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REVIEW ARTICLE

# Interaction between stomatal, mesophyll and chloroplast regulation for plant photosynthetic acclimation to drought

**Zaffar Mahdi Dar[1*], Farooq Ahmad Khan[1], Malik Asif Aziz[1], Mushtaq Ahmad Malik[1], Mohammad Maqbool Mir[2], Khalid Hussain Bhat[1], Wajid Waheed[1], Amjad Masood[1] & Prajal Dey[1]**

[1]Division of Basic Sciences and Humanities, Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Srinagar 190 025, Jammu and Kashmir, India

[2]Division of Fruit Sciences, Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K), Srinagar 190 025, Jammu and Kashmir, India

*Correspondence email - zaffar_123@yahoo.com

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## Abstract

Drought triggers a series of physiological and biochemical changes at the stomatal, mesophyll and chloroplast levels, which are linked and integrated into a finely tuned regulatory network that determines plant photosynthetic acclimation and drought tolerance. In this regard, stomatal regulation acts as the primary control, regulating aperture adjustment, density and kinetics, helping to establish the level of carbon dioxide ($CO_2$) influx in response to hydraulic signals, abscisic acid (ABA) signalling and root-to-shoot chemical messengers. Besides stomata, mesophyll conductance ($g_m$) is considered a decisive factor for $CO_2$ diffusion influenced by changes in cell wall porosity, membrane permeability, aquaporin (AQP) activity and chloroplast positioning. At the chloroplast level, drought modulates ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activation by dissipating energy through the reorganisation of the thylakoid electron transport, use of alternative electron sinks and non-photochemical quenching (NPQ) to prevent photoinhibition and oxidative damage. The coordinated interplay between these regulatory levels allows plants to adjust their photosynthetic rate (Pn) by dynamically balancing the trade-off between carbon assimilation and water conservation. Gaining insight into this multi-scale integration is critical not only for forecasting plant responses to the escalating drought but also for conceiving the targeted interventions. This article goes a step further in integrating present knowledge on stomatal-mesophyll-chloroplast communication to illustrate how the literature collected can be utilised for breeding and other strategies to enhance water-use efficiency (WUE) and drought resilience in crop systems.

**Keywords:** acclimation; chloroplast; drought; mesophyll; photosynthetic; stomata

## Introduction

Drought, with predicted increases in frequency, duration and intensity in the coming years, is a major abiotic stressor negatively impacting ecosystems and crop yields worldwide (1). Disturbance of the carbon assimilation-to-transpiration ratio in plants under drought has been reported as the main driver of reduced molecular and cellular functions, eventually resulting in reduced crop growth and yield (2, 3). Out of all the physiological processes, photosynthesis is the one that is affected first by drought, mainly because of its dependence on leaf water potential (Ψw), cell turgor and efficient gas exchange. Futhermore, drought stress reduces ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activity, disrupts photosynthetic electron flow and increases the production of reactive oxygen species (ROS), further exacerbating the decline in photosynthetic efficiency (4, 5). In addition, limitations to carbondioxide ($CO_2$) diffusion imposed by stomatal closure and reduced mesophyll conductance ($g_m$) are key factors in the observed inhibition of ATP synthesis, chloroplast metabolism and photosystem II (PS II) activity (6–8). Conventional explanations propose that the decrease in photosynthetic rate (Pn) under drought is attributed to the closure of stomata and limited $CO_2$ diffusion to the chloroplast (6). However, increasing evidence indicates that drought-induced changes in Pn are a complex process involving interactions and communication among different plant parts at the stomatal, mesophyll and chloroplast levels (9, 10). Stomata regulate $CO_2$ intake and water loss, thereby directly affecting leaf water balance and $CO_2$ concentration. On the other hand, the mesophyll layer regulates internal $CO_2$ conductance and diffusion efficiency which, depending on drought severity, can be severely affected by cell wall porosity, aquaporin (AQP) activity and chloroplast positioning (11, 12). At the chloroplast level, the plants are confronted with a drop in photochemical efficiency, redox balance and metabolic fluxes to maintain the energy balance and avoid photoinhibition (7, 8). These regulatory levels are interconnected via a complex signal network, including abscisic acid (ABA), reactive oxygen species (ROS), calcium ions ($Ca^{2+}$), nitric oxide (NO) and redox mediators, which collectively orchestrate carbon capture and water conservation (13). This hierarchical yet integrated control enables plants to optimise photosynthetic performance and prevent

irreversible oxidative damage under fluctuating water availability.

Despite such constraints, plants' plasticity can alter their structural, biochemical and molecular physiological networks to maintain carbon assimilation (14, 15). These adjustments include not only temporary regulatory mechanisms, e.g., changes in stomatal aperture, osmotic adjustment, non-photochemical quenching (NPQ) and antioxidant activity, but also long-term responses, such as changes in leaf anatomy, higher water use efficiency (WUE) and reprogramming of photosynthetic metabolism (16, 17). Hence, an in-depth understanding of the cross-talk among stomatal, mesophyll and chloroplast processes is crucial for unravelling mechanisms of drought acclimation and improved crop WUE (5, 16). Therefore, this review intends to combine the recent developments in understanding the interaction of the three interdependent regulatory modules during drought-induced photosynthetic adjustments. Moreover, unravelling the physiological, biochemical and molecular mechanisms that underpin photosynthetic acclimation to drought, the following sections of the review explore the potential of this knowledge for integrating crop enhancement programs aimed at producing climate-smart and resource-efficient agriculture in the future.

## Literature search

Thorough literature search was carried out by using the major scientific databases like Web of Science, Scopus, PubMed and Google Scholar to collect relevant papers on plant photosynthetic responses to drought. The search was carried out through combinations of the main keywords like "stomatal conductance" and "mesophyll conductance" and "chloroplast regulation" and "drought stress" along with the related terms such as photosynthetic acclimation, WUE, ABA signalling and hydraulic regulation. The search primarily covered publications from 1990–2025, with a special focus on the most recent advances. Only peer-reviewed articles published in English, which include original research papers and review articles were considered. Conference abstracts and other forms of non-peer-reviewed sources were excluded. The initial literature pool was subjected to a screening process to determine the level of their relevance to the integration of stomatal, mesophyll and chloroplast processes under drought conditions. About 100 major references were selected based on their high scientific standards, accuracy of methods, impact of their citations and their direct relevance to the subject matter of this review.

## Stomatal regulation under drought

Stomatal regulation is among the most sensitive physiological responses and the first line of defence, working to reduce water loss and $CO_2$ uptake during the initial stages of drought (2). Decreased leaf Ψw induces stomatal closure under drought through a combination of hydraulic and chemical signals, primarily mediated by ABA accumulation in guard cells followed by activation of secondary messengers such as $Ca^{2+}$, ROS and NO initiating ion efflux through guard cell membrane located key channel proteins i.e., slow anion channel-associated 1 (SLAC1) and $K^+$ inward-rectifying channel 1 (KAT1) resulting in turgor loss followed by pore closure (18, 19).

### Signalling mechanism of stomatal closure

Stomatal closure under drought involves a complicated and highly integrated network of hormonal (ABA), ionic ($Ca^{2+}$), redox (ROS, NO) and hydraulic signals that operate independently as well as synergistically to fine-tune guard cell behaviour to enable plants to perceive drought rapidly, translate them into bio-chemophysical signals to maintain optimal plant WUE (20, 21). The ABA synthesised in the plant root and shoot system under drought is transported via xylem to guard cells, where it binds to a family of ABA receptors, i.e., pyrabactin resistance/PYR-like/regulatory component of ABA receptor (PYR/PYL/RCAR) (22). To integrate root-to-shoot communication this binding inhibits protein phosphatase such as ABA insensitive 1 and 2 (ABI1 and 2), thereby activating sucrose non-fermenting 1 (SNF1) related protein kinase 2 (SnRK2/OST1), which phosphorylates and activates the slow anion channel-associated 1 and 3 (SLAC1 and SLAH3), resulting in membrane depolarisation, potassium efflux through gated outwardly-rectifying $K^+$ channel (GORK) and osmotic water loss that drives stomatal closure (21, 23, 24). Further, ABA triggers cytosolic $Ca^{2+}$ elevations by activating $Ca^{2+}$-permeable plasma membrane and tonoplast-located channels, which activate $Ca^{2+}$-dependent protein kinases (CDPKs) that phosphorylate SLAC1 and regulate guard cell ion fluxes (25, 26). These $Ca^{2+}$ oscillations exhibit stimulus-specific frequencies, encoding signal strength and duration in a phenomenon termed "$Ca^{2+}$ signature" (27). Another major component in stomatal signalling is the generation of ROS, particularly hydrogen peroxide ($H_2O_2$), which acts downstream of ABA via activation of plasma membrane-localised NADPH oxidases like respiratory burst oxidase homolog D and F (RBOHD and F), leading to opening of $Ca^{2+}$ channels, to support $Ca^{2+}$-dependent feedback and amplify the closure response (28, 29). Moreover, interaction between ROS and NO signalling modulates ion channel activity and synergises with ABA to induce stomatal closure (30). Futhermore, biochemical signalling, hydraulic regulation is also an early and fast response system. When drought persists, the drop in leaf Ψw first reduces guard cell and epidermal turgor, leading to mechanical shrinkage and passive stomatal closure even before ABA levels rise (18, 19). The hydropassive stomatal closure thus complements the hydroactively -mediated ABA pathway; together, they ensure the stomatal aperture is controlled in the short- and long-term. Additionally, very recent works reveal that plasma membrane intrinsic proteins (PIP2; 1, PIP2; 2), termed as AQP, are involved in guard cell hydraulic conductance, thereby associating the water permeability with the osmotic regulation under stress (31). The interaction between ABA and hydraulic feedback, shown in Fig. 1, allows continuous adjustment of $g_s$ in accordance with drought severity and time. Such complex signalling patterns not only depict plant stress physiology more deeply but are also focal points for crop breeding or biotechnological improvement to achieve better drought tolerance (13, 21).

Further, blue light-induced stomatal opening, which is mostly controlled by phototropins (PHOT1 and PHOT2), is a key mechanism in regulating stomatal aperture. This mechanism involves the activation of plasma membrane $H^+$-ATPases and results in changes to the turgor of the guard cells. In well-watered conditions, this mechanism encourages stomatal opening for the maximum uptake of $CO_2$ and photosynthesis. However, the signalling of blue light pathway is hardly changed by its interaction with ABA-dependent mechanisms during drought stress (2, 5). Through secondary messengers like $Ca^{2+}$, ROS and ABA signalling, counteract blue light-induced stomatal opening by blocking $H^+$-ATPase activity, stimulating ion efflux and generating stomatal closure (2, 3, 32). This interaction signifies a very important nexus

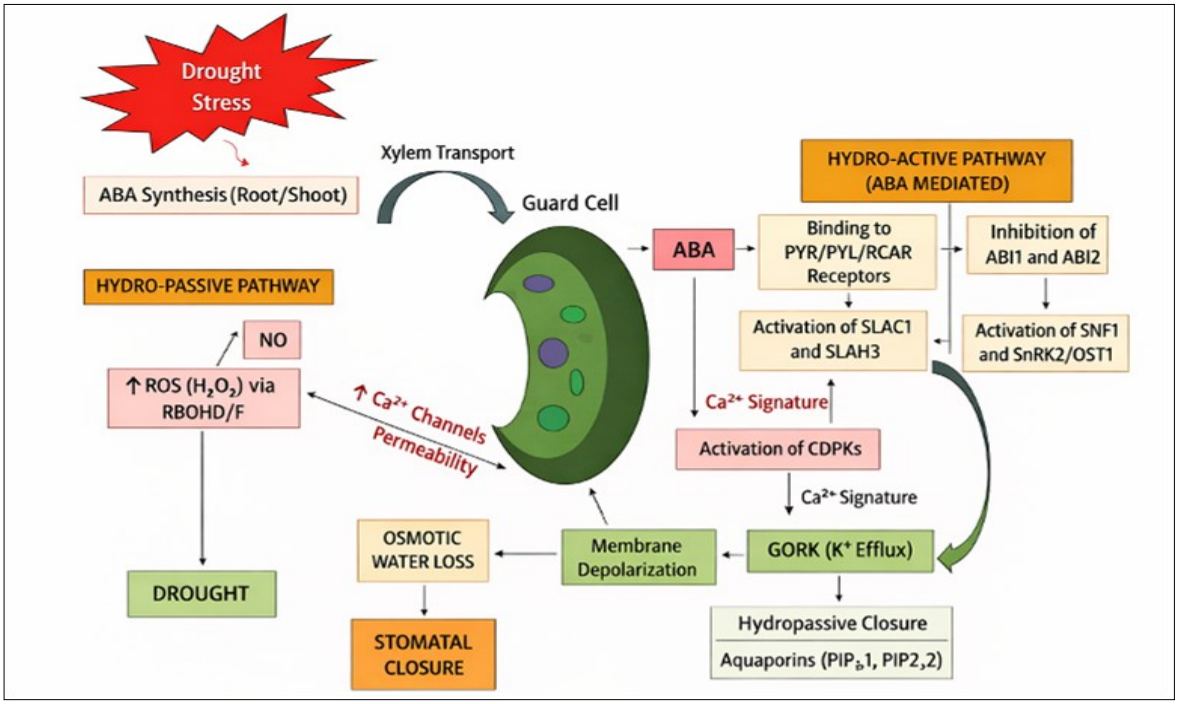



**Fig. 1.** Guard cell signalling network underlying stomatal closure during drought stress.

integrating environmental and endogenous signals which together regulate stomatal behaviour most efficiently with plant water status. Within the framework of the stomatal, mesophyll, chloroplast continuum, stomatal opening triggered by blue light actually increases $CO_2$ diffusion to the mesophyll and chloroplasts, which in turn allows photosynthetic electron transport to operate more effectively. On the other hand, when plants are exposed to drought, the pathway that is suppressed by ABA limits the supply of $CO_2$, which might result in elevated excitation pressure at the chloroplast and therefore the plant will have to activate photoprotective mechanisms such as NPQ and alternative electron sinks more strongly (2, 3).

Several studies recently suggest that the relative contribution of phototropin signalling and ABA sensitivity to a plant's response to drought could possibly explain the different behaviours of isohydric and anisohydric plants. In terms of plant breeding, by focusing on the blue light signalling pathway components, in addition to ABA signalling pathways, one can achieve a balanced stomatal behaviour and, significantly enhance WUE in plants, without drastically reducing carbon assimilation (5, 32). Therefore, the incorporation of blue light-regulated stomatal control as one of the mechanisms of drought response will greatly enhance our knowledge of how different signalling pathways are interrelated in governing the coordination of stomatal, mesophyll and chloroplast functions during water stress.

**Optimisation of water use efficiency under drought**

Stomata are crucial in determining plant WUE under drought stress. Essentially, WUE is the ratio of the amount of carbon dioxide absorbed for photosynthesis to the amount of water lost via transpiration (20, 32, 33). The balance between stomatal sensitivity to Ψw and its regulation efficiency determines how well plants can adapt to drought by sensing water availability (18, 19). In addition to stomatal control, changes at the mesophyll level, such as improved mesophyll conductance ($g_m$), increased Rubisco activity and elevated antioxidant levels, help maintain photosynthetic carbon uptake (9). On the other hand, the plant's long-term response to drought comprises structural and biochemical changes, such as decreased leaf area, thickened leaves, chloroplast protection and enhanced carboxylation efficiency to maximise carbon gain per unit of water lost (15). Plants also regulate their WUE through spatial and temporal regulation of stomatal conductance, photosynthetic capacity and leaf nitrogen (N) distribution, strategies evolved for survival in dry and semi-arid habitats (34). According to the optimal stomatal conductance model and other contemporary physiological models, plants adjust stomatal opening in real time to maximise carbon gain and minimise water loss (35). Those plants that inherently have a higher WUE usually exhibit a stomatal regulation pattern that is more sensitive and coordinated to the internal carbon demand. However, drought-resistant varieties and xerophyte species generally display isohydric behaviour, in which tight stomatal control prevents excessive water loss by maintaining Ψw within safe physiological limits, thereby avoiding xylem embolism (36). Conversely, anisohydric species tend to allow bigger variations in leaf Ψw, thus maintaining longer photosynthetic activity during drought at a greater risk of hydraulic damage. Isohydric and anisohydric responses rely on different physiological and molecular regulatory systems that can directly affect the coordination among stomatal, mesophyll and chloroplast processes during water stress. The stomata of isohydric plant species close quickly as a result of increased sensitivity to ABA that is regulated by several factors including the ABA biosynthetic genes (e.g. NCED), signalling receptors (PYR/PYL/RCAR), protein phosphatases (PP2Cs) and SnRK2 kinases (2, 5, 32). The stomatal control is so strict that it maintains the leaf Ψw but at the same time, it may cause a significant reduction in $CO_2$ diffusion which in turn limits $g_m$ and lowers the amount of $CO_2$ in chloroplasts. Therefore, chloroplasts in isohydric plants under drought are more risk of becoming overexcited and photoinhibited, which is the reason they need to exhibit strong photoprotective capabilities such as NPQ and utilising alternative electron sinks (2, 3). In contrast, anisohydric species keep stomata open for a longer time after soil water content has started to decline. This ability is supported by various hydraulic traits such as higher xylem conductivity, bigger hydraulic capacitance and continued AQP activity (5, 9). Besides, these plants have less robust ABA response and use osmotic adjustment mechanisms with compatible solutes more than isohydric plants (2, 5). Hence,

diffusion of $CO_2$ to mesophyll and chloroplast continues for a longer time, which enables photosynthesis to go on but also elevates the chance of hydraulic failure if the drought is very severe (5, 32). This is often linked at the chloroplast level with a better-balanced redox state and late activation of photoprotective mechanisms with respect to isohydric types (2, 3). It should be also noted that these two strategies are combined through shared signalling networks comprising ABA, ROS, $Ca^{2+}$ and hydraulic clues, which synchronise stomatal behaviour with mesophyll diffusion capacity and chloroplast electron transport (2, 5). In terms of breeding, these revelations pinpoint ABA sensitivity adjustment, AQP expression optimisation, mesophyll conductance enhancement and photoprotective capacity improvement as drought resilience improvement targets (5, 32). Thus, consideration of the genomic and physiological underpinning of isohydric, anisohydric behaviour gives a mechanistic basis linking water usage strategies with photosynthetic acclimation and provides workable paths for crop development.

**Long-term acclimation of stomatal density and morphology**

Acclimation of stomatal density and morphology to drought stress over a long period represents an adaptive multi-dimensional response of stomata not only to balance among different physiological processes but also influences development, anatomy and genetic aspects of the plant (20, 37–39). One of these alterations is the decrease in stomatal density accompanied by a reduction in the size of stomatal pores and guard cells. This modification is among the most widely observed adaptations that improve stomatal kinetics by enhancing the speed and sensitivity of stomatal responses, resulting in precise control of gas exchange in response to environmental fluctuations (32, 37, 40). A decrease in stomatal density, combined with guard cell shrinkage, can increase hydraulic safety margins by reducing transpiration, thereby reducing the risk of cavitation and embolism under continued drought stress. Stomatal density usually varies with leaf anatomical characteristics such as mesophyll porosity, chloroplast distribution and epidermal cell size, suggesting that drought acclimation involves coordinated remodelling of the entire leaf system rather than isolated trait adjustments (41). Acclimation and stomatal development at the molecular level are under the control of a very complicated network of transcriptional and hormonal signals like the transcription factors DNA SPEECHLESS (SPCH), master regulator of guard mother cell formation (MUTE) and the final regulator of guard cell differentiation (FAMA) synchronise the progression of guard cell lineage while EPIDERMAL PATTERNING FACTOR (EPF 1 and 2) peptides, negatively regulate stomatal initiation to modulate density in response to environmental stress (42, 43). It has been reported that drought-induced ABA accumulation suppresses SPCH activity and upregulates EPF2 signalling, thereby reducing stomatal biosynthesis during leaf development in both *Arabidopsis* and rice (44, 45).

New developments in CRISPR/Cas-based gene editing of stomatal regulators, such as EPF1, SPCH and SLAC1, reveal the potential to produce crops with a customised set of stomatal traits for climate-resilient agriculture (46). Moreover, epigenetic modifications, such as DNA methylation and histone acetylation, are currently considered mediators of stress memory, enabling plants to "remember" drought experiences and to precondition stomatal development for the next generation, thereby allowing them to tolerate drought better (47).

From an ecological and evolutionary perspective, stomatal plasticity provides plants living in heterogeneous environments with the advantage of adaptability by facilitating dynamic regulation of carbon gain and hydraulic stress (20). Both desert shrubs and alpine perennials can alter stomatal density and stomatal aperture kinetics in line with changes in root-zone water availability, indicating the evolutionary development of morphological and physiological integration. This plasticity is correlated with higher WUE and drought recovery potential traits, which are increasingly selected in crop breeding programs. Thus, unlocking the potential of this plasticity proves the prospects of crop resilience engineering against drought and other environmental stresses.

**Integration of root-to-shoot and hydraulic signalling**

Response to drought-induced stomatal closure is a highly coordinated systemic process, rather than a simple leaf-localised response. It involves integrating root-derived hydraulic and chemical signals with photosynthetic and hormonal feedback from the leaves. This integration results in a tightly regulated communication loop between the plant parts above and below ground, which pre-emptively adjusts stomatal opening before any significant drop in leaf water potential occurs, thus preventing plant hydraulic failure (48, 49). One of the earliest root-to-shoot signalling events is modulated xylem-borne signalling molecules such as ABA, pH changes, hydraulic tension and ROS, which together propagate the drought signal (soil drying). When soil water potential drops, water uptake by roots diminishes, which leads to a decrease in xylem pressure and this signal is rapidly sent to the leaves, where it is detected by guard and bundle sheath cells, thus hydroactive stomatal closure is initiated-a purely physical signal, independent of biochemical messengers as claimed by the hydraulic signal hypothesis (50, 51). A major chemical signal is root-derived ABA, generated under dry soil conditions and subsequently transported via the xylem to leaves, where it acts as a long-distance signal to trigger stomatal closure (52). This signal from the xylem-transported ABA influences ion channels in the guard cells through stimulation of cytosolic $Ca^{2+}$ oscillations and activation of $K^+$ channels of the S-type and SLAC1-type anion channels, thereby losing turgor and causing stomatal closure (13, 21). Moreover, drought-triggered alkalinisation of the xylem cavity also facilitates ABA availability in the apoplast by decreasing its conjugation to ABA glucose ester, thus increasing stomatal responsiveness (53). In addition, ABA, cytokinins, jasmonates, strigolactones and the CLAVATA3/EMBRYO SURROUNDING REGION-related (CLE) peptides transported via xylem also affect stomatal response by changing leaf hydraulic conductance and gene expression patterns (54, 55).

Recent studies highlight that AQPs facilitate water transport across cell membranes and play a dual role in long-distance signalling. On the one hand, they can modulate hydraulic conductance and on the other, they can influence signal transmission efficiency (56, 57). These proteins are like molecular valves that accept hydraulic and biochemical signals and, in response, regulate stomatal opening to the optimal level (31). The AQP in root cortical cells, xylem parenchyma and bundle sheath cells modulate their permeability flexibly in response to drought stress signals, including changes in turgor, pH and ROS generation. For example, in Arabidopsis, PIP1; 2 and 2; 1 are rapidly phosphorylated in response to mild drought, thereby enhancing root hydraulic conductivity and allowing transpiration to continue during the initial drought stage (58, 59). Conversely, under severe drought conditions, AQP gating is suppressed by dephosphorylation and oxidative

modification, limiting water supply to leaves and activating a water-saving mode (60). The harmonious downregulation of AQPs in the root and upregulation in leaf bundle sheath and guard cells imply that there is differential hydraulic regulation across plant parts during progressive drought, ensuring that water flow is primarily sustained to the photosynthetically active tissues (61, 62). A change in AQP activity gives a plant a flexible hydraulic architecture, allowing it to recover stomatal conductance and photosynthesis under altered water status rapidly. Integration of root-to-shoot signalling and AQP-mediated hydraulic adjustments accounts for the "hydraulic memory" phenomenon - whereby plants that have gone through previous drought conditions close their stomata faster upon re-experiencing the stress (63). This memory is associated not only with ABA biosynthetic genes but also with AQP transcriptional reprogramming and xylem anatomical modifications that render the plant more responsive to drought.

## Mesophyll conductance and internal $CO_2$ diffusion constraints

Carbondioxide diffusion from intercellular spaces to the chloroplast stroma, referred to as $g_m$, is a process that largely influences photosynthetic efficiency under drought conditions (64–66). In contrast to $g_m$, which is concerned with the regulation of $CO_2$ uptake through the stomata, $g_m$ is responsible for the intracellular diffusion pathway through various compartments such as cell walls, plasma membranes, cytosol and chloroplast envelopes (64). As a consequence of drought stress, $g_m$ generally declines significantly, which is explained by factors such as cell shrinkage, reduced exposure of chloroplast surfaces to intercellular airspaces, thickening of cell walls and reduced membrane and AQP permeabilities (65, 66). Through such structural and biochemical changes, resistance to $CO_2$ transfer increases, thereby limiting photosynthetic carbon fixation even when stomata are not completely closed. The reduction of $g_m$ is not merely an involuntary reaction; there are communications between the chloroplast metabolism and hydraulic signals that are involved. This controlled adjustment of $g_m$ underscores its critical role in photosynthetic acclimation, as it affectively regulates stomatal behaviour, chloroplast function and leaf hydraulics under conditions of water scarcity.

### Drought-induced leaf internal structural changes as factors affecting mesophyll conductance ($g_m$)

As shown in Fig. 2 and 3 drought triggers a multitude of changes at the anatomical and ultrastructural levels of mesophyll cells, generally aimed at reducing water loss and sustaining cellular integrity even when $CO_2$ diffusion and consequently, its fixation are compromised (64, 67). Altered composition of mesophyll cell walls followed by their thickening due to the deposition of lignin, cellulose and suberin polymers is among the most spectacular reactions of plants to drought, which, along with decreasing water permeability, increases their mechanical strength (68, 69). Although these adjustments help resist turgor loss and desiccation, they also increase the resistance of the $CO_2$ diffusion pathway, leading to a reduction in $g_m$ to attain high water retention (70). Another important feature of drought-associated structural changes includes variations in the volume of intercellular air spaces (IAS), which serve as the medium for gaseous diffusion within the leaf. As water is lost, mesophyll cells shrink, leading to a drastic decrease in the fraction of IAS and leaf porosity. The path for $CO_2$ diffusion becomes longer, thus $g_m$ gets reduced even in leaves where stomatal apertures are still partially open (70, 71). The disappearance or shrinkage of IAS not only interferes with gas exchange but also alters light scattering and the internal distribution of $CO_2$, leading to unequal chloroplast $CO_2$ availability (72). Some species, especially drought-resistant shrubs and conifers, have such structural plasticity that enables them to partially retain the IAS volume even under dehydration by strengthening the middle lamella or producing specialised intercellular lacunae, which maintain the airspace geometry (70, 73).

In addition, drought strongly affects the organisation of chloroplasts and their positioning within mesophyll cells, which is the most important structural factor determining internal $CO_2$ diffusion (69). Under normal conditions, chloroplasts position themselves along the cell wall facing the IAS, thereby increasing the surface area exposed for $CO_2$ diffusion into the sites of carboxylation

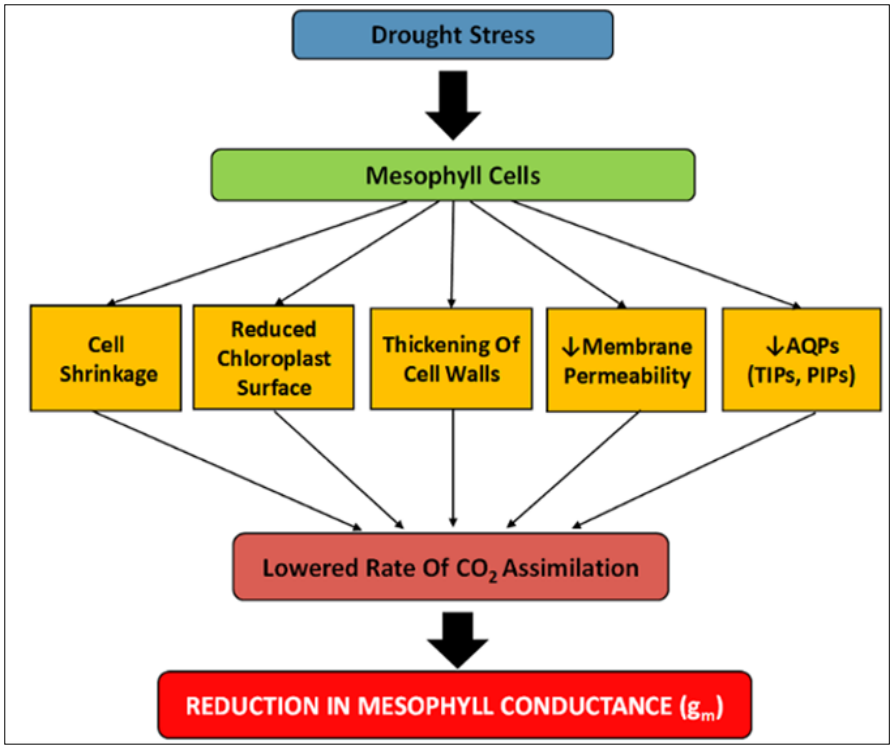



**Fig. 2.** Drought induced reduction in mesophyll conductance.

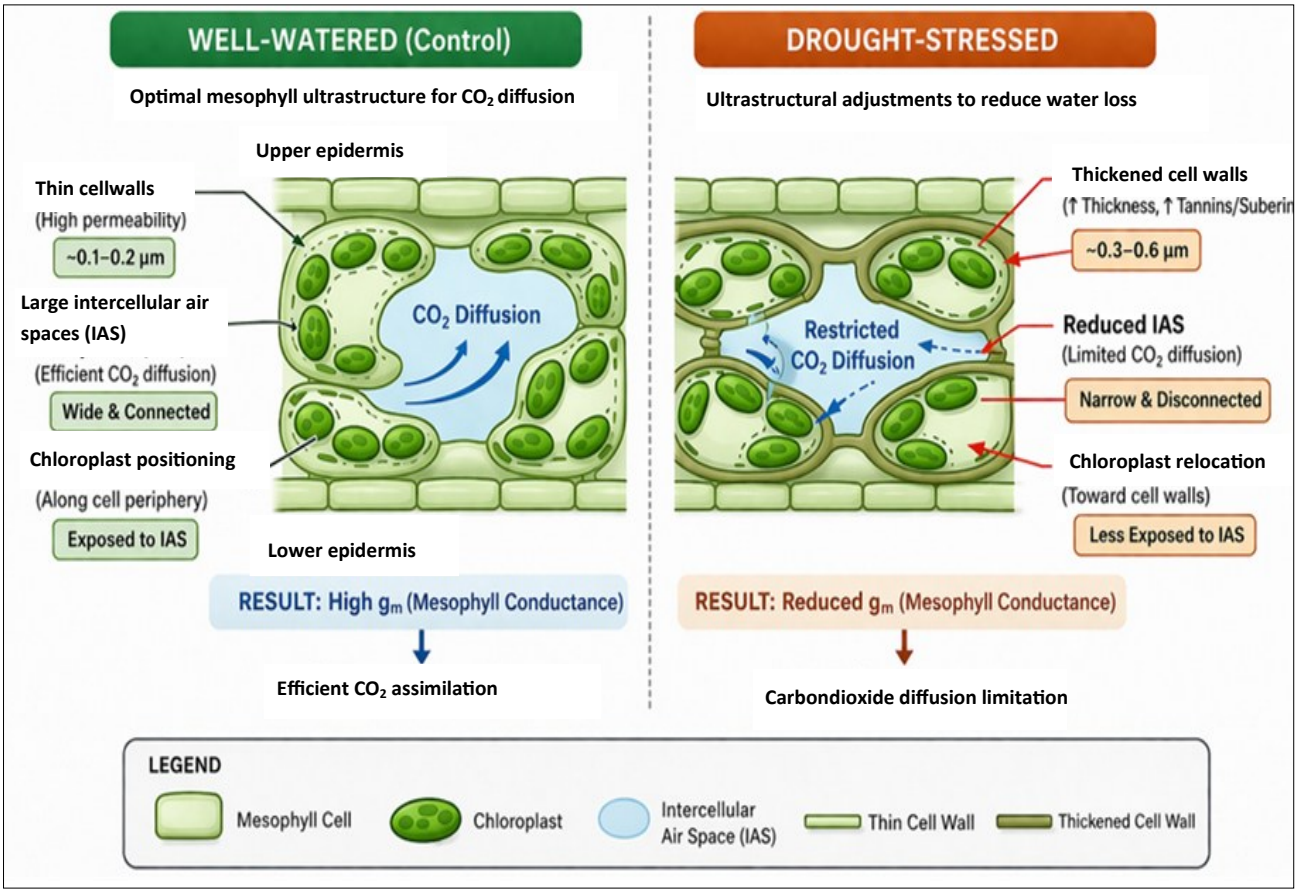



**Fig. 3.** Structural modifications of mesophyll tissue under well-watered and drought stressed conditions.

(68). On the contrary, during drought, loss of turgor and cytoplasmic shrinkage result in the relocation of chloroplasts to the cytoplasmic interior, thereby increasing the diffusion path in the cytoplasm and decreasing $g_m$ and consequently, photosynthesis (64, 70). Studies show that, under such conditions, chloroplast thylakoid membranes can undergo structural changes, aggregate, or be partially deformed, affecting the photochemical and biochemical phases of photosynthesis (70). In summary, cell wall thickening and compositional changes, decline in intercellular airspace and chloroplast retraction are among the drought-induced modifications that constitute a coordinated, balanced structural strategy in plants, allowing them to conserve water while still carrying out some photosynthetic activity. These alterations are not just passive effects of water shortage, but they also represent anatomical and physiological changes that accompany the leaves in developing the capacity to supply the chloroplasts with minimal $CO_2$ and preserve the leaf mechanical and hydraulic functionality at the same time.

**Role of aquaporins and metabolic regulation in maintaining carbondioxide diffusion**

Typically, drought leads to a reduction in $g_m$ to limit the entry of $CO_2$ into the leaf. However, a significant number of plants still maintain photosynthetic activity through $g_m$ optimisation, achieved through AQPs (10, 73). The AQPs, particularly plasma membrane intrinsic protein (PIPs) and tonoplast intrinsic proteins (TIPs), create selective channels that allow bidirectional movement of water and $CO_2$ across biological membranes; thus, they connect the plant's hydraulic and photosynthetic systems (74). In mild drought, some PIP isoforms, such as *Nicotiana tabacum* aquaporin 1 (NtAQP1), PIP1;2 and PIP2;1, can remain active or be upregulated to maintain $CO_2$ permeability and internal diffusion, thereby preventing a significant drop in $P_n$ (75, 76). On the other hand, under very severe drought conditions, ABA signalling via phosphorylation and cytosolic acidification leads to the deactivation of AQP, reducing membrane permeability to $CO_2$ to prevent water loss while maintaining a minimum metabolic flux (61). This integration at the hydraulic and metabolic level, mainly controlled by hormonal and redox signalling pathways, is a function of the ABA action that acts as the master regulator, coordinating AQP gene expression and post-translational gating through reversible phosphorylation, which is frequently brought about by sucrose non-fermenting-1-related protein kinase 2 (SnRK2) and open stomata 1 kinase (OST1) (31). Furthermore, the $Ca^{2+}$ concentration increase induced by ABA, along with ROS, modulates trafficking and opening of AQPs and thus, water and $CO_2$ fluxes are dynamically regulated as drought intensity fluctuates. Similarly, redox signalling pathways provide a fundamental basis for regulating AQP activity and mesophyll-chloroplast metabolism, ensuring that $CO_2$ diffusion towards Rubisco remains efficient. Reactive oxygen species, mainly $H_2O_2$, which is formed in the apoplast and chloroplasts by NADPH oxidases, is the regulatory signal that fine-tunes the control of AQP through oxidation of key cysteine residues as well as a ROS-dependent phosphorylation cascade mediated by ABA-OST1/SnRK2 pathways. These redox-mediated changes in water and $CO_2$ permeabilities of AQP provide plants with the ability to fine-tune hydraulic conductivity to $g_m$ on a single-cell basis (77). Simultaneously, redox signals influence the metabolic operations that influence $CO_2$ diffusion, including the activity of carbonic anhydrases (CAs), isoforms such as βCA1 and βCA4 (chloroplast-localised) and βCA2 and βCA3 (cytosolic forms) which establish the steep $CO_2/HCO_3^-$ gradients between the cytosol and stroma (78) as well as chloroplast electron transport, which functions to stabilise NADPH, ATP and stromal redox potential for Rubisco activation and RuBP regeneration (79). When it comes to drought or changing light, coordinated redox reactions, together with $Ca^{2+}$

transients and ABA signalling, work in concert to stabilise $CO_2$ supply while preventing over-reduction and oxidative stress.

**Coordination between $g_m$ and $g_s$ in optimising photosynthetic performance**

The coordination of stomatal conductance ($g_s$) and mesophyll conductance ($g_m$) under drought stress conditions is a key regulatory mechanism that controls how the plant balances carbon assimilation with water conservation (80, 81). The two types of conductances work one after another to determine the diffusion route of $CO_2$ starting from the atmosphere through the stomatal pores into the intercellular airspaces and finally to the chloroplast stroma, where carbon fixation occurs (66). When plants are watered adequately, a very close coordination between $g_s$ and $g_m$ is responsible for maintaining an optimal internal concentration of $CO_2$ ($C_i$) along with a steady $C_i/C_a$ ratio that supports high enzymatic efficiency for carboxylation and also keeps photosynthetic rate significantly above the $CO_2$ compensation point (Γ), which is the level of $CO_2$ at which photosynthesis and photorespiration are balanced (64, 65).

Drought stress, however, interrupts this coordination and the more severe it is the greater the effect. For example, in case of a moderate drought the changes in $g_s$ are usually larger than those in $g_m$ on proportional basis, which results in a decrease of $C_i/C_a$ ratio and increase of diffusional limitation. At this point, mesophyll conductance helps to offset lowered stomatal influx to some extent, chloroplast $CO_2$ concentration ($C_c$) and photosynthetic rates remain at least partially unchanged. As a result, carboxylation efficiency is dropped only a little and the plants can carry out photosynthesis still above Γ. On the other hand, during a severe or long-lasting drought $g_m$ is likely to be decreased not only in terms of function but also structurally more than $g_s$, for instance, due to changes in the mesophyll cell wall and less chloroplast surface area being in contact with the intercellular airspaces, down-regulation of AQPs and carbonic anhydrases (CAs) occur which results in an impeded $CO_2$ diffusion in the mesophyll tissue (67, 73). This change results in an even greater decrease in $C_i$ compared to $C_i$ which, in addition to $g_s$ and $g_m$ becoming uncoupled, carboxylation efficiency is reduced and photorespiration increases so that the apparent $CO_2$ compensation point is elevated. Besides, this coordination between $g_s$ and $g_m$ can be even more fine-tuned by highly intricate signalling networks comprising ABA, ROS and hydraulic cues (81). Once soil water potential (Ψw) decreases due to drought, the roots and the vascular tissues biosynthesise ABA, while the hormone is then transported via the xylem to the leaves. In guard cells, ABA recognition is done through PYR/PYL/RCAR receptors, which then induce PP2C inhibition and SnRK2 activation. Subsequent ion fluxes ($Ca^{2+}$ $K^+$, $Cl^-$), ROS production and the activation of anion channels such as SLAC1 lead finally to stomatal closure and lowering of transpiration. Meanwhile, in mesophyll cells, ABA and ROS modulate AQP activity, cytosolic $Ca^{2+}$ fluctuations and chloroplast redox balance, causing a decrease in $g_m$ and an adjustment in photosynthetic electron transport in order to prevent oxidative damage under $CO_2$-limited conditions.

Meanwhile, other than through ABA, leaf wilt and xylem tension increase can rapidly trigger hydraulic signals that can travel along vascular tissues and affect the stomatal and mesophyll of the leaf. Apoplastic and xylem-originated ROS play dual roles here as they enhance ABA signalling and at the same time, being secondary messengers, they help coordinate vascular, epidermal and mesophyll responses. Feedback from redox status of the mesophyll back to the vascular tissue results in the regulation of the transport and distribution of ABA. As a whole, this combined ABA, ROS, hydraulic signalling system synchronises stomatal and mesophyll conductance in a perfectly harmonised manner that maintains the carboxylation efficiency to a certain extent, thus, provides WUE and photosynthetic capability climax under drought stress.

**Differential regulation of the stomatal-mesophyll-chloroplast continuum in $C_3$ and $C_4$ plants under drought**

Conducting a comparative study of $C_3$ and $C_4$ plants offers a good opportunity to understand how the stomatal, mesophyll, chloroplast network is regulated in response to drought stress. In $C_3$ plants, a decrease in $g_s$ is first responsible for reducing $CO_2$ levels. If this is combined with a reduction in the $g_s$, the concentration of chloroplastic $CO_2$ is lowered even more, which results in an increase in photorespiration (3, 64). This close interaction between the closing of stomata and the diffusion of mesophyll limits results in the chloroplast electron transport being restricted, which causes more excitation pressure. As a result, photoprotective processes like NPQ and alternative electron sinks are required to release surplus energy and keep photosynthesis working properly (2, 3). On the other hand, $C_4$ plants have a different pattern of the association between those regulatory elements due to their $CO_2$-concentrating mechanism. Since $CO_2$ is initially fixed by phosphoenolpyruvate carboxylase in mesophyll cells and then $CO_2$ is released from the $C_4$ acids in the bundle sheath chloroplasts, Rubisco has a high concentration of $CO_2$ around it, which not only keeps the rate of photorespiration at a low level but also makes the chloroplast metabolism less dependent on stomatal limitations (2, 3). Consequently, under low $g_s$, $C_4$ plants are capable of maintaining the photosynthetic electron transport and carbon assimilation and, on top of that, they can be a source of higher plant intrinsic WUE (5, 9).

The significance of $g_m$ is substantially different between $C_3$ and $C_4$ plants. In $C_3$ plants, $CO_2$ diffusion through mesophyll represents a major limiting step, especially during drought, where both $g_s$ and $g_m$ are restricted, thus limiting $CO_2$ supply to chloroplasts. On the other hand, $C_4$ metabolism changes the way diffusional constraints operate since these plants depend on an exchange of metabolites between mesophyll and bundle sheath cells, thereby making their direct reliance on intercellular $CO_2$ diffusion minimal (5, 9). Another interesting aspect is that physiological responses of chloroplasts are divided between mesophyll and bundle sheath cells in $C_4$ plants. This leads to a differential control of electron transport and photoprotective mechanisms than in $C_3$ species. More importantly, although there are huge differences, both types of plants, $C_3$ and $C_4$, activate their stomatal behaviour through a harmonious interplay of signalling pathways that include ABA, ROS, $Ca^{2+}$ and hydraulic signals in conjunction with mesophyll and chloroplast functions during drought (2, 5). Without a doubt, possessing a carbon-concentrating mechanism enables $C_4$ plants to change the main limitation target under extreme drought conditions from $CO_2$ diffusion to biochemical and metabolic activities. Therefore, a side-by-side examination of both $C_3$ and $C_4$ systems shows how major evolutionary changes in carbon assimilation pathways have led to the complete transformation in the interplay among stomatal, mesophyll and chloroplast functions. At the same time, such analysis uncovers the potential to develop crops that can withstand

drought and utilise water more effectively.

## Chloroplast structural and functional adjustments under drought

Chloroplasts are specialised structures within cells where photosynthesis, as well as redox metabolism, takes place, depending on the morphological integrity and operational adaptability of the organelles (82, 83). In addition to changes in energy balance, chloroplast shape, thylakoid organisation, electron transport pathways and photoprotective responses, plants under drought conditions also suffer from osmotic and oxidative stress (3). These changes, on the one hand, limit the photooxidative damage and, on the other, enable the plant to use the available carbon efficiently (84). The following paragraphs provide a detailed description of the structural and functional adaptations in plants under water stress.

### Changes in chloroplast size, shape and positioning

Drought stress causes changes to the size, shape and arrangement of chloroplasts in the mesophyll cells (3, 5). These changes as shown in Fig. 3 highlight the chloroplast's central role in maintaining the photochemical system's stability when cells lose water. Under drought, the resulting loss of cell turgor leads to compression of the intracellular spaces, followed by a reduction in chloroplast size and an elongated, flattened, or irregular shape to suit the smaller cell volume (5, 85). In addition, changes in osmotic potential of the chloroplast stroma, membrane tension and cytoskeletal anchoring collectively contribute to modifications in chloroplast geometry (86). These structural changes serve to extend membrane stability while minimising ROS production (87). It has further been reported that chloroplasts also change their position within the mesophyll cell (88). As reported, in cells with optimal water conditions, chloroplasts position themselves along the periclinal cell walls to absorb maximum light. But under drought accompanied by high-light exposure, chloroplasts actively relocate to the anticlinal walls in a process called chloroplast avoidance movement (89). By doing this, the cell's cross-sectional area exposed to incoming light is reduced, preventing overexcitation of PS II and limiting photooxidative damage.

Further blue light, mainly through phototropins (PHOT1/PHOT2), plays a crucial role in plant stomatal regulation by stomatal, mesophyll, chloroplast regulatory continuum. It first triggers phototropins inside the guard cells. This, in turn, sets off a series of events involving BLUS1 kinase, PP1 and finally the activation of plasma membrane $H^+$-ATPases. The result of these changes is membrane hyperpolarisation, $K^+$ uptake and stomatal opening. Since this light-driven path depends on photosynthetic demand, it effectively links stomatal conductance with chloroplast activity (27, 40). Nevertheless, during water deficit stress, this stomatal opening brought about by blue light is largely regulated by ABA signalling. In particular, ABA build-up triggers SnRK2 kinases like OST1, enhances ROS and $Ca^{2+}$ signalling and initiates anion channels such as SLAC1 ultimately leading to stomatal closure (21, 23, 34, 35). It is also noteworthy that studies show a reciprocal functional relationship between phototropin-mediated opening and ABA-induced closure pathways, where ABA is able to suppress blue light responses by preventing $H^+$-ATPase activation and the related ion transport activities (21, 22). From an integrative point of view, this interaction is crucial for maximising WUE during drought. For example, blue light induces stomatal opening which results in enhanced $CO_2$ intake and photosynthetic assimilation of carbon, while ABA restricts water loss through the imposition of hydraulic limitations. The equilibrium of these antagonistic signals will determine stomatal kinetics and will have an immediate effect on $g_m$ and chloroplast $CO_2$ supply (38, 39, 42). Besides that, signals produced by chloroplasts such as ROS and retrograde signalling molecules are also integrated in the regulation of guard cells, affecting the phototropin sensitivity and the ABA responsiveness (90–92). Therefore, blue light signalling is an important element of the intertwined network that links light perception, hydraulic signalling and photosynthetic acclimation under drought stress.

### Alterations in thylakoid membrane organisation

Thylakoid membranes are severely affected under drought on the molecular as well as architectural level owing to dehydration-driven loss of membrane plasticity, ROS-mediated lipid oxidation and destabilisation of pigment protein supercomplexes (29, 91). It has been reported that drought decreases stromal hydration and ionic balance, weakening the electrostatic forces and Van der Waals interactions that maintain grana stacking (92, 93), leading to partial or complete destacking of grana, loosening of membrane appressions and dilation of stromal lamellae (94, 95). These changes are tightly linked to drought-induced decrease in galactolipids, mainly monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG), essential not only for maintaining the membrane curvature and bilayer stability but also for ensuring proper assembly of PS II and LHCII complexes as well (96). In addition to excessive ROS production, drought leads to lipid peroxidation of thylakoid polyunsaturated fatty acids, thereby reducing membrane fluidity, inducing thylakoid fragmentation and promoting vesiculation (21). From a proteomic perspective, drought breaks the PS II-LHCII supercomplexes, leading to PSI instability, a decrease in the levels of cytochrome $b_6f$ complexes, thus inhibiting linear electron flow, plastoquinone redox cycling and production of ATP and NADPH caused by $D_1$ protein degradation through chloroplast-localised FtsH and Deg proteases (26, 31, 42). Reports suggest that impaired grana stacking disrupts the energy balance between PSI and PS II, shifting more of it to PSI, to drastically reduce the energy transfer to PS II, therefore enhancing the NPQ pathways to protect the photosynthetic apparatus (20, 50). These photoprotective modifications, including PS II subunit S (PsbS) energy-dependent quenching (qE) and intensified xanthophyll cycle activity, dissipate excess excitation energy as heat to prevent photodamage, even under reduced $CO_2$ availability (81). In addition, drought stress affects the thylakoid-associated antioxidant machinery, comprising the ascorbate-glutathione cycle and thylakoid-bound peroxidases. Still, their capacity is often limited even in the early stages of drought, leading to photoinhibition, grana swelling and chloroplast senescence (76). Finally, the interplay of galactolipid remodelling, oxidative membrane damage, decreased protein complex stability and enhanced NPQ transforms the chloroplast from a highly efficient photosynthetic state into a structurally protected state. Still, a metabolically downregulated state is the main mechanism by which drought acts as a photosynthetic performance constraint and reduces plant productivity (89, 92).

### Engagement of alternative electron sinks

Owing to reduced concentration of $CO_2$ inside the leaf, the Calvin-Benson cycle slows down drastically under drought conditions (3, 5), leading the electron transport chain in the chloroplast to become over-reduced. As a result, the photosynthetic apparatus

has to recruit several alternative electron sinks to maintain redox balance and minimise ROS production (79) (Fig. 4 and 5). The most significant electron sink among the alternative ones is photorespiration, which is by far the most dominant pathway and is greatly increased under low $CO_2$ conditions (7, 15). Although photorespiration is an energy-consuming pathway, requiring extra ATP and reducing power to reassimilate photorespiratory intermediates and leading to a net loss of previously fixed carbon, it is indispensable for photoprotection. By consuming surplus ATP and NADPH, photorespiration stops over-reduction of the photosynthetic electron transport chain and ROS formation, thus keeping the redox state of chloroplasts balanced (90, 94). This essential and protective role of photorespiration is also very well aligned with the malate, aspartate shuttle that makes it possible for the chloroplasts, mitochondria and cytosol to exchange reducing equivalents. In fact, during drought-induced stomatal closure, when $CO_2$ is less and NADPH accumulates, an excess of reducing power can be transported from the chloroplast in the

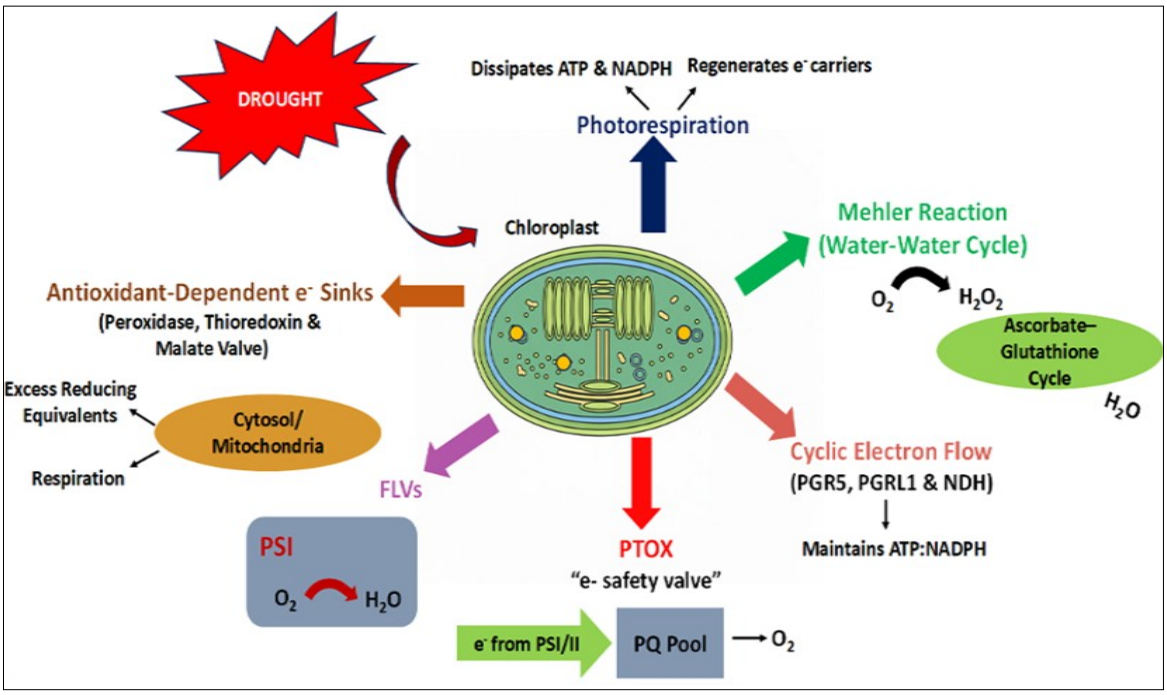



**Fig. 4.** Photoprotective alternative sinks in drought stressed chloroplast.

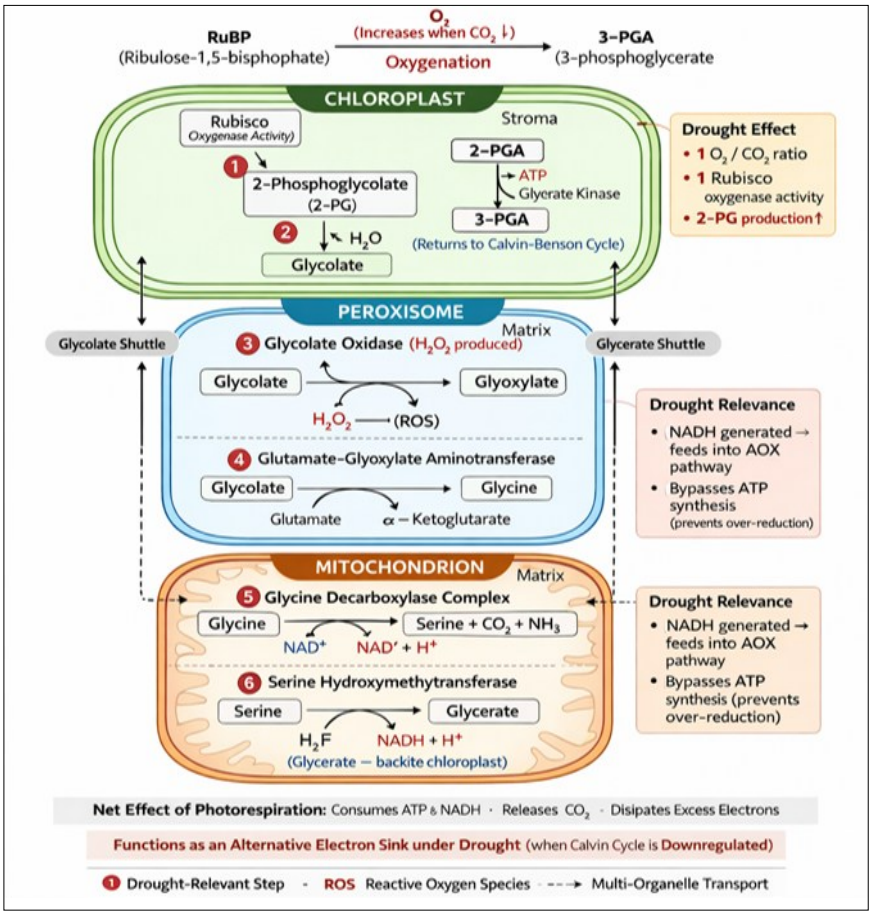



**Fig. 5.** Photorespiration ($C_2$ cycle) as an alternative electron sink under drought.

form of malate via the malate valve. This action aids mitochondrial respiration and photorespiratory metabolism, where NADH is oxidised to regenerate $NADP^+$, which is necessary for sustained electron flow in the chloroplast. At the same time, the interconversion of malate and oxaloacetate, together with aspartate transamination reactions, makes it possible to efficiently redistribute carbon skeletons and redox equivalents among different cellular compartments (78, 79). In fact, photorespiration and the malate, aspartate shuttle work together very closely to mitigate cellular redox imbalances and thereby maintain electron transport activity and metabolic flexibility during drought stress. Although they come at a cost to metabolism, this desynchronised system is a lifeline for the plant in protecting the photosynthetic apparatus and surviving under conditions of reduced $CO_2$ and oxidative stress (87, 90).

Furthermore, photorespiration, the Mehler reaction, also referred to as the water-water cycle, is the main source of rapid electron outlet, where electrons from PSI reduce oxygen to superoxide ($O_2^-$) ions, followed by its conversion to $H_2O_2$, which is later removed by the ascorbate-glutathione cycle (18, 23). In addition to scavenging the excess electrons, the Mehler reaction also strengthens the change in thylakoid membrane pH to support ATP synthesis and enhance NPQ under drought (46). In addition, cyclic electron flow around PSI is one of the most important photoprotection mechanisms that is turned on under drought, especially the pathways including proton gradient regulation 5 (PGR5), PGR5-Like 1 (PGRL1) and chloroplast NADH dehydrogenase-like complex (NDH) pathways that locally produce ATP without NADPH formation, to maintain the ATP/NADPH ratio needed for metabolic homeostasis, lumen acidification for qE formation and protect PSI from over-reduction and irreversible photodamage (45). Over-reduction of PSI under drought is further prevented by elevated plastoquinone terminal oxidase (PTOX) levels, which serve as an "electron safety valve" in chloroplasts by removing electrons from the plastoquinone pool and delivering them to oxygen (25).

In the same way, flavodiiron proteins (FLVs), which are almost absent in angiosperms, act as highly effective alternative sinks in basal land plants and a few crop species by directly reducing oxygen to water at PS I, thereby decreasing ROS production and stabilising PS I redox state during drought. In addition to these processes, chloroplasts are more prone to the engagement of antioxidant-dependent electron sinks, which, among others, include peroxidase-catalysed pseudocyclic electron transport, thioredoxin-mediated redox buffering and the malate valve that removes the excess of reducing equivalents to the cytosol and mitochondria, where they can be dissipated by respiratory metabolism (71). These alternative electron sinks thus constitute a multilayered and tightly linked photoprotective network that not only protects PS I and PS II from damage but also limits ROS production, stabilises the thylakoid proton motive force, facilitates NPQ, maintains energy balance and prevents photoinhibition.

#### Induction of non-photochemical quenching

Non-photochemical quenching rapidly activated under drought conditions is amongst the most important photoprotective mechanisms within the chloroplast to avoid the overexcitation of PS II and the production of $O_2^-$ generated by the reduction of $O_2$ by the overexcited electron transport chain (25, 46). Its induction represents a dynamic and highly regulated acclimation response that safeguards photosynthetic integrity under drought-induced stomatal closure, fluctuating light and other environmental stressors. Thus, it is a major factor in plant drought tolerance and photosynthetic resilience and it can act as a mediator of drought-induced photoprotection (75, 89). The rapid and most dominant component of NPQ, i.e., qE (energy-dependent quenching), is activated when drought or high-light conditions reduce $CO_2$ availability, leading to proton accumulation and acidification of the thylakoid lumen (84, 97, 98), causing conformational changes in the pH-sensing PS II subunit S (PsbS) protein and stimulating violaxanthin de-epoxidase activity, driving the conversion of violaxanthin to zeaxanthin within the xanthophyll cycle (18, 29). Zeaxanthin, together with protonated PsbS and reorganised LHCII complexes, facilitates efficient thermal dissipation of excess excitation energy. Additionally, slower NPQ components, i.e., zeaxanthin-dependent NPQ component (qZ), state-transition quenching (qT) and photoinhibitory quenching (qI), contribute to sustained photoprotection through zeaxanthin stabilisation, state transitions and repair-linked photoinhibition. The different components of NPQ thus constitute a single unit of a thermal dissipation system that can be activated deeply or shallowly depending on the duration and severity of the drought.

### Signaling networks linking the three regulatory levels

At the whole-plant level, drought initiates an extensive signalling process involving an interconnected systemic network of regulatory functions in stomata, mesophyll cells and chloroplasts that governs the plant's photosynthetic acclimation as indicated in Fig. 6 and 7 (87, 99). As a result of a fall in soil $\Psi_w$, communication in the form of ABA is initiated in the plant roots and transported to leaves via xylem (45, 78), followed by ion flux changes through PYR/PYL-PP2C-SnRK2 signalling modules, resulting in stomatal closure and limiting the $CO_2$ uptake (67, 71). Mesophyll cells detect the reduced $CO_2$ concentration in the intercellular spaces to regulate their conductance ($g_m$) through the interaction of AQPs, cell wall modifications and chloroplast relocation through actin cytoskeleton dynamics (64, 71, 78). These changes are further supported by signals originating from chloroplasts, thus constituting a feedback network that adjusts $CO_2$ diffusion. An imbalance in electron transport within chloroplasts due to drought releases redox signals, most notably through changes in the plastoquinone (PQ) pool, the $NADPH/NADP^+$ ratio, thioredoxin activity and ROS (100). These retrograde signals convey the energetic status of the chloroplast to the nucleus and cytosol, thereby regulating the expression of stress-responsive genes, antioxidant enzymes and metabolic pathways (67, 78). In addition, $H_2O_2$ is capable of moving through membrane-bound AQP to act as a mobile chemical signal that harmonizes mesophyll and stomatal responses by altering guard cell sensitivity to ABA and $Ca^{2+}$ oscillations (89, 91). Calcium waves initiated by chloroplast ROS and plasma membrane channels spread over mesophyll cells and affect ion channel activity in guard cells as well as chloroplast gene regulation, resulting in a synchronised calcium-dependent signalling network (86, 89).

On the other hand, drought stress further increases the production of chloroplast-localised metabolites, such as β-cyclocitral, methylerythritol cyclodiphosphate (MEcPP) and 3′-phosphoadenosine-5′-phosphate (PAP) (56, 57). Beta-cyclocitral, which is produced by the oxidative cleavage of beta-carotene under high levels of singlet oxygen, serves as a signalling molecule under

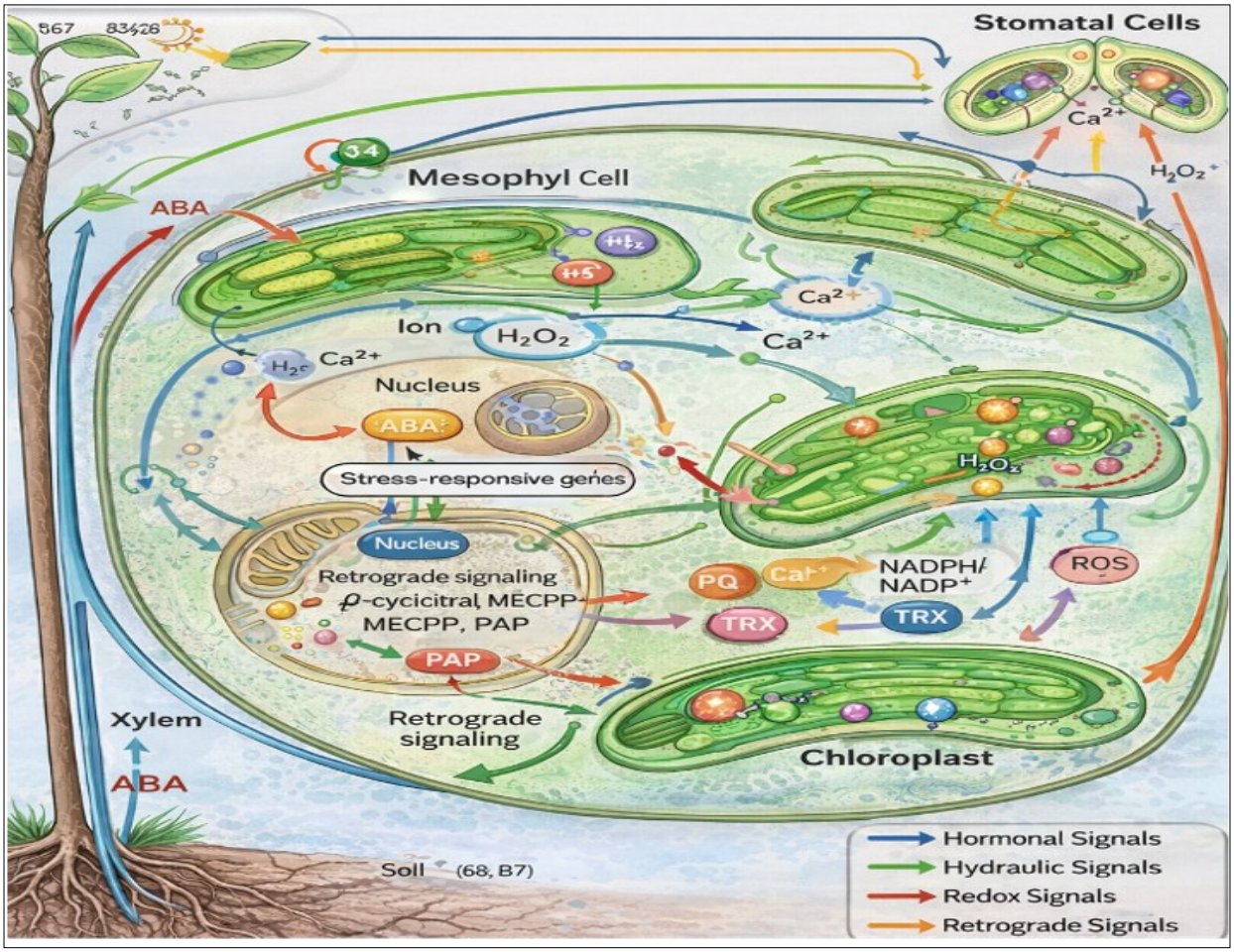



**Fig. 6.** Whole plant signalling network under drought stress.

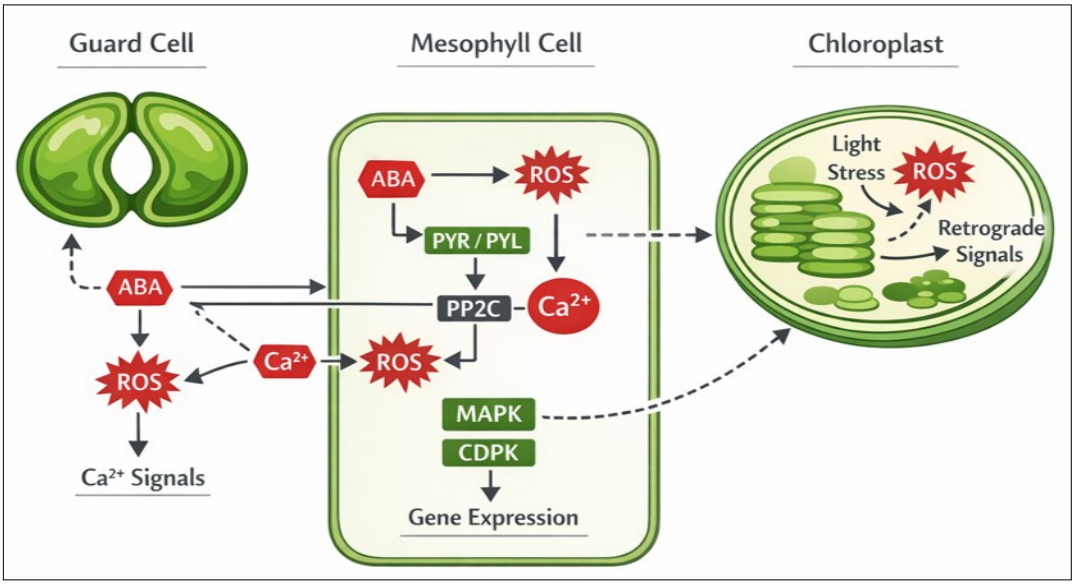



**Fig. 7.** Integrated signalling network linking guard cells and chloroplast under drought.

drought stress to initiate the expression of nuclear genes that are a part of the antioxidant defence, detoxification pathways and PS II repair system, to enhance the plant resistance to photooxidative stress (101, 102). On the other hand, MEcPP, a late product of the methylerythritol phosphate (MEP) pathway, accumulates in plants under drought and high light conditions that regulates the nuclear transcription through changing the expression of stress-responsive genes, including those connected to salicylic acid signalling, redox homeostasis, plastid metabolic status for stress acclimation pathways (103, 104). The PAP, a nucleotide metabolite coming from chloroplasts, works by inhibiting nuclear exoribonucleases (XRNs) and results in the stabilisation of transcripts responding to stress and the induction of ABA-dependent gene expression, especially those involved in stomatal closure and drought-responsive signalling (104, 105). This retrograde signalling is strongly linked to changes in the redox state of the photosynthetic electron transport chain and is generally initiated under the conditions of over-reduction. Together, these signalling pathways coordinate the changes at the whole plant level by regulating genes related to stomata development and their opening/closure, release of AQPs that control $g_m$, antioxidant systems and rearrangements of light-harvesting complexes. In this way, β-cyclocitral, MEcPP and PAP through their co-action connect

the perception of chloroplast stress to the cell-wide response, enabling the initiation of photoprotective processes such as NPQ, alternative paths of electron flow, as well as the photosystem components being downregulated in a controlled manner to avoid photodamage (104, 106). Therefore, these metabolites, in addition to be stress markers, are key regulators which coordinate the stomatal activity, mesophyll metabolism and chloroplast functions during drought stress.

Latest research indicates that drought responses at the whole-plant level are achieved through systemic acquired acclimation (SAA), which refers to the process where stress signals initially produced in one part of the plant are quickly communicated to other parts, allowing those parts to change their physiology before they get stressed. In fact, SAA is a complex integration of hydraulic, electrical and chemical elements, with ABA, ROS and $Ca_2^+$ waves among the chemical signals. This results in a very well-coordinated network linking stomatal control to mesophyll and chloroplast function (107–109). Besides, it has been observed from recent studies that SAA is more than just signal transmission; it also includes gene expression changes and metabolic reprogramming in different tissues, which together strengthen the plant's drought resistance on a whole-plant level (110). In this regard, mobile RNA species, among them small RNAs (miRNAs, siRNAs), mRNAs, long non-coding RNAs (lncRNAs) and RNA fragments, have been recognised to play a key role in long-distance drought signalling. Through plasmodesmata and the vascular system, these molecules can coordinate gene expression in different organs simultaneously, thus linking local drought stress perception to systemic physiological responses (111–113). Their function as mobile signals allows plants to synchronise the processes of stomatal closure, osmotic adjustment, antioxidant defences and photosynthetic regulation in tissues exposed to water deficit.

Moreover, the studies illustrate that non-coding RNAs responding to drought can regulate important signalling pathways of hormones, redox homeostasis and carbon metabolism. In this way, they can have a direct impact on $g_s$, $g_m$ and chloroplast electron transport (114, 115). Also, experiments indicate that mobile mRNAs assist in systemic metabolic reprogramming that comprises photosynthetic regulation and stress-responsive pathways at remote locations. This sheds light on their significance in the coordination of the stomatal, mesophyll, chloroplast continuum during drought (113). Within the concept of photosynthetic acclimation, SAA and mobile RNA signalling are two of the key components that make sure that the two types of responses, distant and local, are synchronised both in time and space. For example, systemic signals are able to prepare the stomatal sensitivity to ABA, influence the mesophyll diffusion capacity and change chloroplast photoprotective mechanisms even before the plant is finally exposed to a very severe stress. In this way, the plant is able to reduce the excitation pressure and at the same time enhance photosynthetic stability. As it turns out, this kind of anticipatory coordination is one of the most important factors for the plant's tolerance to drought and its ability to recover efficiently (109, 110).

On the other hand, hydraulic signals emanating from reduced leaf $\Psi_w$ and turgor changes affect guard cell mechanics, mesophyll elasticity and thylakoid membrane conformation as they spread to all three regulatory levels (67, 87, 91). Collectively, these hydraulic, hormonal, redox, calcium and metabolite-dependent networks constitute a complex, interconnected regulatory matrix that synchronises stomatal closure, mesophyll $CO_2$ diffusion and chloroplast photochemistry (5, 21). It is this coordinated effort of the plant at different levels that allows photosynthesis, energy balance and protective mechanisms to be maintained at close to optimal levels across different drought intensities and durations, thus ultimately preserving the plant's capacity for carbon assimilation and survival under water-limited conditions (20, 34).

Moreover, the drought-affected regulatory network activates major changes in how cellular energy is managed and osmotic regulation to keep the photosynthesis working. When the plant cells have restricted access to water, they increase the production and storage of compatible solutes like proline, soluble sugars and glycine betaine which function to maintain cellular osmotic balance and also to stabilise proteins and membranes in the mesophyll and chloroplast compartments (17). These osmoprotective molecules are not only capable of protecting the photosynthetic apparatus structurally, but they also play a role in maintaining the enzymatic activities for carbon fixation and electron transport. Concurrently, the plants adjust the distribution of ATP and reducing equivalents among photosynthesis, photorespiration and stress defence routes to avoid metabolic overload and oxidative damage (5, 6). These biochemical changes which closely align with the signalling networks ensure the metabolic demand of stress tolerance is affectively balanced with the energy supply. Hence, this coupled metabolic and energetic restructuring significantly enhances plant's ability to preserve photosynthetic performance, protect cellular structures and support growth during extended drought periods (3).

## Conclusion

Drought-induced photosynthetic changes are mediated by a very coordinated and hierarchical regulatory continuum including stomatal, mesophyll and chloroplast processes rather than working as separate parts. The integration of the stomatal-mesophyll-chloroplast axis is a systems-level property involving dynamic feedback between hydraulic signals, abscisic acid (ABA), reactive oxygen species (ROS), calcium ($Ca^{2+}$) waves and chloroplast-derived retrograde signalling to regulate gas exchange, internal $CO_2$ diffusion, electron transport and photoprotection in a synchronised manner. Initially, stomatal regulation acts as the primary limitation of photosynthesis through quick hydraulic and ABA-dependent signals leading to stomatal closure, changes in guard cell ion channels, lower $g_s$ and improved water use efficiency (WUE). On the other hand, $g_m$ represents the next level of regulation, which depends on both the structural and biochemical features of the cells such the properties of the cell walls, the layout of intercellular air spaces, AQP levels and the positioning of chloroplasts. At the chloroplast level, drought results in the rearrangement of thylakoids, generation of additional electron acceptors and upregulation of non-photochemical quenching (NPQ), leading to redox homeostasis and the prevention of photodamage. Close association of these regulatory mechanisms at different levels results in harmonised constraint and protection of the photosynthetic machinery under drought. Understanding this integrated regulatory network at a deeper level will form an excellent basis to enhance plant drought resilience and WUE. Research in the future ought to focus on breeding and biotech strategies that not only target individual traits but also their well-coordinated combinations including highly sensitive stomata, better mesophyll diffusion and stronger

chloroplast protection mechanisms to sustain the balanced relationships among $g_s$, $g_m$ and electron transport even in drought. Additionally, genome editing technologies like CRISPR/Cas provide accurate means to alter very important regulatory genes such as EPF1/EPF2, SPCH, SLAC1, specific AQP isoforms and NPQ-related proteins like PsbS, which will help produce plants with better water usage and enhanced photoprotection. Most especially, integrated multi-omics approaches covering transcriptomics, proteomics, metabolomics and ionomics should be used in order to dig into the complicated, multilevel regulatory networks handling drought reactions and to pinpoint new molecular targets. Combining these data with sophisticated physiological and hydraulic modelling that take into account stomatal actions, $g_s$, chloroplast activities and processes at the whole canopy level, will drastically improve prediction abilities and quicken the process of selecting favourable phenotypes. Moreover, future investigations must take into account natural changes and evolutionary viewpoints as a way to enhance the knowledge of drought adaptation tactics. Studies comparing different species with opposing water-use characteristics, for example, isohydric versus anisohydric water regulation, $C_3$ versus $C_4$ photosynthetic pathways and xerophytic versus glycophytic adaptations, will offer very valuable insights into the range and adaptability of the integrated photosynthetic response to drought. Exploiting this diversity will be crucial for the climate-resilient crops development and the crops that will be able to adapt to different agro-ecological conditions.

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## Authors' contributions

ZMD originated the idea of the study, planned the structure and wrote the first very draft of the manuscript. FAK and MAA critically analysed and refined the stomatal and mesophyll regulatory parts of the manuscript. MAM and MMM performed a literature survey, synthesised data and integrated drought physiology concepts. KHB and WW helped with the interpretation of the chloroplast regulation and photosynthetic acclimation mechanisms of the manuscript. AM and PD handled the manuscript editing, figure conceptualisation and formatting besides were responsible for coordination, reference management and overall manuscript organisation. All authors have read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interest to declare.

**Ethical issues:** None

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