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PHOTOSYNTHESIS AND WATER STRESS: MOLECULAR MECHANISMS, PHOTOCHEMICAL REGULATION, AND INTEGRATIVE STRATEGIES OF PLANT DROUGHT TOLERANCE – A REVIEW

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ABSTRACT

In this review, water deficit remains one of the major constraints on plant productivity under rising temperatures, increasing atmospheric aridity, and the growing frequency of extreme weather events. Photosynthesis responds to drought not simply as a consequence of reduced water availability, but as a systemic disturbance of hydraulic, carbon, energy, and redox balance. At the early stage of stress, the decline in net CO_2 assimilation is determined mainly by stomatal closure and reduced stomatal conductance; however, as water deficit intensifies, the roles of mesophyll conductance, biochemical capacity for CO_2 assimilation, ribulose-1,5-bisphosphate regeneration, and thylakoid membrane function become increasingly important. In parallel, reduced chloroplastic CO_2 availability enhances photoprotective processes, alters ATP/NADPH balance, and increases the importance of photorespiration, mitochondrial respiration, and antioxidant defense. This review synthesizes current concepts of ABA -dependent and ABA - independent stomatal closure signaling, non -stomatal limitations of photosynthesis, photochemical regulation, and the roles of photorespiration and redox homeostasis, with particular emphasis on the diagnostic value of integrating gas exchange with PAM - fluorometry. Drought tolerance is interpreted as a multicomponent trait emerging from the coordination of molecular, physiological, morphological, and recovery related processes across levels of plant organization.

INTRODUCTION

Water is simultaneously a structural, transport, metabolic, and thermoregulatory component of the leaf; therefore, its deficit rapidly affects all major components of the photosynthetic system. Drought has become an increasingly important constraint under current climatic conditions because it rarely occurs in isolation and is often accompanied by high temperature and elevated vapor pressure deficit, which together intensify disturbances in plant carbon and water relations. For this reason, water stress should be regarded not merely as an episodic environmental disturbance, but as one of the principal factors determining agroecosystem resilience, crop productivity, and yield stability (Lawlor et al., 2002; Chaves et al., 2009; Grossiord et al., 2020; López et al., 2021). Its significance is especially evident in agricultural systems, where even moderate but recurrent water limitation can reduce biomass accumulation, impair reproductive development, and destabilize yield formation. Under field conditions, drought usually develops as a dynamic process differing in timing, duration, and intensity, and its effects depend strongly on developmental stage, canopy position, leaf age, and the interaction with thermal and atmospheric load. As a consequence, plant responses to water deficit cannot be adequately interpreted as a simple binary contrast between “stress” and “no stress”; rather, they must be viewed as a continuum of physiological adjustments and, if stress exceeds the limits of compensation, of progressive dysfunction.

The classical scheme of plant response to drought begins with a decline in water potential and the redistribution of water along the soil – root – xylem – leaf continuum. This results in decreased turgor, altered hydraulic conductivity, and partial stomatal closure. Because stomata simultaneously regulate CO_2 influx and water loss by means of

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transpiration, their response occupies a central position in the physiological adjustment of plants to water deficit. Accordingly, an early decline in stomatal conductance (*gs*) is commonly accompanied by reduced transpiration and a lower net rate of CO_2 assimilation (Passioura, 1982; Davies et al., 2002; Buckley, 2005, 2019; Chaves et al., 2009). However, contemporary research has clearly shown that the decline in photosynthesis under drought cannot be explained solely by stomatal closure. As stress intensifies, limitations progressively extend beyond stomata and involve mesophyll conductance, chloroplastic CO_2 availability, the activity of Calvin-Benson cycle enzymes, ribulose - 1,5 - bisphosphate (RuBP) regeneration, thylakoid energy partitioning, and cellular redox homeostasis. Thus, the reduction in net photosynthetic rate under water deficit reflects not a single limiting event, but a dynamic shift from predominantly diffusive limitation to mesophyll, biochemical, and, under severe or combined stress, photochemical limitation (Flexas et al., 2004; Baker, 2008; Chaves et al., 2009).

This complexity has important methodological consequences. No single trait can serve as a universal indicator of drought tolerance. High intrinsic water use efficiency, elevated antioxidant enzyme activity, or even a relatively stable maximum quantum yield of PSII after dark adaptation (*Fv/Fm)* value do not necessarily indicate superior productivity or true stress tolerance. The same apparent phenotype may arise from fundamentally different physiological causes: for example, reduced assimilation may reflect stomatal regulation, loss of mesophyll conductance, biochemical impairment, or the onset of photochemical dysfunction. Conversely, a short-term maintenance of one parameter may conceal growing instability in another regulatory domain. Therefore, correct interpretation requires an integrative framework linking plant hydraulics, stomatal dynamics, mesophyll CO_2 diffusion, gas exchange, chlorophyll fluorescence, respiratory metabolism, photoprotection, and recovery capacity after rewatering (Baker, 2008; Chaves et al., 2009; Murchie et al., 2013).

This issue is particularly important for physiological phenotyping and breeding. In practical work, drought tolerance is often inferred from a limited number of measurements, such as leaf water status, stomatal conductance, chlorophyll fluorescence, or antioxidant activity. Yet, the diagnostic value of each of these parameters depends strongly on stress severity, temporal scale, and the coordination among different levels of regulation. A genotype that conserves water rapidly may display high instantaneous water use efficiency but lose carbon gain too early; another may maintain assimilation longer, but at the cost of accelerated dehydration and thermal load. Consequently, the biologically meaningful question is not which isolated response is strongest, but how effectively hydraulic regulation, carbon metabolism, photoprotection, and recovery are coordinated within the whole plant system.

Against this background, drought tolerance should be regarded as a systemic property emerging from the coordination of water transport, carbon metabolism, membrane photochemistry, redox regulation, and structural plant traits. The aim of this review is to integrate current knowledge on the molecular, physiological, and photochemical mechanisms by which water deficit alters photosynthesis, with particular emphasis on the transition from stomatal to non - stomatal limitations, the regulation of photoprotection and redox balance, and the value of combining gas exchange and PAM - fluorescence approaches for drought phenotyping and the identification of stress resilient genotypes. Accordingly, the following sections consider, in sequence, the hydraulic and stomatal basis of the response, the metabolic and biochemical restrictions on carbon assimilation, the membrane and photochemical mechanisms of energy dissipation, the role of photorespiration and redox homeostasis, the contribution of structural traits, and the integrative value of modern physiological phenotyping.

WATER AS A SYSTEMIC FACTOR AND THE TRANSITION FROM STOMATAL TO NON - STOMATAL LIMITATIONS

The earliest and most sensitive manifestation of water deficit in the leaf is a decline in stomatal conductance (Buckley, 2005; Davies et al., 2002; Chaves et al., 2009). Even a moderate reduction in soil and leaf water potential disturbs the hydraulic status of the soil – plant - atmosphere continuum and leads to partial stomatal closure (Figure 1) (Buckley 2005; Buckley, 2019; Davies et al., 2002). From a physiological perspective, this response is initially adaptive: it restricts water loss through transpiration, helps preserve leaf water status, and delays the onset of severe dehydration. At the same time, however, stomatal closure reduces CO_2 entry into the intercellular air spaces and initiates a cascade of subsequent limitations to photosynthesis (Lawlor et al., 2002; Chaves et al., 2009; Buckley, 2019).

At the initial phase of stress, the decline in net CO_2 assimilation (*A*) is often explained mainly by reduced *gs* and a lower intercellular CO_2 concentration (*Ci*) (Lawlor et al., 2002; Flexas et al., 2004; Chaves et al., 2009). If mesophyll conductance and enzymatic capacity are still largely maintained, the restriction of CO_2 supply is predominantly diffusional in nature (Flexas et al., 2004; Keenan et al., 2010; Niinemets et al., 2009). Under these conditions, the decrease in photosynthesis reflects an early limitation of substrate availability rather than a collapse of the biochemical machinery itself. This stage is usually accompanied by a moderate decline in the effective quantum yield of photosystem II photochemistry (ΦPSII, Y(II), Yield), whereas *Fv/Fm* remains relatively stable, indicating that the structural integrity of photosystem II (PSII) is still preserved and that regulatory rather than injurious processes predominate (Maxwell et al., 2000; Baker, 2008; Murchie et al., 2013).

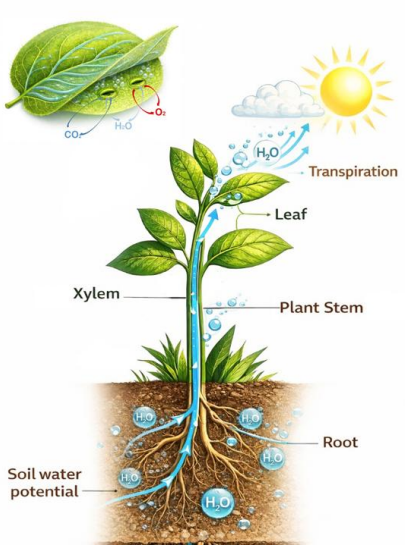

Figure 1. Schematic representation of the soil – plant – atmosphere hydraulic continuum and the early adaptive stomatal response to water deficit. A decline in soil and leaf water potential disturbs plant hydraulic status, promotes partial stomatal closure, restricts transpirational water loss, and helps preserve leaf water status at the initial stage of drought

This early stomatal phase is especially important because it illustrates a general principle of drought physiology: the first measurable reduction in carbon gain does not necessarily imply irreversible damage. On the contrary, in many cases it represents a regulated shift of the system toward water conservation. Nevertheless, even at this stage, the physiological cost of such regulation is already evident, since lower *gs* not only restricts CO_2 diffusion but also reduces transpiration driven cooling and progressively alters the thermal and energetic state of the leaf (Buckley, 2005; Buckley, 2019; Chaves et al., 2009; Grossiord et al., 2020). For this reason, stomatal limitation should be regarded not as an isolated event, but as the beginning of a broader systemic transition.

As drought intensifies, the simple scheme "less water → lower gs → lower A" becomes insufficient (Lawlor et al., 2002; Flexas et al., 2004; Chaves et al., 2009). Reduced transpiration weakens evaporative cooling, leaf temperature (Tleaf) rises, and the latter imposes additional strain on the enzymatic and membrane components of photosynthesis (Grossiord et al., 2020; Qiao et al., 2024; Chaves et al., 2009). At the same time, the diffusion pathway inside the leaf also changes: the properties of cell walls and membranes are altered, mesophyll conductance (gm) begins to decline, and the CO_2 concentration in chloroplasts (Cc) decreases more strongly than would be expected from changes in Ci alone (Flexas et al., 2004; Niinemets et al., 2009; Keenan et al., 2010). It is at this stage that the transition toward non- stomatal limitation begins (Lawlor and Cornic 2002; Flexas et al., 2004; Chaves et al., 2009). This logic can be expressed in the general form:

$$A = f(gs, gm, Vcmax, Jmax, TPU, Rd) \quad (1)$$

where *A* is the net assimilation rate jointly determined by stomatal and mesophyll CO_2 diffusion, the carboxylation capacity of RuBisCo (EC 4.1.1.39) (V*cmax*), RuBP regeneration (*Jmax*), triose - phosphate utilization (TPU), and respiratory losses (*Rd*) (Farquhar *et al*. 1980; Flexas et al., 2004; Chaves et al., 2009).

In this sense, *gs* should be regarded not merely as a symptom of drought, but as a central switch, that transfers the system from a state of relatively normal coupling between water and carbon fluxes to a state of multicomponent limitation (Buckley 2005; Buckley 2019; Chaves et al., 2009). Once this transition has started, photosynthetic performance can no longer be interpreted in terms of stomatal aperture, because internal diffusion, biochemical capacity, and the energetic state of the chloroplast increasingly contribute to the observed decline in *A*.

An important practical consequence follows from this: assessing drought tolerance on the basis of *gs* or *Ci* alone may be seriously misleading (Flexas et al., 2004; Keenan et al., 2010; Niinemets et al., 2009). Two genotypes exhibiting a similar reduction in *gs* may differ substantially in the stability of *gm* (Keenan et al., 2010), the maintenance of RuBP regeneration (Baker, 2008), the pattern of non-photochemical quenching of chlorophyll fluorescence (NPQ), and the ability to restore *A* after rehydration (Xu et al., 2009; Jahan et al., 2023). Thus, similar stomatal behavior does not necessarily imply similar functional outcomes. What appears superficially as the same degree of "stomatal limitation" may in fact correspond to different levels of mesophyll, biochemical, and photochemical resilience.

This distinction is particularly important for physiological phenotyping. If the analysis is limited to stomatal traits alone, adaptive regulation may easily be confused either with superior tolerance or, conversely, with the onset of damage. In reality, the biological meaning of stomatal closure depends on how effectively it is coordinated with mesophyll CO_2 transfer, thermal balance, energy dissipation, and subsequent recovery of assimilation after rehydration (Flexas et al., 2004; Baker, 2008; Keenan et al., 2010; Xu et al., 2009; Jahan et al., 2023). Therefore,

the stomatal response represents only the first stage of the overall process rather than its complete explanation (Lawlor et al., 2002; Chaves et al., 2009; Qiao et al., 2024).
At the same time, the fact that stomata occupy this early and pivotal position in the drought response raises a further question: how is stomatal closure initiated, regulated, and diversified among genotypes? Answering this requires moving from the functional level of gas exchange and hydraulic imbalance to the molecular and signaling mechanisms that control guard cell behavior. This transition leads to the next section on stomatal closure signaling and its genetic determinants.

STOMATAL CLOSURE SIGNALING AND GENETIC DETERMINANTS OF THE STOMATAL RESPONSE

The transition from hydraulic disturbance to functional stomatal limitation necessarily raises the question of how stomatal closure is initiated and regulated at the cellular level. The molecular control of the stomatal response to drought is currently understood as a multi-level regulatory network in which abscisic acid (ABA) plays a central, though not exclusive, role (Hsu et al., 2021; Kim et al., 2010). As water potential declines in plant tissues, including both roots and leaves, ABA biosynthesis, redistribution, and signaling increase, and the hormone activates a characteristic guard cell signaling cascade (Nambara et al., 2005; Schachtman et al., 2008). At the core of this response lies the canonical module comprising PYR/PYL/RCAR receptors, PP2C phosphatases, SnRK2/OST1 kinases, and downstream ion channels (Hsu et al., 2021; Kim et al., 2010).
ABA binding to its receptors suppresses PP2C activity and thereby releases OST1 from inhibition (Hsu et al., 2021; Kim et al., 2010). Activated OST1 phosphorylates effector proteins, including SLAC1 - type anion channels, which are essential for stomatal closing responses (Lee et al., 2009; Geiger et al., 2009). The resulting anion efflux promotes plasma membrane depolarization, K^+ loss, osmotic adjustment, and water efflux from guard cells (Geiger et al., 2009; Hsu et al., 2021). As guard cell turgor declines, stomatal aperture decreases and gs falls rapidly (Lee et al., 2009; Geiger et al., 2009). This pathway is fundamentally important because it connects changes in plant water status with the active cellular control of gas exchange rather than with passive mechanical closure alone.
At the same time, the ABA -dependent pathway should not be viewed as a simple linear sequence. Its operation involves several amplification and modulation loops in which reactive oxygen species (ROS) and calcium ions (Ca^{2+}) play essential roles. The production of H_2O_2 by NADPH oxidases, oscillations in cytosolic Ca^{2+}, and the activation of Ca^{2+} dependent kinases increase the flexibility, sensitivity, and context dependence of the stomatal response (Sierla et al., 2016; Hsu et al., 2021; Kim et al., 2010). Consequently, the extent and kinetics of stomatal closure are determined not only by ABA concentration per se, but also by the responsiveness of the signaling network and by the capacity of guard cells to integrate multiple signals into a coordinated output (Sierla et al., 2016; Kim et al., 2010).
Stomatal regulation under drought is further shaped by signals that are partly ABA - independent or that interact with ABA signaling in complex ways (Jalakas et al., 2021; Merilo et al., 2018; Assmann et al., 2016). These include hydraulic perturbation, increasing vapor pressure deficit (VPD), CO_2/bicarbonate signaling, and the light environment (Jalakas et al., 2021; Xue et al. 2011; Assmann et al., 2016). Elevated VPD, for example, may induce partial stomatal closure even before the classical hormonal response is fully expressed, although the molecular basis of this effect remains debated (Merilo et al., 2018; Jalakas et al., 2021). Likewise, shifts in Ci and Cc associated with reduced assimilation can influence anion channel activity and thereby reinforce stomatal closure (Xue et al., 2011; Kim et al., 2010). Light normally promotes stomatal opening, but under drought combined with high photosynthetically active radiation (PAR), increasing photochemical imbalance and ROS production may shift the regulatory balance toward closure instead (Assmann et al., 2016; Sierla et al., 2016). Thus, stomatal behavior during drought emerges from the integration of hormonal, hydraulic, atmospheric, and metabolic cues rather than from a single regulatory input.
From a phenotypic and ecophysiological perspective, genetic variation in the kinetics of stomatal movement is especially important. Drought tolerance is not determined simply by the minimum or maximum *gs* attained under stress, but by how rapidly, appropriately, and reversibly stomata adjust to changing environmental conditions (Lawson et al., 2014; Faralli et al., 2019). Genotypes with faster and better coordinated responses may reduce excessive water loss more effectively while avoiding an unnecessarily strong decline in carbon assimilation, although the actual adaptive value depends on the environmental context and on the combination of other physiological traits (Lawson et al., 2014; Faralli et al., 2019). This has increased interest in stomatal density, size, and patterning traits, as well as in genes regulating stomatal development, including SPCH, MUTE, FAMA, and EPF (Chua et al., 2024; Hughes et al., 2017; Faralli et al., 2019).
However, early stomatal closure should not automatically be interpreted as the optimal drought strategy. An excessively conservative response may increase intrinsic water use efficiency (*iWUE*), yet restrict carbon gain too early and thereby reduce overall productivity. Conversely, a more permissive stomatal phenotype, in which stomata remain open for longer, may sustain assimilation for a greater period, but at the cost of enhanced dehydration risk and weaker thermal buffering. For this reason, the most drought resilient genotypes are not necessarily those with

the strongest stomatal closure, but those in which stomatal regulation is appropriately coordinated with hydraulic capacity, photochemical stability, and the leaf's ability to tolerate declining CO_2 availability without rapidly entering irreversible dysfunction (Faralli et al., 2019; Lawson et al., 2014; Saradadevi et al., 2017).

This interpretation has an important methodological consequence. Stomatal traits are highly informative, but they cannot be treated as self-sufficient markers of drought tolerance. The biological value of rapid closure depends on what follows after closure: whether mesophyll CO_2 transfer remains functional, whether biochemical capacity is preserved, and whether the leaf can maintain energy balance and recover after rehydration. In this sense, stomatal signaling defines the early regulatory trajectory of the stress response, but does not by itself explain the full decline in photosynthetic performance.

Accordingly, once the stomatal phase has been established, the analysis must move beyond guard cell regulation toward the internal constraints that increasingly determine carbon assimilation under progressive drought. This leads directly to the next level of analysis: the non-stomatal limitations of photosynthesis, including changes in mesophyll conductance, carboxylation capacity, electron transport, and metabolic reorganization.

NON-STOMATAL LIMITATIONS OF PHOTOSYNTHESIS: *gm*, V*cmax*, J*max* AND METABOLIC REORGANIZATION

Following the stomatal phase, stress development becomes increasingly determined by non-stomatal limitations of photosynthesis (Chaves et al., 2009; Lawlor et al., 2009; Urban et al., 2017). At this stage, the decline in carbon assimilation can no longer be interpreted solely as a consequence of reduced stomatal aperture, because important constraints arise within the leaf itself. A key concept here is mesophyll conductance, gm, i.e. the conductance for CO_2 diffusion from the intercellular air spaces to the chloroplast stroma (Flexas et al., 2008; Niinemets et al., 2009). This parameter links intercellular CO_2 concentration to the actual substrate availability of CO_2 for RuBisCo:

$$Cc = Ci - \frac{A}{gm} \quad (2)$$

This relationship shows that even when the decrease in *Ci* is only moderate, the decline in *Cc* may be substantial if *gm* decreases in parallel (Niinemets et al., 2009; Warren, 2008; Keenan et al., 2010). As a result, the chloroplast may experience a more severe substrate limitation than would be inferred from gas phase measurements alone.

The decline in *gm* during drought is associated with several interacting factors, including dehydration of cell walls and the resulting changes in their diffusion properties, reorganization of chloroplast position relative to the intercellular air spaces, and reduced activity of certain aquaporins involved in CO_2 transport under water and redox stress (Flexas et al., 2008; Flexas et al., 2012; Groszmann et al., 2017; Miyazawa et al., 2008). For this reason, interpretation of photosynthetic limitation on the basis of Ci alone often overestimates the contribution of stomata and underestimates the mesophyll component (Flexas et al., 2004; Warren 2008; Urban et al., 2017). Physiologically, this marks a critical transition, because the decline in assimilation is no longer explained only by the amount of CO_2 entering the leaf, but increasingly by the efficiency with which that CO_2 reaches the chloroplast and can be metabolically utilized there.

Methodologically, this has an important consequence. Once *gm* becomes unstable, the use of *Ci* alone as an indicator of substrate availability becomes increasingly insufficient, especially when comparing genotypes or stress severities. Similar values of *Ci* may correspond to markedly different values of *Cc*, and therefore to different degrees of biochemical restriction within the chloroplast. This is precisely why drought induced limitation cannot be partitioned reliably without taking the mesophyll component into account (Flexas et al., 2004; Niinemets et al., 2009; Keenan et al., 2010; Urban et al., 2017). In practical terms, the transition from stomatal to non-stomatal limitation is not just a shift in location, but a shift in the physiological meaning of the measured parameters.

The next level of limitation is associated with carboxylation capacity and with the biochemical organization of the Calvin - Benson cycle. Within the Farquhar model, photosynthesis can be described by at least three potential limitations:

$$A = \min(Ac, Aj, Ap) - Rd \quad (3)$$

where carboxylation limitation (*Ac*) depends on the carboxylation capacity of RuBisCo, RuBP -regeneration limitation (*Aj*) depends on electron transport and RuBP regeneration, and triose-phosphate-utilization limitation (*Ap*) depends on the use of triose phosphates (Farquhar et al., 1980; McClain et al., 2019). Under real drought conditions, the relative contribution of these limitations changes over time and depends on stress intensity, duration, and the degree to which diffusive restriction has already altered chloroplast metabolism (Chaves et al., 2009; Lawlor et al., 2009; Wang et al., 2018).

A decrease in V*cmax* may arise either from a regulated decline in carboxylation demand or from direct impairment of the enzymatic machinery (Parry et al., 2002; Galmés et al., 2011; Perdomo et al., 2017). A particularly sensitive component is RuBisCo activase, which is required to maintain RuBisCo in an active state and is highly vulnerable to the combined effects of water deficit, high temperature, and oxidative stress (Crafts-Brandner et al., 2000;

Salvucci et al., 2004; Perdomo et al., 2017). Therefore, in the intermediate and late stages of drought, a reduction in V*cmax* often reflects not only diminished CO_2 supply, but also a genuine loss of biochemical capacity within the leaf (Galmés et al., 2011; Lawlor et al., 2009; Perdomo et al., 2017). In other words, carbon assimilation becomes limited not merely because less substrate is available, but because the enzymatic system itself becomes less able to process that substrate.

In parallel, J*max* may also decline (Chaves et al., 2009; Wang et al., 2018). This is associated not only with changes in electron transport, but also with impaired RuBP regeneration, redistribution of fluxes toward cyclic electron transport, shifts in the ATP/NADPH balance, and inhibition of enzymes in the regenerative segment of the Calvin cycle (Tezara et al., 1999; Huang et al., 2012; Wang et al., 2018). Consequently, the leaf progressively loses the capacity to sustain a high rate of carboxylation even when *Cc* is temporarily or partially restored. This point is important because it shows that restoration of CO_2 availability alone is not necessarily sufficient to restore photosynthetic performance once biochemical restrictions have become established.

A further component is triose phosphate utilization (Farquhar et al., 1980; McClain et al., 2019). Under drought and disturbed source sink relations, the risk of feedback inhibition of photosynthesis increases: slowed sugar export, limited phosphate regeneration, and the accumulation of photosynthetic products in the leaf can intensify energetic constraints (McClain et al., 2019; Lemoine et al., 2013; Salmon et al., 2019). Inorganic phosphate deficiency additionally restricts ATP synthesis, so carbon and energy limitations become tightly interconnected (McClain et al., 2019; Tezara et al., 1999). Thus, drought progressively transforms the photosynthetic system from one primarily constrained by diffusion into one increasingly constrained by the coordination of substrate supply, metabolic turnover, and energy balance.

At the same time, these non-stomatal limitations do not emerge simultaneously or with equal weight throughout stress development. In moderate drought, reduced *gm* may dominate the decline in chloroplast CO_2 availability while the core biochemical machinery remains only partly affected. Under more severe or prolonged stress, however, biochemical restriction becomes more prominent, and reductions in V*cmax*, J*max*, and phosphate - dependent metabolism increasingly shape the decline in *A* (Chaves et al., 2009; Lawlor et al., 2009; Wang et al., 2018). This temporal dimension is important because it shows that non- stomatal limitation is not a single event, but a progressive restructuring of photosynthetic control.

An important and still debated question is which process should be considered primary: the decline in *gm*, the loss of V*cmax*/J*max*, or the emergence of energy imbalance (Lawlor et al., 2009; Urban et al., 2017; Wang et al., 2018). In practice, these mechanisms form a network of reinforcing feedbacks. A decline in *Cc* increases photorespiratory and redox load; Calvin cycle limitations alter chloroplast energetic state; redox shifts affect membranes and enzymes; and enzymatic deterioration, in turn, further amplifies the decline in *A* (Lawlor et al., 2009; Wang et al., 2018; Qiao et al., 2024). For this reason, non-stomatal limitations should not be interpreted as isolated lesions, but as a dynamic reorganization of the photosynthetic system under progressive stress (Lawlor et al., 2009; Urban et al., 2017).

From a physiological and breeding perspective, the most valuable genotypes are those that, even with reduced *gs,* maintain a relatively high *gm*, lose less V*cmax* and J*max*, and avoid entering deep biochemical suppression too early (Flexas et al., 2016; Keenan et al., 2010; Perdomo et al., 2017). It is precisely this coordination that determines the difference between short term regulatory adjustment and actual deterioration of the photosynthetic apparatus (Flexas et al., 2016; Urban et al., 2017). At the same time, once biochemical restrictions intensify and energy consumption by carbon metabolism declines, the absorbed light energy itself becomes increasingly difficult to balance safely. This is why the analysis of non-stomatal limitation naturally leads to the next level of response: membrane regulation, photoprotection, and the limits beyond which regulatory energy dissipation gives way to photoinhibition.

MEMBRANE RESPONSES, PHOTOPROTECTION AND THE PHOTOINHIBITION LIMITS

The light phase of photosynthesis under water deficit is not merely a passive background to metabolic limitation, but an independent regulatory level of the stress response (Baker, 2008; Lawlor et al., 2009; Urban et al., 2017). As the Calvin - Benson cycle slows the consumption of NADPH and ATP declines, whereas the flux of absorbed excitation energy may remain high. This creates a mismatch between the rate of photosystem excitation and the rate at which reductants and ATP can be utilized, i.e. a state of energy imbalance (Baker, 2008; Lawlor et al., 2009). Under these conditions, the photosynthetic membrane system is forced to reallocate energy between photochemistry, thermal dissipation, and, if protective capacity is exceeded, photodamage.

The functional state of PSII is one of the most sensitive indicators of this reorganization; however, correct interpretation requires distinguishing between parameters that answer different physiological questions (Maxwell et al., 2000; Baker, 2008; Murchie et al., 2013). The maximum quantum yield of PSII after dark adaptation reflects the potential efficiency of open reaction centers and is therefore particularly informative for diagnosing chronic photoinhibition and the degree of recovery of the photosynthetic apparatus (Maxwell et al., 2000; Baker, 2008). By contrast, the effective quantum yield of PSII in the light reflects the fraction of absorbed energy that is actually

used in photochemistry under a given combination of PAR, CO_2 availability, leaf temperature, and stomatal limitation (Baker, 2008; Murchie et al., 2013):

$$\Phi\text{PSII} = \frac{Fm' - Fs}{Fm'} \quad (4)$$

For this reason, under moderate drought, ΦPSII usually declines earlier and more strongly than *Fv/Fm*. This discrepancy does not necessarily indicate structural damage. Rather, it shows that although PSII remains largely intact, the actual efficiency of photochemistry is reduced because the demand for electron acceptors has fallen, reaction centers become more reduced, and energy is redistributed toward protective pathways (Baker 2008; Murchie et al., 2013; Urban et al., 2017). In physiological terms, this is a hallmark of functional down regulation rather than injury. It reflects the plant's attempt to maintain membrane stability under conditions in which carbon metabolism is no longer able to consume absorbed light energy at the former rate.

A central regulatory response to this condition is the increase in non -photochemical quenching (Müller et al., 2001; Ruban, 2016):

$$\text{NPQ} = \frac{Fm - Fm'}{Fm'} \quad (5)$$

An increase in NPQ reflects the activation of thermal energy dissipation, especially through the energy dependent quenching *(qE)* component, which depends on ΔpH, the PsbS protein, and the xanthophyll cycle (Müller et al., 2001; Li et al., 2002; Ruban, 2016). At this stage, enhanced NPQ should not be interpreted as a sign of damage, but as evidence of functional photoprotection (Müller et al., 2001; Ruban, 2016). By dissipating excess excitation energy as heat, the leaf lowers the probability of excessive excitation pressure and reduces the risk of uncontrolled photogeneration of reactive oxygen species (ROS) (Müller et al., 2001; Ruban, 2016). In this sense, NPQ is not simply a fluorescence derived parameter, but a direct indicator of how actively the photosynthetic apparatus is defending itself against imbalance.

At the same time, the protective capacity of NPQ is not unlimited. If water deficit is combined with high irradiance and elevated temperature, the rate of damage to the D1 protein and other PSII components may exceed the rate of repair (Aro et al., 1993; Murata et al., 2007; Tikkanen et al., 2014). Under such conditions, regulatory down regulation gives way to true photoinhibition. This threshold is physiologically important because it marks the point at which the system is no longer merely redistributing energy safely, but begins to lose structural and functional integrity.

This shift is best captured by the behavior of *Fv/Fm* after dark adaptation:

$$\frac{Fv}{Fm} = \frac{(Fm - F0)}{Fm} \quad (6)$$

Unlike ΦPSII, this parameter is less dependent on the current light regime and on immediate diffusive restriction, and is therefore more informative for detecting already established photoinhibition (Maxwell et al., 2000; Baker, 2008; Murchie et al., 2013). A decline in *Fv/Fm* indicates a transition from regulatory reorganization to structural impairment of PSII, whereas incomplete recovery after rehydration is evidence of chronic or only partially reversible damage (Baker, 2008; Murchie et al., 2013; Urban et al., 2017). This is why *Fv/Fm* is especially valuable not for diagnosing the earliest phase of drought stress, but for determining whether the photosynthetic apparatus has crossed the boundary between adaptive adjustment and lasting injury.

Another important compensatory component is cyclic electron flow around photosystem I (CEF) (Yamori et al., 2016; Huang et al., 2012). This pathway supports additional ATP formation without further accumulation of NADPH, increases ΔpH across the thylakoid membrane, and thereby reinforces photoprotective energy dissipation (Yamori et al., 2016; Tikkanen et al., 2014). Under drought, this becomes especially important because photorespiration and protective processes may increase ATP demand precisely when reductant consumption by carbon fixation is constrained. Therefore, enhanced CEF should be regarded as one of the principal components of membrane adaptation to stress rather than as a minor auxiliary pathway (Huang et al., 2012; Yamori et al., 2016).

These membrane responses must not be considered in isolation from carbon metabolism. A decline in *Cc* restricts carboxylation; reduced carboxylation alters the redox state of the thylakoids; and redox changes enhance photoprotective responses and may influence nuclear gene expression through retrograde signaling (Lawlor et al., 2009; Gollan et al., 2015). Thus, PSII status and NPQ dynamics not only reflect the depth of stress, but also contribute to shaping its subsequent trajectory (Gollan et al., 2015; Baker, 2008). This point is important because it shows that membrane regulation is not simply a passive consequence of metabolic limitation, but a central part of the overall adaptive strategy.

From a methodological point of view, *Fv/Fm* and ΦPSII/Yield are not interchangeable. *Fv/Fm* is most useful after dark adaptation for assessing whether the system has shifted from regulatory loss of efficiency to structural photoinhibition and how fully it recovers after stress (Maxwell et al., 2000; Baker, 2008; Murchie et al., 2013). ΦPSII/Yield, by contrast, is more informative under actinic light for tracking the current depth of photochemical limitation, comparing it with *A*, *gs*, and NPQ, and estimating electron transport through PSII (ETR) (Baker, 2008; Murchie et al., 2013; Urban et al., 2017). This distinction is especially important in phenotyping, because an

apparent decline in fluorescence yield may represent either reversible photoprotective adjustment or the early development of deeper dysfunction, depending on the behavior of the accompanying parameters.
Accordingly, the most typical sequence under drought is the following: ΦPSII decreases first, while *Fv/Fm* remains close to the control level; NPQ then increases as protective heat dissipation becomes more active; and only when the limits of photoprotection are exceeded does *Fv/Fm* begin to decline. It is precisely this divergence between effective and maximum quantum yield that allows one to distinguish adaptive reorganization from developing damage (Baker, 2008; Murchie et al., 2013; Ruban, 2016). At the same time, once carbon fixation becomes restricted and membrane regulation is increasingly driven by redox constraints, the role of alternative sinks and oxidative balance becomes more prominent. This naturally leads to the next level of analysis: photorespiration, mitochondrial respiration, and redox homeostasis under drought.

PHOTORESPIRATION, MITOCHONDRIAL RESPIRATION AND REDOX HOMEOSTASIS

As drought progressively restricts carboxylation and alters the balance of energy use within the chloroplast, the role of alternative metabolic pathways becomes increasingly important. Under these conditions, photorespiration can no longer be regarded merely as an unproductive loss of previously fixed carbon (Wingler et al., 2000; Bauwe et al., 2010). When chloroplastic CO_2 concentration declines, the stromal O_2/CO_2 ratio increases, and RuBisCo more frequently catalyzes its oxygenase reaction. This leads to the formation of 2-phosphoglycolate and activation of the photorespiratory pathway, which functionally links the chloroplast, peroxisome, and mitochondrion into a coordinated metabolic network (Bauwe et al., 2010; Peterhansel et al., 2010).
From the standpoint of net carbon gain, photorespiration is indeed associated with CO_2 release and energetic cost (Bauwe et al., 2010; Peterhansel et al., 2010). However, under conditions in which carboxylation is restricted, its physiological significance is much broader. Photorespiration provides an alternative sink for electrons and reductants, helps maintain photosynthetic electron transport, and lowers the risk of excessive over reduction of the chloroplast electron transport chain (Wingler et al., 2000; Kozaki et al., 1996). In this sense, it functions as a metabolic safety valve that reduces excitation pressure and helps delay the onset of photoinhibition (Wingler et al., 2000; Kozaki et al., 1996).
This dual character is central to its interpretation under drought. On the one hand, photorespiration reduces carbon use efficiency; on the other, it stabilizes the photosynthetic system when CO_2 assimilation is constrained. Therefore, abrupt suppression of photorespiratory flux may reduce carbon loss only at the price of greater redox imbalance, stronger oxidative pressure, and higher vulnerability of PSII (Kozaki et al., 1996; Wingler et al., 2000). This is why plants with impaired photorespiratory metabolism often suffer more severe photooxidative damage under stress (Kozaki et al., 1996; Peterhansel et al., 2010). A functionally stable drought response, therefore, does not consist in minimizing photorespiration at any cost, but in maintaining an appropriate balance between carboxylation and oxygenation fluxes (Wingler et al., 2000; Bauwe et al., 2010).
At the same time, photorespiration should be viewed not as an isolated chloroplastic consequence, but as part of inter organelle metabolic coordination. Because the pathway spans chloroplasts, peroxisomes, and mitochondria, its operation directly influences cellular redox balance, energy turnover, and the processing of stress induced metabolites. For this reason, the significance of photorespiration under drought lies not only in the fate of carbon, but also in its contribution to metabolic continuity when the normal balance between light reactions and carbon fixation has been disrupted.
Mitochondrial respiration also assumes a modified role under drought. During the early or short-term phase of stress, dark respiration may increase because the plant requires additional ATP and metabolic flexibility to maintain ion homeostasis, protein repair, synthesis of protective metabolites, and processing of photorespiratory intermediates (Lawlor et al., 2009; Pinheiro et al., 2011). Under prolonged or severe stress, however, respiration often declines as a consequence of general metabolic depression, substrate limitation, and progressive disruption of cellular function (Lawlor et al., 2009; Pinheiro et al., 2011). Thus, respiratory behavior under drought is dynamic and should not be interpreted as uniformly increasing or decreasing, but as shifting according to stress severity and metabolic state.
Alternative oxidase (AOX) is especially important in this context (Vanlerberghe, 2013). By allowing electron flow through a non- phosphorylating branch of the respiratory chain, AOX decreases over reduction of mitochondrial electron carriers and lowers the probability of electron leakage to oxygen, thereby limiting ROS formation in mitochondria (Vanlerberghe, 2013). Although this pathway is less efficient in terms of ATP yield, under drought its major significance is protective rather than energy conserving. In other words, AOX contributes to redox buffering and metabolic stability precisely when maximizing ATP production is less important than preventing oxidative damage.
The role of ROS in this system is likewise dual (Mittler, 2002; Foyer et al., 2005). Under moderate drought, reactive oxygen species should not be viewed exclusively as toxic byproducts. They also function as signaling molecules that participate in adaptive reorganization of metabolism, membrane behavior, and stress response

pathways (Mittler, 2002; Foyer et al., 2005; Gill et al., 2010). Chloroplasts, peroxisomes, and mitochondria become the principal intracellular sources of ROS, and the actual redox state of the cell depends not only on the rate of ROS generation, but also on the efficiency of detoxification and compartmental coordination (Mittler, 2002; Gill et al., 2010). For this reason, it is more accurate to speak not simply of oxidative stress, but of redox homeostasis as a central axis of the drought response (Foyer et al., 2005; Gill et al., 2010).

The antioxidant system includes both enzymatic and non-enzymatic components. Superoxide dismutase (SOD) (EC 1.15.1.1) convert superoxide into H_2O_2; catalase (CAT) (EC 1.11.1.6) and the ascorbate - glutathione cycle participate in peroxide detoxification; carotenoids and tocopherols help protect membranes; and proline together with other compatible osmolytes contributes to stabilization of proteins and membranes (Mittler, 2002; Gill et al., 2010; Foyer et al., 2005). However, high antioxidant activity should not be interpreted in itself as a direct equivalent of drought tolerance. In many cases, it simply reflects high stress intensity. A more informative trait is the plant's ability to maintain redox balance while preserving relatively stable photochemical and gas exchange performance (Gill et al., 2010; Urban et al., 2017).

This point is methodologically important. A drought tolerant genotype should not be defined as one that invariably exhibits the highest activities of SOD, ascorbate peroxidase (APX) (EC 1.1.1.11), or CAT, but rather as one that maintains *Fv/Fm*, ΦPSII, *A*, and *gm* within a functional range, shows a controlled increase in NPQ, and does not show a pronounced decline after rehydration (Baker, 2008; Murchie et al., 2013; Urban et al., 2017; Flexas et al., 2016). Antioxidant protection is therefore best understood as a component of overall system coordination rather than as an independent indicator of tolerance (Urban et al., 2017; Flexas et al., 2016).

Taken together, photorespiration, mitochondrial respiration, AOX activity, ROS signaling, and antioxidant defense form an integrated redox regulatory network that helps maintain functional continuity of the photosynthetic apparatus under drought. This network does not eliminate the cost of stress, but it strongly influences whether the plant remains within the zone of reversible adjustment or proceeds toward deeper damage. At the same time, the effectiveness of this regulation depends not only on metabolism itself, but also on the structural organization of the leaf and the plant as a whole. This naturally leads to the next level of analysis: the morphological and anatomical strategies through which drought tolerance is expressed at the tissue, organ, and whole plant levels.

MORPHOLOGICAL STRATEGIES AND THE INTEGRATIVE NATURE OF DROUGHT TOLERANCE

The physiological response to drought is inseparably linked to plant morphology and anatomy (Tomás et al., 2013; Bertolino et al., 2019; Comas et al., 2013). Structural traits do not merely accompany the stress response; they strongly influence how rapidly water deficit is translated into hydraulic disturbance, stomatal limitation, mesophyll restriction, and thermal imbalance. Cuticle thickness, the properties and organization of the wax layer, stomatal density and size, mesophyll structure, chloroplast positioning, leaf thermal regime, and root system architecture all affect the rate and extent to which diffusive and non- stomatal limitations develop (Tomás et al., 2013; Bertolino et al., 2019; Comas et al., 2013; Teskey et al., 2015). In this sense, morphology should be viewed not as a passive background, but as a structural regulator of drought response.

These features cannot be interpreted separately from molecular and biochemical regulation; rather, they represent its structural expression at the tissue, organ, and whole plant levels (Bandurska, 2022; Martínez-Vilalta et al., 2017). The same drought signal may produce different functional outcomes depending on how leaf and root traits constrain water flux, CO_2 diffusion, and energy balance. Therefore, structural organization is important not only because it affects plant form, but because it determines the physical framework within which physiological regulation operates.

At the leaf level, stomatal and mesophyll traits are particularly influential. Small stomata often provide faster opening and closing kinetics, whereas stomatal density and spatial patterning affect the potential range and responsiveness of gas exchange (Lawson et al., 2014; Tomás et al., 2013). Mesophyll structure, including the arrangement of cells and intercellular air spaces, influences the magnitude of *gm* and the extent to which *Cc* declines when *gs* decreases (Tomás et al., 2013; Lawson et al., 2014). Chloroplast positioning is also relevant, because it affects the effective path of CO_2 diffusion and the exposure of the photosynthetic machinery to changing light and thermal conditions. Consequently, leaf anatomical traits shape not only the amount of carbon acquired under drought, but also the speed with which the system shifts from diffusive limitation to deeper metabolic restriction.

The cuticle and epicuticular wax layer add another level of control. They do reduce residual transpiration, but the magnitude of this effect depends on the chemical composition, spatial organization, and barrier properties of the surface rather than on simple thickness alone (Bertolino et al., 2019; Grünhofer et al., 2023). For this reason, the rule "the thicker, the better" is physiologically misleading. Likewise, leaf thermal regime is not a secondary trait: the capacity to limit overheating reduces the probability of secondary heat injury, especially under combined drought and heat stress (Teskey et al., 2015). Thus, external leaf structure affects not only water conservation, but also the thermal and energetic environment in which photosynthesis continues under stress.

At the whole plant level, root system architecture and hydraulic organization are equally important. A well-developed root system, high hydraulic conductance, and the ability to maintain water flow delay the onset of critical water deficit in leaves and help sustain transpiration, cooling, and carbon acquisition for longer (Comas et al., 2013; Shekoofa et al., 2018; Martínez-Vilalta et al., 2017). However, the value of root investment cannot be interpreted independently of shoot demand. Drought performance depends not simply on having "more roots," but on effective root to shoot coordination, continuity of water transport, and the balance between water uptake, transport capacity, and evaporative demand. Structural drought tolerance is therefore not a property of isolated organs, but of the organization of the plant as an integrated hydraulic and photosynthetic system.

This helps explain why drought tolerance at the whole plant level can be described as a combination of avoidance, tolerance, and recovery strategies (Bandurska, 2022; Xu et al., 2010). Avoidance is associated with limiting water loss and improving water acquisition; tolerance involves the ability to maintain photochemical stability, redox balance, and metabolic function under low water potential; recovery refers to the rate at which *A*, *gs*, and hydraulic parameters return after rehydration (Bandurska, 2022; Xu et al., 2010). In practice, the most viable genotypes are usually those that combine these strategies rather than relying predominantly on only one of them (Bandurska, 2022). A highly conservative water saving strategy may prolong survival, yet reduce carbon gain too strongly; conversely, a more productive phenotype may maintain assimilation longer, but at the cost of faster hydraulic exhaustion. The most functionally successful plants are therefore not those that maximize a single trait, but those that achieve the most effective coordination among structural protection, physiological regulation, and post stress recovery.

In this context, it is especially important not to overestimate intrinsic water use efficiency:

$$iWUE = A/gs \quad (7)$$

A high *iWUE* may reflect either a truly economical and well-coordinated regime or simply an excessively strong reduction in *gs* when assimilation is already suppressed. Therefore, in both physiological and breeding contexts, *iWUE* should be interpreted together with absolute values of *A*, the capacity for post stress recovery, and final productivity (Lawson et al., 2014; Flexas et al., 2016). Strictly speaking, *iWUE* should be treated as an informative but partial index of functional regulation, not as a universal equivalent of drought tolerance (Lawson et al., 2014; Flexas et al., 2016).

Thus, drought tolerance is not a static trait, but a multidimensional systemic property that emerges at the intersection of hydraulics, stomatal dynamics, mesophyll CO_2 diffusion, metabolic stability, photochemical regulation, and plant structural organization (Bandurska, 2022; Martínez- Vilalta et al., 2017; Flexas et al., 2016). This interpretation is essential because it shifts the analysis from descriptive trait listing to an understanding of how traits interact functionally across scales. Precisely for this reason, the assessment of drought tolerance requires integrative phenotyping approaches capable of linking gas exchange, chlorophyll fluorescence, hydraulic behavior, and structural traits within a common physiological framework (Comas et al., 2013; Bertolino et al., 2019; Flexas et al., 2016).

INTEGRATION OF GAS EXCHANGE AND PAM FLUOROMETRY IN PHENOTYPING

Modern analysis of the photosynthetic response to water deficit requires the integration of two complementary methodological platforms: gas exchange and PAM fluorometry. Gas exchange reveals the extent to which CO_2 influx, transpiration, and net carbon assimilation are limited, whereas fluorometry reflects the functional state of PSII, the partitioning of absorbed energy, and the depth of photoprotective reorganization. Considered separately, each approach provides only part of the picture. Considered together, they make it possible to distinguish early diffusive limitation from fully developed biochemical and photochemical dysfunction (Urban et al., 2017; Baker, 2008; Murchie et al., 2013).

This integrative approach is especially important because drought does not affect a single step of photosynthesis. Rather, it progressively alters CO_2 diffusion, chloroplast substrate availability, carboxylation capacity, electron transport, energy dissipation, and post stress recovery. For this reason, physiologically meaningful phenotyping cannot be based on one parameter in isolation. It requires a coordinated interpretation of variables that describe both carbon metabolism and the functional state of the photosynthetic apparatus.

Analysis of *A* - *Ci* curves and the estimation of V*cmax*, J*max*, and, where possible, also *gm* and *Cc* allow quantitative separation of diffusive and biochemical contributions to photosynthetic limitation. In this context, it is particularly important to recognize that the use of *Ci* alone as an indicator of substrate CO_2 availability often leads to error, because *gm* may change under drought independently of *gs*. Without evaluating the mesophyll component, the role of stomatal limitation may be overestimated, while the contribution of internal dysfunction of the photosynthetic system may be underestimated (Farquhar et al., 1980; Flexas et al., 2004, 2008; Urban et al., 2017). Thus, the integration of gas exchange with mesophyll related interpretation is essential not only for parameter estimation, but also for correct physiological diagnosis.

Fluorescence parameters provide different, but equally important, types of information. *Fv/Fm* after dark adaptation and ΦPSII (light adapted Yield) answer different physiological questions and should therefore be

interpreted together: the former reflects the maximum potential efficiency of PSII and the degree of photoinhibition, whereas the latter reflects the current operational efficiency of photochemistry under the prevailing conditions. NPQ characterizes thermal dissipation, while photochemical quenching *(qP)* and fraction of open reaction centers *(qL)* provide information on the proportion of open reaction centers and the redox state of QA. On the basis of ΦPSII, electron transport through PSII is commonly estimated as:

$$ETR = \Phi PSII \times PAR \times \alpha \times \beta \quad (8)$$

This parameter is informative, but it must be interpreted cautiously, because α and β are often treated as constants even though they may vary with leaf structure, pigment content, and plant physiological status under stress (Maxwell et al., 2000; Baker, 2008; Kramer et al., 2004; Murchie et al., 2013; Urban et al., 2017). Thus, ETR is most useful not as an absolute value taken at face value, but as part of a broader comparative framework.

What is often most informative is not the individual value of a given parameter, but the pattern of relationships among parameters. For example, a decline in *A* accompanied by nearly unchanged *Fv/Fm* and only a moderate rise in NPQ generally indicates the predominance of diffusive and biochemical limitations while the core PSII structure remains preserved. A divergence between ETR and *A* may indicate increased reliance on alternative electron sinks, enhanced photorespiration, or the development of energetic imbalance. By contrast, a sharp decline in ΦPSII together with reduced *Fv/Fm* and strongly elevated NPQ points toward a more advanced state approaching combined photooxidative injury (Baker, 2008; Murchie et al., 2013; Urban et al., 2017). The diagnostic value therefore lies not in isolated numbers, but in the physiological configuration, they form together. This point is crucial for drought phenotyping. The most practically valuable traits include the ability to maintain relatively high *A* under a moderate decline in *gs*, the relative stability of *gm* and the absence of a pronounced drop in *Cc* as stress intensifies, the maintenance of *Fv/Fm* and ΦPSII together with a controlled increase in NPQ, a physiologically reasonable relationship between ETR and *A* without signs of chronic over reduction of the electron transport chain, and the speed and completeness of recovery of gas exchange and photochemistry after rehydration (Flexas et al., 2016; Urban et al., 2017; Xu et al., 2010). Such combinations of traits are more informative than any single marker because they reflect coordination among water relations, carbon metabolism, membrane regulation, and stress recovery.

Conversely, several popular indicators require careful interpretation. High *iWUE* may reflect productive water economy, but it may also simply result from severe stomatal closure. Elevated antioxidant enzyme activity often indicates not tolerance, but high stress intensity. Even a stable *Fv/Fm* does not guarantee maintenance of productivity if *A* and *gm* decline simultaneously. For this reason, practical criteria should be based on combinations of traits and on their physiological coherence rather than on an isolated marker taken out of context (Flexas et al., 2016; Baker, 2008; Urban et al., 2017).

In breeding and physiological monitoring, one of the most promising directions is the shift from isolated measurements to profiling of functional trajectories. A plant should be evaluated not only by a single value of *gs*, *gm*, *A*, ΦPSII, NPQ, or T*leaf*, but by how these parameters change over time and how they relate to one another during stress development and recovery (Urban et al., 2017; Xu et al., 2010). This dynamic approach makes it possible to identify where the dominant limitation of the system is located at a given stage: CO_2 diffusion, biochemical capacity, membrane photochemistry, or redox regulation. In other words, it transforms phenotyping from a static trait inventory into an analysis of physiological strategy.

An additional strength of this approach is its comparative value across genotypes, environments, and developmental stages. Because drought tolerance is inherently multidimensional, meaningful comparison requires not only standardized measurements, but also a shared interpretative framework linking gas exchange, fluorescence, thermal status, and recovery responses. The integration of gas exchange and PAM fluorometry provides precisely such a framework, because it connects light absorption, electron transport, carboxylation, photorespiration, respiratory support, and protective energy dissipation within a common physiological language (Urban et al., 2017; Baker, 2008; Murchie et al., 2013).

Thus, the integration of gas exchange and PAM - fluorometry should be regarded not simply as a combination of instrumental techniques, but as a conceptual model for the analysis of drought tolerance. Its main value lies in the ability to move from separate measurements toward a mechanistic understanding of how limitations arise, interact, and recover. For modern plant physiology and drought-oriented phenotyping, this is one of the most productive ways to assess tolerance in a form that is biologically interpretable and practically useful for both research and breeding (Urban et al., 2017; Baker, 2008; Murchie et al., 2013).

CONCLUSIONS AND PERSPECTIVES

Water stress limits photosynthesis not through a single mechanism, but through a sequential and partially overlapping network of processes. At the early stages, stomatal limitations predominate and are closely associated with the maintenance of tissue water status. As water deficit intensifies, the contribution of mesophyll conductance, biochemical carboxylation capacity, RuBP regeneration, and triose phosphate utilization becomes increasingly

important. Under conditions of sustained high irradiance, energy and redox imbalance develops, activating photoprotective mechanisms, photorespiration, mitochondrial respiration, and antioxidant defense. When these regulatory processes are no longer sufficient, the system shifts from adaptive reorganization to photoinhibition and deeper metabolic destabilization.

The principal conclusion of this review is that drought tolerance is determined not by extreme values of individual parameters, but by the degree of coordination among hydraulic status, stomatal dynamics, mesophyll CO_2 diffusion, chloroplast biochemical stability, photochemical regulation, and recovery capacity. In this sense, tolerance should be viewed primarily as a property of physiological coordination. A plant is not drought tolerant simply because one protective mechanism is more strongly expressed, but because multiple levels of regulation operate in concert and prevent the system from shifting toward irreversible damage.

For experimental plant physiology, this implies the need to move from recording individual stress symptoms toward an integrative analysis of functional processes. The most informative strategy is the combined assessment of gas exchange parameters, mesophyll conductance, chlorophyll fluorescence, leaf thermal status, microclimatic conditions, and recovery dynamics after rehydration. Only such an approach makes it possible to distinguish early adaptive reorganization, chronic restriction of carbon metabolism, and fully developed photooxidative damage.

In practical terms, experiments designed to assess drought tolerance should be conceived as multidimensional and dynamic. It is not sufficient to measure only water potential, only stomatal conductance, or only fluorescence parameters. Simultaneous control of PAR, leaf temperature, vapor pressure deficit, *gs* dynamics, changes in *gm*, photochemical status, and post stress recovery after rewatering is required. Particular attention should be paid to plant developmental stage, leaf position within the canopy, duration of stress exposure, and the interaction of drought with heat load. Without this context, adaptive regulation may easily be confused with damage, whereas short term compensation may be mistaken for true tolerance.

For breeding programs, a similarly important conclusion follows. The search for drought tolerant forms should be based on the coordination of physiological, morphological, and ultimately productivity related traits, and in the future also on their integration with molecular markers. The most promising genotypes are those that, under moderate water limitation, maintain an acceptable assimilation rate, avoid an early collapse of *gm* and ΦPSII, sustain a controlled increase in NPQ, and rapidly restore photosynthetic activity after rehydration. Under field conditions, where stress more often develops in waves than as a single constant episode, the combination of tolerance and reversibility of response is especially important.

Looking ahead, one of the most promising directions is the integration of high-resolution physiological phenotyping, molecular markers, root architecture analysis, remote sensing, and mathematical modeling of carbon water relations. Such an approach would make it possible to move from the description of individual mechanisms toward prediction of genotype behavior in real environments. The more closely molecular biology, physiology, ecology, and applied breeding are integrated, the greater the likelihood of developing cultivars that not only survive drought, but maintain functional productivity under conditions of climatic instability.

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