentration, thereby limiting photosynthetic carbon assimilation. This decrease negatively impacts the Calvin cycle, a crucial process in photosynthesis where CO_2 conversion into glucose occurs. Consequently, reduced Calvin cycle activity promotes accumulation of ROS within the chloroplasts, which can cause oxidative stress and damage to cellular components. Rice breeding lines that maintain intermediate stomatal responsiveness show better coupling of CO_2 assimilation to transpiration, thereby supporting stable photosynthesis and grain yield under drought, consistent with the positive correlations reported for yield under drought QTL *qDTY12.1* (Tseng *et al.*, 2026).

ABA is the central hormonal regulator of stomatal movement under water-deficit conditions. ABA, produced in the vascular system and transported to guard cells, activates PYR/PYL/RCAR receptors, triggering a signaling cascade involving PP2C phosphatases

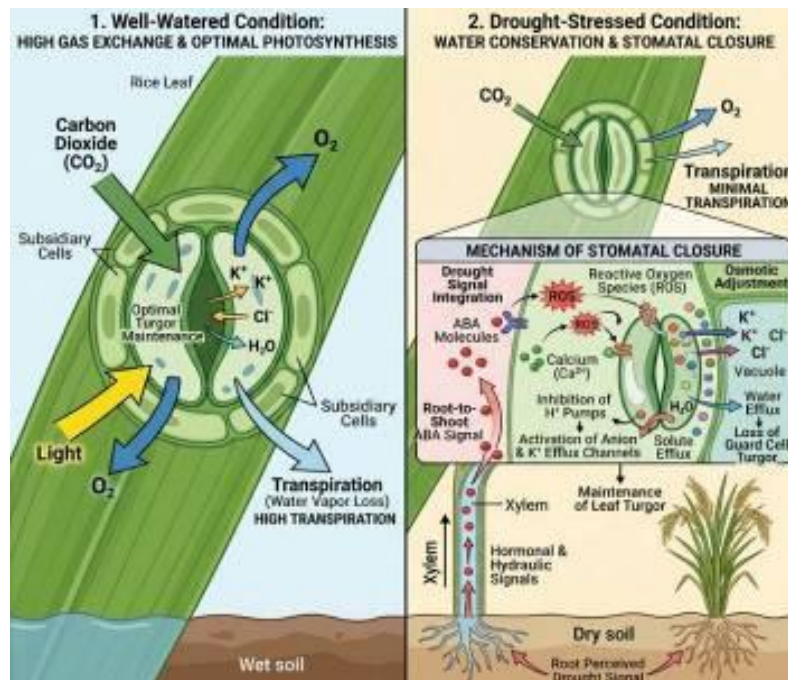


Figure 2. Comparative functional roles of rice stomata under well-watered and drought stress.

and ion channels such as SLAC1 and KAT1. This causes K^+ and anion efflux, leading to guard-cell turgor loss and stomatal closure (Liu *et al.*, 2022). In rice, enhanced expression of the ABA receptor *OsPYL9* improves drought tolerance by strengthening stress response pathways. Additionally, downstream bZIP transcription factors *OsABF2* and *OsbZIP2* play an important role in transmitting ABA signals and activating drought-responsive genes, thereby contributing to adaptive responses under water-deficit conditions.

During drought stress, specific tissues and guard cells accumulate compatible solutes. This process allows for osmotic adjustment, which helps the plant maintain its turgor pressure despite the lack of water. These compatible solutes help stabilize cellular membranes and proteins while still permitting partial stomatal reopening during periods of mild stress or post-stress recovery, thereby preserving photosynthetic capacity while minimizing further water loss.

Stomatal closure during drought undergoes further regulation by a synergistic ROS- Ca^{2+} signaling loop. ABA signaling regulates stomatal closure under drought by

inducing ROS production and Ca^{2+} influx into guard cells (Liu *et al.*, 2022). Therefore, breeding for improved stomatal responsiveness and plasticity represents a promising strategy for enhancing drought adaptation and yield stability in rice.

Genotypic and phenotypic variability

Gas-exchange performance primarily relies on the stomatal density (SD), stomatal size (SS), and aperture (SA). Rice often displays reduced SD and decreased pore sizes under drought stress. These features help to minimize excessive transpirational water loss at the cost of decreased availability of CO_2 for crucial fixation (Phunthong *et al.*, 2024). More broadly, across multi-environment breeding trials, drought-tolerant breeding lines, such as *Sahbhagi Dhan*, have combined lower stomatal density with maintained stomatal function, pointing to dynamic regulation of stomatal behavior as a key component of their drought performance.

The SD-SS balance regulation results from developmental regulators, including *OsEPFL9* and *OsSPCH2*, which determine

stomatal initiation and spacing. EPFL9/STOMAGEN positively regulates stomatal development by competing with inhibitory EPF peptides, enhancing stomatal lineage progression and epidermal differentiation. Under drought, it directly influences stomata density, transpiration rate, and water-use efficiency (Karavolias *et al.*, 2023) and increases CO₂ assimilation capacity under non-stressed conditions (Caine *et al.*, 2023). Collectively, these studies demonstrate that stomatal adaptation to water deficits garners control from both structural (SD/SS/SA) and regulatory (EPF/EPFL/SPCH) components. Natural variation in stomatal density, size, and aperture among rice cultivars corresponds with different drought responses. The contrasts between upland and lowland rice were evident in fewer but larger stomata in upland rice genotypes. This facilitates WUE compared to higher stomatal density and thus better photosynthetic performance under well-watered conditions in the lowland areas (Pitaloka *et al.*, 2022). Stomatal traits differ significantly among rice ecotypes, which translate to adaptations to sequentially opposite hydrological conditions and entail significant functional implications for gas exchange and water use. Lowland rice grown under well-watered conditions generally exhibits higher stomatal density, and this enhances maximum stomatal conductance (g_s) (Table 1). This characteristic correlates with higher photosynthetic capacity and carbon assimilation, as well as yield potential under optimal water supply. In contrast, upland rice adapted to aerobic and drought-prone environments exhibits a more conservative stomatal phenotype (Table 1). Intrinsic water-use efficiency (iWUE) is understood as the ratio of net CO₂ assimilation rate (A_n) to stomatal conductance (g_s), expressed as A_n/g_s , representing the efficiency with which a plant fixes carbon relative to water lost through stomatal conductance under prevailing atmospheric conditions (Ma *et al.*, 2023). Unlike instantaneous WUE, which reflects actual evapotranspiration, iWUE captures the leaf's intrinsic capacity to balance carbon gain against water loss independently of vapor pressure deficit, making it a stable

physiological indicator for comparing drought tolerance across genotypes and environments. Reduced stomatal density and efficient gas-exchange control showed an association with increased iWUE and drought tolerance in upland rice cultivars (Phunthong *et al.*, 2024), while *OsEPF1* overexpression reduces stomatal density, enhancing WUE and drought tolerance (Karavolias *et al.*, 2023).

QTLs and candidate genes for stomatal traits

Substantial progress has succeeded in fine mapping of QTLs for stomatal traits and drought tolerance in rice. Numerous QTLs affecting stomatal density, size, and responsiveness have been mapped in different genetic backgrounds (Table 2). In addition, GWAS and functional genomics have identified important stomatal conductance and drought-responsive regulators in rice, including *OsEPF1*, *OsbZIP23*, and ABA-receptor *OsPYL9*, which regulate stomatal behavior under water-deficit conditions (Zong *et al.*, 2020).

The genetic architecture of stomatal and drought-related traits in rice remains polygenic. Recent genome-wide association and QTL analyses have identified numerous loci associated with stomatal density, stomatal size, water-use traits, canopy temperature, leaf rolling, and drought tolerance across multiple chromosomes (Phunthong *et al.*, 2024). Recent QTL and GWAS studies indicate that genomic regions influencing stomatal traits can co-localize with loci for drought-related yield traits, canopy temperature, and water-use efficiency, supporting coordinated genetic control of drought adaptation (Li *et al.*, 2024). Several of these loci contain genes involved in epidermal development, peptide signaling, hormone perception, and transcriptional regulation (Caine *et al.*, 2023). Importantly, many stomatal QTLs exhibit moderate individual effects but contribute substantially to drought adaptation when combined, supporting the use of genomic selection to capture cumulative genome-wide effects rather than relying solely on MAS for individual loci (Varshney *et al.*, 2021).

Table 1. Comparative stomatal and physiological traits across rice ecotypes.

Traits	Upland (e.g., Vandana, Way Rarem)	Lowland (IR64, Takanari)	Functional Consequences	
			Upland	Lowland
Stomatal Density	Low	High	Conserves water	maximizes CO ₂ flux
Stomatal Size	Larger	Smaller	Larger stomata/slower but conserve water	smaller/enable rapid gas exchange
Stomatal Conductance (gs)	Lower (conservative)	Higher (maximal)	Limits water loss	supports high photosynthesis
Stomatal Responsiveness	Slower opening, faster closure	Faster opening/slower closure	Prioritizes survival	prioritizes productivity
Intrinsic WUE	High (efficient)	Moderate to low	Little biomass per unit water	more biomass per unit water
Transpiration Rate	Low to moderate	High	Conserves soil moisture	enhances evaporative cooling
Canopy Temperature	Higher (warm canopy)	Lower (cool canopy)	Higher heat stress	uses transpiration to reduce heat stress
Photosynthetic Capacity (A _n)	Moderate	High	Lower yield potential	higher yield potential
Mesophyll Conductance	Moderate	High	Impair internal CO ₂ diffusion	enhances internal CO ₂ diffusion
Hydraulic Conductivity	Moderate	High	Against high transpiration demand	supports high transpiration demand
Leaf Anatomy (Thickness, SLA)	Thicker leaves, lower SLA	Thinner leaves, higher SLA	Reduces water loss	maximizes light capture
Stomatal Distribution (Adaxial/Abaxial)	More balanced or reduced	Often higher on both surfaces	Minimize gas exchange capacity	maximizes gas exchange
Cuticular Wax / Leaf Surface Traits	Higher wax deposition	Lower wax	Reduces non-stomatal water loss	increases non-stomatal water loss
Plasticity of Stomatal Traits	High plasticity under stress	Lower plasticity	Adjusts better to differing environments	adapts better to varying environments

Note: SLA, Specific leaf area

Table 2. Reported QTLs and candidate genes linked to stomatal morphology and canopy temperature regulation under water deficit conditions.

QTL/Region (as reported)	Stomata Trait (surface)	Candidates locus / supported by study	Population type	Genetic material	Reference
GWAS SD locus (panel) <i>OsEPFL9/STOMAGEN editing</i>	Stomatal density SD tuning	Trehalose-6-phosphate synthase (TPS) EPFL9 paralog editing tunes SD	GWAS panel Gene editing (CRISPR)	Rice diversity panel Rice cultivars	Phetluan <i>et al.</i> , 2023 Karavolias <i>et al.</i> , 2023
SS-SD architecture	SS & SD	Negative-SS-SD correlation	Multi-study synthesis	Multiple germplasm	Caine <i>et al.</i> , 2023
Low SD mutants	SD & SS	Low SD + small SS → ↑WUE, ↑biomass	Mutant population	EMS / induced mutants	Pitaloka <i>et al.</i> , 2022
Drought-selected mutants	SD	Reduced SD → ↑WUE under drought	Mutant selection	Rice mutant lines	Phunthong <i>et al.</i> , 2024
<i>OsBC1L1</i> / <i>OsBC1L8</i>	Stomatal patterning	Double mutant → clustering; OE → abnormal patterning	Mutant / transgenic	Rice lines	Li <i>et al.</i> , 2021
RSD1 (patterning factor)	SD / spacing	RSD1 regulates patterning EPF1/2	Functional genetics	Rice lines	Yu <i>et al.</i> , 2020

SD; stomatal density, SS; stomatal size, WUE; water use efficiency

Developmental regulators of stomatal formation

The developmental program controlling stomatal formation is highly conserved among flowering plants. In rice, stomatal lineage initiation begins with *OsSPCH* (*SPEECHLESS*), a basic helix-loop-helix (*bHLH*) transcription factor that promotes asymmetric epidermal cell divisions and initiates stomatal precursor cells (Zhou *et al.*, 2025). Subsequent differentiation entailed regulation sequentially by *OsMUTE* and *OsFAMA*, which specify guard-cell identity and promote guard-cell maturation (Yu *et al.*, 2020).

Additional regulators, including *OsSCR* (*SCARECROW*) and *OsSHR* (*SHORTROOT*), coordinate subsidiary cell differentiation and stomatal spacing in rice. Functional studies show mutations in these transcription factors disrupt epidermal patterning and stomatal development, leading to abnormal stomatal arrangement and altered stomatal function (Hughes and Langdale, 2022).

These developmental regulators operate within an interconnected regulatory network involving *EPF/EPFL* peptide signaling, receptor-like kinases including *OsERECTA*, and downstream transcriptional cascades that integrate developmental and environmental cues to establish final stomatal density and patterning on each leaf surface (Caine *et al.*, 2023).

Integration of phenotyping with genomics and gene editing

Along with CRISPR/Cas9 gene editing, high-resolution phenotyping and RNA-seq have confirmed several candidate genes controlling stomatal development and drought tolerance. The *OsEPF1*-edited/low stomatal density rice lines exhibited dramatically lower transpiration under drought stress (Phunthong *et al.*, 2024). Likewise, compared with the wild type, *OsPYL9* overexpression lines showed faster stomatal closure under ABA treatment, lower transpiration water loss, and enhanced drought tolerance, leading to yield stability (Usman *et al.*, 2023).

Recently, such networks' mapping can have high accuracy with multi-omics approaches, including transcriptomics, proteomics, and metabolomics. These integrated strategies are essential for breeding genotype-by-environment interactions and enhancing high-yielding, climate-resilient rice genotypes with efficient stomatal regulation.

Stomatal traits and drought-responsive phenotyping

Variation in stomatal morphology, density, size, aperture, and conductance has shown close relationships to the functional performance of rice drought tolerance. These stomatal properties are critical determinants of WUE and photosynthetic rate and potential indicators of drought tolerance (Pitaloka *et al.*, 2022; Karavolias *et al.*, 2023). Recent improvements in imaging technologies, QTL mapping, and high-throughput phenotyping have made it feasible to accurately characterize stomatal traits, enabling their incorporation into rice breeding programs for increased tolerance. Advances in automated stomatal phenotyping, leveraging computer vision and deep learning frameworks, such as StoManager1, have replaced manual quantification methods, enabling high-throughput, precise measurement of stomatal traits to support large-scale genetic studies (Li *et al.*, 2023).

Breeding for stomatal optimization

Developing drought-resilient rice requires integrating conventional selection with molecular tools, including MAS for resilience QTLs and genome editing to refine stomatal traits. Optimizing stomatal function balances CO₂ uptake and transpiration, thereby improving photosynthetic performance while reducing excessive water loss under drought conditions. Effective stomatal regulation enhances WUE by maintaining adequate gas exchange during drought periods (Pitaloka *et al.*, 2022). This adaptive mechanism ultimately contributes to improved plant growth, drought tolerance, and yield stability under drought conditions (Pitaloka *et al.*, 2022).

Historically, drought-tolerance breeding focused on root morphology, osmotic adjustment, and stress-induced yield. Stomatal traits, including density, aperture plasticity, and conductance, have since emerged as important selection criteria for drought-tolerant rice varieties (Fernando *et al.*, 2025). By combining high-throughput phenotyping with molecular markers, breeders can quickly select for complex traits indirectly. This approach reduces reliance on unpredictable field data, making the overall process of developing new cultivars much more efficient.

The identification of QTLs governing stomatal density, leaf rolling, and stomatal conductance has provided molecular targets for marker-assisted selection (MAS), accelerating the development of drought tolerance in rice varieties. Specific QTLs, such as *qDaba3.1* and *qLada3.1*, regulate stomatal orientation, though their consistent identification remains challenging (Pitaloka *et al.*, 2022). Combining MAS and GS leverages both large- and small-effect loci and has been shown to improve predictive accuracy. Key molecular targets include *OsEPF1* and *OsEPFL9*, which inversely regulate stomatal density to optimize WUE (Karavolias *et al.*, 2023), as well as *OsPYL* ABA receptors, which enhance drought tolerance when overexpressed.

Transgenic approaches provide functional validation of stomatal-regulatory genes. Overexpression of *OsEPF1* reduces stomatal density by up to 67%, increasing iWUE with minimal yield penalty under well-watered conditions (Caine *et al.*, 2023). Knockout of the positive regulator STOMAGEN similarly reduces stomatal density and *gs*, improving WUE under drought, though warranting caution to avoid limiting CO₂ capture (Shahbaz *et al.*, 2025). Collectively, targeting stomatal density, size, and aperture through EPF/EPFL peptides, ABA receptors, and transcription factors offer precise tools for engineering drought-resilient rice.

High-throughput phenotyping

Infrared thermography

Infrared thermal imaging has emerged as a powerful non-destructive approach for assessing stomatal conductance on a large scale. Given that transpirational cooling reduces leaf surface temperature, canopy thermal signatures provide an indirect measure of stomatal aperture status. Thermal imaging has appeared to accurately capture stomatal opening and closure kinetics under dynamic field conditions, offering a non-invasive means of tracking real-time stomatal behavior without damaging plant tissues.

Drone-mounted infrared sensors now enable canopy-scale monitoring across large breeding nurseries, allowing evaluation of thousands of genotypes within a single season. Thermal imaging has reached extensive adoption in wheat drought breeding, with an increasing application in rice to identify genotypes with efficient stomatal regulation and superior water-use efficiency (Chaplin *et al.*, 2025).

Chlorophyll fluorescence imaging

Since stomatal closure restricts CO₂ diffusion, chlorophyll fluorescence imaging provides complementary information on the photosynthetic consequences of altered stomatal behavior. Modern imaging systems simultaneously quantify photosystem II efficiency (F_v/F_m), quantum yield, electron transport rate, and non-photochemical quenching, enabling researchers to evaluate spatial heterogeneity across the leaf surface and distinguish stomatal limitations from biochemical impairment of photosynthesis (Moustakas *et al.*, 2021). Integration of fluorescence and thermal imaging provides a more comprehensive assessment of drought tolerance than either technique alone.

Hyperspectral and UAV-based phenotyping

Hyperspectral imaging measures reflectance across hundreds of narrow spectral bands, enabling non-destructive estimation of leaf water content, chlorophyll concentration, and stomatal conductance. Machine-learning models trained on hyperspectral datasets can predict drought stress before visible symptoms appear, and when integrated with genomic models, these approaches substantially improve predictive accuracy for complex, quantitatively inherited stress-response traits.

Unmanned aerial vehicles (UAVs) equipped with RGB, multispectral, thermal, and LiDAR sensors enable rapid field-scale assessment of canopy temperature, vegetation indices, and drought stress responses under realistic conditions. This brought ease where drought commonly interacts with heat stress and fluctuating vapor pressure deficit to shape overall plant performance.

Programs at the International Rice Research Institute (IRRI) have increasingly incorporated UAV-based phenotyping into genomic selection pipelines to accelerate drought-tolerance breeding (Varshney *et al.*, 2021).

Artificial intelligence and machine learning approaches have substantially reduced manual labor in stomatal phenotyping while enabling extraordinary upscaling across large breeding populations. In rice, Li *et al.* (2023) developed a YOLOv5-based deep-learning model for automatic stomatal detection from leaf epidermal impressions, achieving detection accuracy beyond 90% and reducing quantification time by more than 80% compared with manual methods. This enabled rapid, large-scale characterization of stomatal density and size across diverse crops. Similarly, Gibbs *et al.* (2021) demonstrated that a fully convolutional neural network (CNN) trained on brightfield microscopy images accurately quantified stomatal morphometry, including density, pore length, width, and area. Likewise, it estimated maximal stomatal conductance in wheat and *Arabidopsis*, with outputs strongly correlated with gas exchange measurements, validating AI-derived

phenotypes as physiologically meaningful substitutes for resource-intensive porometry.

In wheat, deep learning has enabled rapid and accurate automated assessment of stomatal traits, with faster R-CNN and U-Net models achieving over 95% accuracy in stomatal index estimation, thereby facilitating high-throughput phenotyping (Zhu *et al.*, 2021). Beyond static morphometry, Sai *et al.* (2023) developed the deep-learning computer vision platform StomaAI (SAI), which automated the quantification of stomatal aperture dynamics and density in *Arabidopsis* and barley, demonstrating that AI tools can capture kinetic stomatal behavior previously inaccessible at scale. In maize, CNN-based flowering-related phenotyping has successful integration with genomic prediction models to estimate stomatal conductance using AI-derived methods. This approach improved the accuracy of genomic estimated breeding values (GEBVs) for drought tolerance compared with marker data alone, offering proof of concept for integrating digital phenotyping with genomic selection pipelines.

Collectively, these advances across rice, wheat, maize, and model species illustrate that AI-based stomatal phenotyping is rapidly transitioning from proof-of-concept to operational deployment in crop improvement programs.

CONCLUSIONS

Stomatal regulation is the primary mechanism balancing CO₂ uptake and water-use efficiency in rice under drought stress, coordinated by master regulators, including NAC, bZIP, and AP2/ERF transcription factors. These regulators provide molecular targets for genome editing and marker-assisted selection (MAS), with SNP markers in *OsSLAC1*, *OsEPF1*, and ABA-responsive genes as strong MAS candidates. Marker-assisted selection and backcrossing combining stomatal and root hydraulic loci have achieved notable field success, pyramiding yield-under-drought QTL markers into elite mega-varieties, producing stable-yielding lines, such as IR64-dty12.1, IR64, Swarna, and Sabitri, which sustain productivity

under reproductive-stage water stress. Comparable progress in wheat, maize, and soybean confirms that stomatal-linked markers translate across cereal and legume species to improve drought resilience. High-throughput phenotyping, including infrared thermography and AI-driven imaging, is accelerating this integration, though future work must still link stomatal behavior with root hydraulics and the rhizosphere microbiome.

REFERENCES

- Caine RS, Harrison EL, Sloan J, Flis PM, Fischer S, Khan MS, Nguyen PT, Nguyen LT, Gray JE, Croft H (2023). The influences of stomatal size and density on rice abiotic stress resilience. *New Phytol.* 237(6): 2180–2195. <http://dx.doi.org/10.1111/nph.18704>.
- Chaplin E, Coleman G, Merchant A, Salter W (2025). FieldDino: Rapid in-field stomatal anatomy and physiology phenotyping. *Plant Cell & Environ.* <http://dx.doi.org/10.1111/pce.15639>.
- Fernando Y, Adams M, Kuhlmann M, Jr VB (2025). Stomatal and non-stomatal leaf traits for enhanced water use efficiency in rice. *Biology* 14(7): 843. <http://dx.doi.org/10.3390/biology14070843>.
- Gibbs JA, McAusland L, Robles-Zazueta CA, Murchie EH, Burgess AJ (2021). A deep learning method for fully automatic stomatal morphometry and maximal conductance estimation. *Front. Plant Sci.* 12: 780180. <http://dx.doi.org/10.3389/fpls.2021.780180>.
- Hassan MA, Dahu N, Hongning T, Qian Z, Yueming Y, Yiru L, Shimei W (2023). Drought stress in rice: Morpho-physiological and molecular responses and marker-assisted breeding. *Front. Plant Sci.* 14: 1215371. <http://dx.doi.org/10.3389/fpls.2023.1215371>.
- Hu WF, Qin YB, Lin JJ, Chen TT, Li SF, Chen YT, Xiong J, Fu GF (2025). Unraveling the nexus of drought stress and rice physiology: Mechanisms, mitigation, and sustainable cultivation. *Plant Stress* 17: 100973. <http://dx.doi.org/10.1016/j.stress.2025.100973>.
- Hughes TE, Langdale JA (2022). SCARECROW is deployed in distinct contexts during rice and maize leaf development. *Development*, 149(7). <https://doi.org/10.1242/dev.200410>.
- Jarin AS, Islam MM, Rahat A, Ahmed S, Ghosh P, Murata Y (2024). Drought stress tolerance in rice: Physiological and biochemical insights. *Int. J. Plant Biol.* 15(3): 692–718. <http://dx.doi.org/10.3390/ijpb15030051>.
- Karavolias NG, Patel-Tupper D, Seong K, Tjahjadi M, Gueorguieva GA, Tanaka J, Gallegos Cruz A, Lieberman S, Litvak L, Dahlbeck D, Cho MJ (2023). Paralog editing tunes rice stomatal density to maintain photosynthesis and improve drought tolerance. *Plant Physiol.* 192(2): 1168–1182. <https://dx.doi.org/10.1093/plphys/kiad183>.
- Li Z, Sun P, Sun P, Liang YK, Ge S (2021). *OsBC1L1* and *OsBC1L8* function in stomatal development in rice. *Biochem. Biophys. Res. Commun.* 576: 40–47.
- Li X, Guo S, Gong L, Lan Y (2023). An automatic plant leaf stomata detection method based on YOLOv5. *IET Image Process.* 17(1), 67–76. <http://dx.doi.org/10.1049/ipr2.12617>.
- Li R, Wang Y, Li D, Guo Y, Zhou Z, Zhang M, Zhang Y, Würschum T, Liu W (2024). Meta-quantitative trait loci analysis and candidate gene mining for drought tolerance-associated traits in maize (*Zea mays* L.). *Int. J. Mol. Sci.*, 25(8), 4295. <https://doi.org/10.3390/ijms25084295>.
- Liu H, Song S, Zhang H, Li Y, Niu L, Zhang J, Wang W (2022). Signaling transduction of ABA, ROS, and Ca²⁺ in plant stomatal closure in response to drought. *Int. J. Mol. Sci.* 23(23): 14824. <http://dx.doi.org/10.3390/ijms232314824>.
- Ma WT, Yu YZ, Wang X, Gong XY (2023). Estimation of intrinsic water-use efficiency from $\delta^{13}C$ signature of C3 leaves: Assumptions and uncertainty. *Front. Plant Sci.*, 13. <https://doi.org/10.3389/fpls.2022.1037972>.
- Matkowski H, Daszkowska-Golec A (2023). Update on stomata development and action under abiotic stress. *Front. Plant Sci.* 14: 1270180. <http://dx.doi.org/10.3389/fpls.2023.1270180>.
- Moustakas M, Calatayud Á, Guidi L (2021). Editorial: Chlorophyll fluorescence imaging analysis in biotic and abiotic stress. *Front. Plant Sci.* 12. <https://doi.org/10.3389/fpls.2021.658500>.
- Phetluan W, Wanchana S, Aesomnuk W, Adams J, Pitaloka MK, Ruanjaichon V, Vanavichit A, Toojinda T, Gray JE, Arikrit S (2023). Candidate genes affecting stomatal density in rice (*Oryza sativa* L.) identified by genome-wide association. *Plant Sci.* 330: 111624.
- Phunthong C, Pitaloka MK, Chutteang C, Ruengphayak S, Arikrit S, Vanavichit A (2024). Rice mutants selected under severe drought stress show reduced stomatal

- density and improved water use efficiency under restricted water conditions. *Front. Plant Sci.* 15: 1307653. <http://dx.doi.org/10.3389/fpls.2024.1307653>.
- Pitaloka MK, Caine RS, Hepworth C, Harrison EL, Sloan J, Chutteang C, Phunthong C, Nongngok R, Toojinda T, Ruengphayak S, Arikrit S (2022). Induced genetic variations in stomatal density and size of rice strongly affect water use efficiency and responses to drought stress. *Front. Plant Sci.* 13: 801706. <http://dx.doi.org/10.3389/fpls.2022.801706>.
- Sadhukhan D, Mukherjee T, Sarkar A, Devi ND, Bisarya D, Kumar V, Jincy M (2024). A comprehensive analysis of drought stress responses in rice (*Oryza sativa* L.): Insights into developmental stage variations from germination to grain filling. *Int. J. Environ. Clim. Change* 14(7): 141–158.
- Sai N, Bockman JP, Chen H, Watson-Haigh N, Xu B, Feng X, Piechatzek A, Shen C, Gilliam M (2023). STOMAAI: An efficient and user-friendly tool for measurement of stomatal pores and density using deep computer vision. *New Phytol.*, 238(2), 904–915. <https://doi.org/10.1111/nph.18765>.
- Shahbaz U, Videau P, Coulonnier E, Papon C, Navarro-Payá D, Valenzuela AV, Matus JT, Malnoy M, Zekri O, Fiorani F, Faralli M (2025). Reduced stomatal density improves water-use efficiency in grapevine under climate scenarios of decreased water availability. *Plant Cell Rep.* 44(9): 1–21. <http://dx.doi.org/10.1007/s00299-025-03577-9>.
- Tseng FY, Chang MH, Jian JY, Tsai YC (2025). Physiological and molecular characterization of the drought tolerance-related QTL *qDTY12.1* in Japonica rice. *Rice*, 19(1). <https://doi.org/10.1186/s12284-025-00871-8>.
- Usman B, Derakhshani B, Jung KH (2023). Recent molecular aspects and integrated omics strategies for understanding the abiotic stress tolerance of rice. *Plants* 12(10): 2019.
- Varshney RK, Bohra A, Yu J, Graner A, Zhang Q, Sorrells ME (2021). Designing future crops: Genomics-assisted breeding comes of age. *Trends Plant Sci.* 26(6): 631–649. <http://dx.doi.org/10.1016/j.tplants.2021.03.010>.
- Yan J, Ninkuu V, Fu Z, Yang T, Ren J, Li G, Yang X, Zeng H (2023). *OsOLP1* contributes to drought tolerance in rice by regulating ABA biosynthesis and lignin accumulation. *Front. Plant Sci.* 14: 1163939. <http://dx.doi.org/10.3389/fpls.2023.1163939>.
- Yu Q, Chen L, Zhou W, An Y, Luo T, Wu Z, Wang Y, Xi Y, Yan L, Hou S (2020). *RSD1* is essential for stomatal patterning and files in rice. *Front. Plant Sci.* 11: 600021. <https://doi.org/10.3389/fpls.2020.600021>.
- 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 regulates stomatal development and drought tolerance in maize. *J. Integr. Plant Biol.*, 67(6), 1487–1500. <https://doi.org/10.1111/jipb.13890>.
- Zhu C, Hu Y, Mao H, Li S, Li F, Zhao C, Luo L, Liu W, Yuan X (2021). A deep learning-based method for automatic assessment of stomatal index in wheat microscopic images of leaf epidermis. *Front. Plant Sci.* 12. <https://doi.org/10.3389/fpls.2021.716784>.
- Zong W, Yang J, Fu J, Xiong L (2020). Synergistic regulation of drought-responsive genes by transcription factor *OsbZIP23* and histone modification in rice. *J. Integr. Plant Biol.* 62(6): 723–729. <http://dx.doi.org/10.1111/jipb.12850>.