

Viewpoints

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Leaf physiological and morphological constraints of water-use efficiency in C₃ plants

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Abstract

The increasing evaporative demand due to climate change will significantly affect the balance of carbon assimilation and water losses of plants worldwide. The development of crop varieties with improved water-use efficiency (WUE) will be critical for adapting agricultural strategies under predicted future climates. This review aims to summarize the most important leaf morpho-physiological constraints of WUE in C₃ plants and identify gaps in knowledge. From the carbon gain side of the WUE, the discussed parameters are mesophyll conductance, carboxylation efficiency and respiratory losses. The traits and parameters affecting the waterside of WUE balance discussed in this review are stomatal size and density, stomatal control and residual water losses (cuticular and bark conductance), nocturnal conductance and leaf hydraulic conductance. In addition, we discussed the impact of leaf anatomy and crown architecture on both the carbon gain and water loss components of WUE. There are multiple possible targets for future development in understanding sources of WUE variability in plants. We identified residual water losses and respiratory carbon losses as the greatest knowledge gaps of whole-plant WUE assessments. Moreover, the impact of trichomes, leaf hydraulic conductance and canopy structure on plants' WUE is still not well understood. The development of a multi-trait approach is urgently needed for a better understanding of WUE dynamics and optimization.

Keywords: Crown architecture; leaf anatomy; mesophyll conductance; minimal conductance; respiration; rubisco; stomata; WUE.

Introduction

Water-use efficiency (WUE) reflects a balance between carbon gain and water loss in plants, introduced more than 100 years ago by Briggs and Shantz (1913). Since then, multiple ways and methods to assess WUE at a different level of organization and temporal resolution were developed and conceptualized (Vadez *et al.* 2014, 2023; Hatfield and Dold 2019; Brendel 2021). Two WUE parameters reflect a momentary state of leaf carbon and water fluxes: intrinsic water-use efficiency (WUE_i) as a ratio of CO₂ assimilation rate (A_n) to water vapour stomatal conductance (g_s), obtained during gas-exchange measurements at leaf level (Petrik *et al.* 2022a). Another closely related variant, instantaneous WUE_i, is calculated as a ratio of A_n and leaf transpiration (Bacon *et al.* 2004). Other WUE

parameters capture the long-term balance between carbon fixation and transpiratory water losses. Biomass-based indices include whole-plant WUE_{bio} as the ratio of biomass accumulation to cumulative transpiration of the plants (Condon *et al.* 2004; Brendel 2021). Furthermore, yield WUE is usually calculated as crop yield per hectare divided by total transpiration or evapotranspiration (Hatfield and Dold 2019; Zahoor *et al.* 2019). The use of growth-based WUE calculated as the ratio of annual basal area increment and cumulative annual transpiration is used in dendrobiology (Szatniewska *et al.* 2022). Moreover, the carbon isotope ratio (δ¹³C) has been extensively used as a proxy of long-term WUE_{13C}, because of the preference for the lighter isotope during physical and chemical processes involved in CO₂ uptake and assimilation (Farquhar *et al.* 1989; Frank *et al.* 2015; Ma *et al.* 2023).

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Ecosystem-wide WUE derived from eddy-covariance measurements (WUE_{GPP}) is a ratio between gross primary production (GPP) of the ecosystem and total cumulative transpiration or evapotranspiration (Yi *et al.* 2019). WUE_{GPP} can be also derived from remote sensing data as the GPP to evapotranspiration ratio (Ahmadi *et al.* 2019). Overall, the individual-level, long-term (vegetation season) based WUE_{bio} is the most precise assessment of real resource utilization of plants as they capture both assimilatory and respiratory balance with productive and unproductive water losses (Brendel and Epron 2022). WUE_{bio} should thus be more commonly used as the standard WUE estimates in agricultural and plant sciences, instead of the WUE_i , which is much easier to measure but represents only one point in time.

The importance of WUE acclimation in plants is due to raising evaporation demands caused by climate change and possible frequent water-deficit stress during seasonal droughts (Ponce-Campos *et al.* 2013; Schuldt *et al.* 2020). Plants with higher WUE will have a competitive advantage in natural ecosystems and economic significance for agricultural production. The momentary WUE_i of plants can be improved either by lower transpiration losses or higher efficiency of carbon assimilation (Flexas *et al.* 2016; Hatfield *et al.* 2019). Understanding of constraining factors of WUE is crucial for crop optimization efforts and the correct assessment of adaptive responses of plant communities (Quan *et al.* 2020; Kang *et al.* 2021). WUE variability is affected by multiple morphological and physiological traits (Figure 1). The size and density of stomata affect the maximal stomatal conductance and stomatal responsiveness to environmental changes (Nunes *et al.* 2022; Pitaloka *et al.* 2022). As stomatal morphology and anatomy can be altered with biotechnological methods for improved WUE, it is a great target for future research (Caine *et al.* 2019; Li *et al.* 2020). The responsiveness of stomata to fluctuating light and drought can also improve long-term WUE_{bio} (Xylogiannis *et al.* 2020; Zhao *et al.* 2021a). Several studies have found a negative correlation between WUE estimates and leaf hydraulic conductance (Wedegaertner *et al.* 2022; Barrera-Ayala *et al.* 2023; Liu *et al.* 2023), but these findings are still inconclusive (Corcuera *et al.* 2012; Sellin *et al.* 2014; Jin *et al.* 2016) and we need a better causal explanation of the relationship. Another important constraint of WUE is the mesophyll conductance (g_m) of CO_2 towards Rubisco (Flexas *et al.* 2016; Zhu *et al.* 2021). Maximization of the g_m/g_s ratio was suggested as a possible goal for improving WUE of crops (Flexas *et al.* 2013a; Fullana-Pericàs *et al.* 2017). The next step of WUE improvement is an optimization of Rubisco carboxylation efficiency (Flexas *et al.* 2016). The long-term WUE_{bio} enhancement could be further achieved by the reduction of respiratory losses and residual water losses during night or drought (Escalona *et al.* 2012; Coupel-Ledru *et al.* 2016). Finally, leaf anatomy, which influences both mesophyll conductance CO_2 and transpiratory losses, can also alter plant WUE (Bramley *et al.* 2013; Trueba *et al.* 2022).

The objective of this review paper was to summarize various morphological and physiological factors, which influence WUE in plants, as a stepping stone for a more holistic approach to the multi-factor assessment of WUE constraints (Figure 1). We also focused on identifying under-represented physiological and morphological traits in current research, which are needed for understanding WUE optimization in plants. Moreover, this review focuses specifically on WUE_i ,

WUE_{13C} and WUE_{bio} to provide the most possibly concise overview of this complex topic at a similar spatial scale. It is worth pointing out that environmental factors such as water availability (Amitrano *et al.* 2019; Zhao *et al.* 2021b), soil structure (Hatfield *et al.* 2001; Rabarijaona *et al.* 2022), air pollution (Hatfield *et al.* 2001; Rabarijaona *et al.* 2022) and nutrients (Dijkstra *et al.* 2016; Gharun *et al.* 2021; Song *et al.* 2022) can also have a significant impact on WUE. However, this falls beyond the scope of the study and is therefore not further discussed.

Water Side of WUE

Stomatal density and trichomes

Plants can influence their transpiratory losses and therefore potentially their WUE via stomatal regulation (Hetherington and Woodward 2003; Bertolino *et al.* 2019). The stomatal adjustment could include changes in stomatal density (SD), stomatal anatomy (size, shape) and stomatal control mechanisms (Sack and Buckley 2016; Petrik *et al.* 2022b). Multiple recent studies, which used genetic manipulation methods to alter SD, have reported improved WUE_i connected to the reduction of SD. A genetic manipulation (*EPF2OE*) approach in a study by Franks *et al.* (2015) led to *Arabidopsis* mutants with lower SD that showed higher WUE_i and long-term WUE_{13C} due to lower stomatal conductance of water vapour (g_s) but unchanged photosynthetic capacity. Similarly, a combination of high-yield rice cultivars with overexpressed OsEPF1 epidermal patterning factor (EPF) led to a reduction of SD, lower g_s , improved WUE_i and overall drought tolerance (Caine *et al.* 2019). The EPF overexpression in bread wheat has led to similar results of reduced SD and improved WUE_i , without yield losses (Dunn *et al.* 2019). Guo *et al.* (2019a) have reported the genetic pathway of EDT1/HDG11, ERECTA, and E2Fa loci, which regulates WUE_i of *Arabidopsis* via modulation of SD. Overexpression of *SITLFP8* (Tubby-like F-box protein 8) reduced SD by 10–20 % in tomatoes and was connected to enhanced WUE_i (Li *et al.* 2020). Similarly, repression of *PuGTL1* via Pu-miR172d overexpression led to a reduction of SD and higher WUE_i in *Populus ussuriensis* (Liu *et al.* 2021). On the other hand, overexpression of STOMAGEN led to higher SD, greater photosynthetic activity (+30 %), but also greater transpiration (+100 %), which resulted in reduced WUE_i (Tanaka *et al.* 2013). Contrary, the study by Bhaskara *et al.* (2022) also reported a positive relationship between SD and WUE_{bio} derived from natural variation in *Arabidopsis* accessions. Moreover, other leaf structures such as trichomes (trichomes/SD ratio) can play a significant role in WUE_i and WUE_{bio} enhancement via lower transpiratory losses due to leaf–air boundary layer resistance (Mo *et al.* 2016; Galdon-Armero *et al.* 2018). For example, Chen *et al.* (2022) observed a doubling in trichome density and a decline in g_s by 85 % between droughted and well-watered *Shepherdia × utahensis* plants. Single gene manipulation efforts, such as *EPF2OE*, could have negative pleiotropic effects on other metabolic processes and should be further explored to avoid these negative side effects (Flexas *et al.* 2016; Husaini *et al.* 2022). It seems that the reduction of SD for improving WUE_i and $WUE_{13C/bio}$ could be a viable option for plant breeding initiatives. Additionally, the incorporation of further leaf structures, such as trichomes, in combination with SD can improve our understanding of WUE constraints.

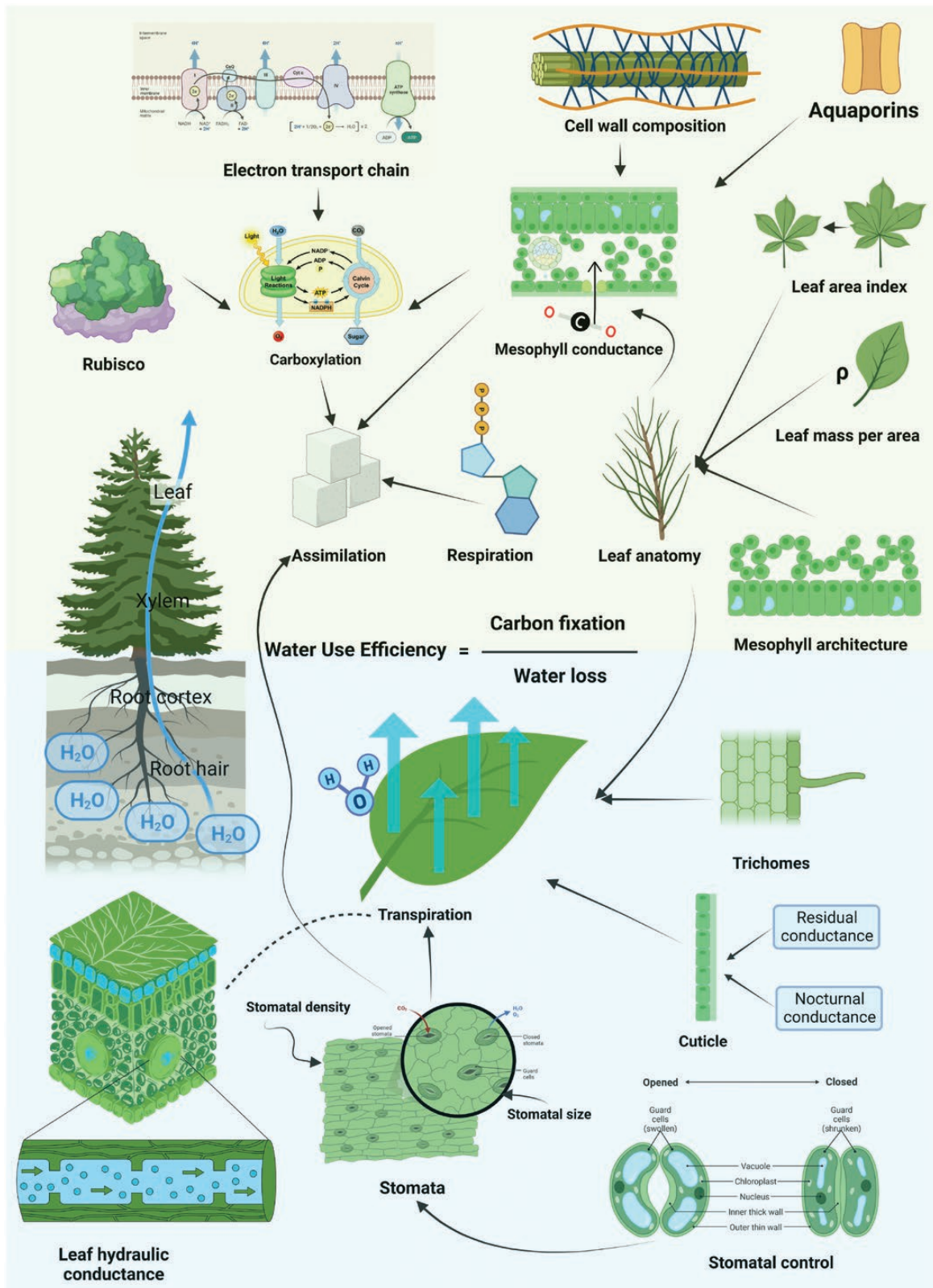


Figure 1. Overview of mechanisms and traits which affect the carbon fixation (upper half) and water loss (lower half) components of water-use efficiency in C_3 plants. Created with BioRender.com and adapted with Canva.com.

Stomatal size and responsiveness

Stomatal control mechanisms include reaction to atmospheric vapour pressure deficit (Grossiord *et al.* 2020), plant water potential (Buckley 2005, 2019; Dayer *et al.* 2020), light conditions (Lawson *et al.* 2010; McAusland *et al.* 2013) and CO₂ concentration (Franks and Beerling 2009). Photosynthetic activity of C₃ plants can adjust in seconds to changes in irradiance, but the lag in stomatal responses limits the CO₂ uptake and therefore constrains photosynthesis and limits WUE (Lawson *et al.* 2012). Several studies have reported that smaller stomata respond faster than larger stomata to changes in environmental conditions (Drake *et al.* 2013; Lawson *et al.* 2014; Kardiman and Raebild 2018; Durand *et al.* 2019). Faster stomatal response in the study by Lawson *et al.* (2014) has been linked to higher WUE_i values under naturally changing irradiance levels. Theoretical maximal stomatal conductance (g_{max}) showed a negative correlation with stomatal size, but smaller stomata showed faster response time to variable irradiance in five *Banksia* species (Drake *et al.* 2013). A study by Lei *et al.* (2023) also found that larger stomata of domesticated rice showed slower response time to fluctuating light and overall lower WUE_{13C}. The genetic manipulation study in rice has found that mutants with small stomatal size showed higher WUE_i, in comparison to mutants with greater stomatal size (Pitaloka *et al.* 2022). Des Marais *et al.* (2014) found that *Arabidopsis* genotypes with larger stomata due to AtMPK12 substitution showed lower WUE_i compared with the common allele. The improved WUE_i of wheat cultivars under water-deficit stress was linked to smaller stomatal size, lower SD and reduced transpiration rates (Li *et al.* 2017). A study by Amitrano *et al.* (2021) showed that a 49 % increase in WUE_i and WUE_{bio} of lettuce has been associated with a reduction of stomatal size under different vapour pressure deficit (VPD) treatments. Moreover, drought stress exposure inhibited stomatal development (smaller stomata) and increased the WUE_i in cotton (Dubey *et al.* 2023). On the other hand, a study by Xiong and Flexas (2020) on ferns, gymnosperms and angiosperms found a negative correlation between stomatal size and g_m , therefore possibly limiting WUE. A comparison of *Quercus robur* genotypes has found a positive correlation between guard cell length and WUE_{13C}, contradicting the majority of results suggesting that smaller stomata promote higher WUE_{13C} (Roussel *et al.* 2009). Liu *et al.* (2018a) have found a quadratic relationship between stomatal size and WUE_i at the community level, across forest ecosystems along the latitudinal transect, with an optimal stomatal size of approximately 400 μm^2 . Smaller stomatal size could be connected to higher WUE in plants, presumably due to faster response to environmental conditions. Nevertheless, there is probably an optimal stomatal size and further reduction can be detrimental due to CO₂ limitations of photosynthesis.

Stomatal control and light sensitivity

Excessive water loss under an impaired state of photosynthetic apparatus (drought, salinity stress) can negatively affect the WUE of plants. Timely stomatal closure is then another major component of WUE optimization of plants under water-deficit stress (Yang *et al.* 2016; Hartmann *et al.* 2021). A study by Yi *et al.* (2019) showed that WUE_{13C} of isohydric species was generally more sensitive to environmental change due to their conservative water potential regulation strategy than WUE_{13C} of the anisohydric species

and increased significantly with rising VPD during periods of water stress. The accumulation of abscisic acid (ABA), which drives the stomatal closure of plants under water deficit, can be considered a key factor for both WUE_i and WUE_{13C} improvement in plants (Negin and Moshelion 2016; Guo *et al.* 2019b; Mukarram *et al.* 2021). Plants capable of fine-tuning their stomatal control with ABA can possess an enhanced WUE_i with sustained biomass or yield gains (Yoo *et al.* 2009; Yao *et al.* 2021). Improved WUE_i in the presence of elevated ABA levels has been demonstrated in transgenic *Arabidopsis* (Zhang *et al.* 2008) and tomato (Thompson *et al.* 2007; Lamarque *et al.* 2020). Exogenous application of ABA showed enhanced WUE_i and WUE_{13C} in *Populus davidiana* (Li *et al.* 2004) and *Marsilea crenata* fern (Tai-Chung *et al.* 2020). French bean and sugar beet plants pretreated with ABA also showed improved WUE_i under water-deficit stress (Pospíšilová and Batková 2004). Enhanced stimulation of ABA signalling of *Arabidopsis* via distinct ABA receptors can result in constitutively high WUE_i (Yang *et al.* 2016). WUE_{13C} of *Arabidopsis* and wheat was also enhanced by modulating ABA responses either by using overexpression of specific ABA receptors or deficiency of ABA coreceptors (Yang *et al.* 2019). ABA receptors from *Populus canescens* were stably introduced into *Arabidopsis* in a study by Papacek *et al.* (2019), which led to enhanced WUE_i. Moreover, overexpression of *PeJAZ2* increased WUE_i of poplar under drought stress by regulating ABA signalling rather than ABA synthesis (Rao *et al.* 2023). Partial root-zone drying can generate a root-to-shoot pressure signal from the dry part of the root zone that also promotes stomatal closure via a drop in cell turgor and enhances WUE_i via ABA utilization (Davies *et al.* 2002; Pérez-Pérez *et al.* 2012; McAdam and Brodribb 2016; Zhang *et al.* 2018; Xylogiannis *et al.* 2020). These results, therefore, suggest great opportunities for WUE optimization in crops with the use of transgenic methods, breeding efforts and biotechnological tools for ABA utilization.

Stomatal sensitivity to light could be another important determinant of plant WUE_i by adjusting the magnitudes of change in g_s as a function of the environment (Viale-Chabrand *et al.* 2016). Part of the stomatal response involves the balance between photosynthetic electron transport and carbon reduction either in guard cells, chloroplasts, or in the mesophyll (Messinger *et al.* 2006). Overexpression of *Photosystem II Subunit S* in tobacco led to lower stomatal opening in response to light, which resulted in a 25 % reduction of water loss and improved WUE_i (Głowacka *et al.* 2018). The desynchronization of A_n and g_s can lead to a surplus in transpiration when A_n is low but g_s is high (e.g. transition from high to low light), hence reducing WUE_i (McAusland *et al.* 2016; Coupel-Ledru 2021). The introduction of a blue light-activated K⁺ ion channel, named BLINK1, to *Arabidopsis*, led to a faster reaction of stomatal aperture under both increasing and decreasing irradiance, which ultimately enhanced the plants' biomass accumulation and WUE_{bio} (Papanatsiou *et al.* 2019). Dynamic plant response to VPD and light fluctuations under natural conditions were suggested to increase plants WUE_{bio} (Gosa *et al.* 2019). Lower stomatal openness and lower g_s under short-term light transitions led to higher WUE_i in chilli pepper treated with "smart glass" compared to the control group (Zhao *et al.* 2021a). A study by Li *et al.* (2023) found that overexpression of *OE-PtrVCS2* in *Populus trichocarpa* led to smaller stomatal aperture under drought stress and overall higher WUE_i than

in the wild type. Greater WUE_i of isohydric Pine species has been also linked to lower stomatal openness under increasing light, while anisohydric Oak species behaved more opportunistically with lower WUE_i (Renninger *et al.* 2015). Reduction of stomatal openness as a reaction to light changes can probably improve the WUE of plants but can lead to a reduction of the total growth and yield of crops. Nevertheless, improving stomatal response time to changing irradiance levels can improve the plants' WUE without a negative impact on assimilation and growth.

Residual and nocturnal conductance

When the stomata are closed (night, drought), plants are still losing water via their cuticle, bark or incompletely closed stomata (Duursma *et al.* 2019; Lintunen *et al.* 2021). Cuticular transpiration has been recognized as a significant factor affecting drought survival rates (Duursma *et al.* 2019) and might affect WUE_{13C/bio} due to residual transpiration (Ni *et al.* 2012; Ávila-Lovera *et al.* 2019). Minimum leaf conductance (g_{\min}) incorporates water loss across the leaf cuticle, bark and through the incompletely closed stomata (Schuster *et al.* 2017; Blackman *et al.* 2019; Duursma *et al.* 2019; Lintunen *et al.* 2021). Minimization of these residual losses during periods of reduced assimilation rate due to stomatal limitations can therefore lead to improved long-term WUE_{13C/bio} (Sevanto 2020). The water loss from leaves of plants under drought is dominated by g_{\min} after stomatal closure. This has been related to the thickness of the cuticular wax layer, which increases in response to water deficit (Jeffree 2006; Shepherd and Wynne Griffiths 2006; Bueno *et al.* 2020). However, a relationship between the thickness of the cuticular wax layer and g_{\min} can be insignificant, both within (Anfodillo *et al.* 2002; Bueno *et al.* 2020) and across species (Riederer and Schreiber 2001). The variability of g_{\min} can be also driven by stomatal morphology (leaky stomata) or chemical composition of cuticle (Duursma *et al.* 2019; Machado *et al.* 2021). In a recent study across 23 genotypes of wheat, cuticular transpiration showed a strong positive correlation with water loss per dry mass unit, which the authors considered as a proxy for WUE_{bio} (Gašparovič *et al.* 2021). A modelling simulation approach by Duursma *et al.* (2019) revealed a theoretical reduction of WUE_i under increasing g_{\min} of plants using the general Ball-Berry model of stomatal conductance. Moreover, hydroponically grown *Festuca arundinacea* exposed to salinity treatment showed enhanced WUE_i and lower g_{\min} compared to the control group (Vandegerer *et al.* 2021). On the other hand, eucalyptus clones under water-deficit treatment showed significant intra-specific differences in cuticular conductance but not in WUE_i (Carignato *et al.* 2019). A study by Clarke *et al.* (1991) also found no significant correlation between minimal conductance and long-term WUE_{bio} in wheat under drought stress. The impact of cuticular conductance or g_{\min} on WUE has not been yet properly quantified and is therefore a great target for future research.

The analogical parameter, nocturnal conductance, is also critical for optimization of long-term WUE_{13C/bio} (Coupel-Ledru *et al.* 2016; Even *et al.* 2019). Excessive water losses during the night (Dawson *et al.* 2007; Forster 2014) decrease long-term WUE as there is no photosynthetic gain during the night. It has been suggested that the low nocturnal conductance of shade-tolerant plant species is consistent with their conservative water-use strategy (Resco de Dios *et al.*

2019). Nocturnal conductance is usually dominated by cuticular transpiration, but incomplete stomatal closure during the night has been observed in C₃ plants (Caird *et al.* 2007; Escalona *et al.* 2012). Reduction of night transpiration can theoretically improve the WUE_{bio} of crops without growth penalties (Tardieu *et al.* 2022). A study by Dayer *et al.* (2021) has shown that night transpiration was linked more to the specific circadian rhythm of the wine cultivars rather than environmental conditions, suggesting strong genetic control. Night transpiration also had a significant impact on total transpiration and WUE_{bio} in a study by Medrano *et al.* (2017) and was recognized as one of the under-explored factors affecting whole-plant WUE. Nocturnal conductance also showed a significant negative correlation with WUE_{bio} among black poplar genotypes exposed to drought stress (Bogeat-Triboulot *et al.* 2019). Differences in the night transpiration between *Pinus contorta* thinning treatments corresponded to differences in WUE under water-deficit stress (Wang *et al.* 2020). Further quantification of the night transpiration effect on the long-term WUE of plants is needed for a proper understanding of the phenomenon. Selection for plants with low cuticular conductance and conservative stomatal control (avoiding leaky stomata) can greatly improve their WUE and drought resistance.

Leaf hydraulic conductance

Leaf hydraulic conductance (K_{leaf}) can be coordinated with higher WUE_i, as observed in several studies (Fichot *et al.* 2009; Andrade *et al.* 2022; Wedegaertner *et al.* 2022). Nevertheless, it is still unknown if the plants with higher WUE develop smaller xylem vessels causing lower K_{leaf} (but greater xylem embolism resistance, cf. Isasa *et al.* 2023) as they have lower hydraulic requirements to maintain leaf gas exchange, or the lower K_{leaf} leads to greater WUE by constraining water supply in leaves. K_{leaf} is tied to leaf assimilation and stomatal conductance rate in a positive linear fashion (Santiago *et al.* 2004; Sellin *et al.* 2014). Reduction of leaf hydraulic conductance via gene manipulation can lead to lower water losses but is also tied with a proportional reduction of assimilation rates and therefore non-significant changes in WUE_{bio} (Zsögön *et al.* 2015). The environmental response of K_{leaf} and its impact on WUE has received more attention in recent studies and has been identified as a major trait that could constrain WUE under changing VPD (Flexas *et al.* 2013a; Xiong *et al.* 2018). However, no consensus has been reached to date regarding the direction of the relationship between K_{leaf} and WUE. On one hand, Yao *et al.* (2021) reported that raising WUE_i of *Caragana* sp. with decreasing water potential was coordinated with decreasing K_{leaf} but also rapid biosynthesis of ABA. The *Solanum* species with significantly lower K_{leaf} showed also significantly higher WUE_{13C} under well-watered conditions (Barrera-Ayala *et al.* 2023), while WUE_i of *Ginkgo biloba* was also negatively correlated with K_{leaf} (Liu *et al.* 2023). Warming treatment in four subtropical tree species led to higher K_{leaf} but lower WUE_{13C} (Wu *et al.* 2020). On the other hand, Jin *et al.* (2016) reported a positive relationship between K_{leaf} and WUE_i among 10 temperate tree species. Similarly, a positive correlation between WUE_{13C} and K_{leaf} was reported for *Pinus pinaster* populations exposed to drought stress (Corcuera *et al.* 2012). Moreover, Sellin *et al.* (2013, 2014) found no significant correlation between WUE_i and K_{leaf} in birch and aspen trees. In conclusion,

the direction of the K_{leaf} -WUE relationship is unclear, and further work must be conducted to assess whether breeding for lower K_{leaf} to reduce water losses possibly leads to improved WUE without a significant reduction of growth. Future experiments with gene manipulation techniques that will not affect other physio-morphological traits are needed to understand the causal link of these correlations.

Carbon Side of WUE

Mesophyll conductance

Improving CO_2 diffusion to the sites of carboxylation without increasing stomatal conductance can enhance WUE_i . This requires improving mesophyll conductance to CO_2 (g_m) and it has been proposed that the ratio g_m/g_s is a relevant breeding trait for improving WUE (Galmés *et al.* 2011; Flexas *et al.* 2013b; Tomás *et al.* 2014a; Flexas 2016). The g_m has been recognized as one of the main limiting factors of WUE in both crops (Leakey *et al.* 2019) and tree species (Zhu *et al.* 2021), potentially due to the close coupling of g_m and K_{leaf} as both share the same pathways of water movement in leaves (Flexas *et al.* 2013b; Xiong *et al.* 2017). A close positive relationship has also been observed between g_m and g_s although the reason for this remains speculative (Guiliani *et al.* 2013; Barbour and Kaiser 2016). However, a study by Fullana-Pericas *et al.* (2017) showed a strong positive correlation between g_m/g_s and WUE_i in Mediterranean tomato landraces. Similarly, WUE_i showed a strong positive correlation with g_m/g_s in tobacco under chloride nutrient treatments (Franco-Navarro *et al.* 2019). The variability of g_m has been linked to leaf anatomy, where cell wall thickness, membrane permeabilities, cytosol and stromal conductance were constraining factors of g_m (Terashima *et al.* 2011; Tomás *et al.* 2013; Ouyang *et al.* 2017). The cell wall conductance to CO_2 can be influenced by cell wall thickness, porosity and tortuosity (Evans *et al.* 2009; Ellsworth *et al.* 2018). A study by Roig-Oliver *et al.* (2020) found a strong negative correlation between cellulose and g_m in grapevine. The hemicellulose to pectin ratio of the cell wall correlated positively with the g_m of tobacco exposed to drought and salinity stress (Clemente-Moreno 2019). Tholen *et al.* (2008) manipulated the chloroplast arrangement in *Arabidopsis* and thus modified g_m through changes in the surface of chloroplasts exposed to the intercellular air spaces (Sc/S). The positive impact of Sc/S on g_m and A_n has been observed also for Mediterranean oak species (Peguero-Pina *et al.* 2017), rice (Xiong *et al.* 2017) and tobacco (Clarke *et al.* 2021). A recent study by Baillie and Fleming (2020) has found that coordination of stomatal and mesophyll development is crucial for the optimization of g_m and therefore WUE. Findings to date suggest that certain stomatal development signalling components, such as TMM, ER and STOMAGEN, may be required for interlayer coordination, and that gas exchange may also regulate mesophyll structure (Dow *et al.* 2017). Acclimation of g_m to changing environmental conditions has been linked to aquaporins and carbonic anhydrase (Flexas *et al.* 2006; Warren 2007). The g_m can be affected by specific genes (e.g. aquaporin *NtAQP1*, *HvPIP2*, *AtBBX21*) and thus targeted by genetic manipulation of crops (Evans *et al.* 2009). Overexpression of aquaporin genes led to increased g_m (Hanba *et al.* 2004) and inhibition of lower g_m in various crops (Flexas *et al.* 2006). Tobacco aquaporin *NtAQP1* aids the trans-membrane transport of CO_2 in plants and thus

contributes to the CO_2 permeability of the plasma membrane of the mesophyll cells (Uehlein *et al.* 2003). Carbonic anhydrase activity has been positively correlated to g_m (Price *et al.* 1994; Momayyezi and Guy 2017) and chloroplast fraction of g_m (Gillon *et al.* 2000). Carbon anhydrase accelerates the interconversion of the dissolved inorganic carbon species, CO_2 and HCO_3^- , which helps optimize the initial stages of photosynthesis. A recent study by Gómez-Ocampo *et al.* (2021) found that overexpression of *AtBBX21* led to enhanced g_m and J_{max} , coupled with higher WUE in potato plants under drought. Moreover, manipulation of heterotrimeric G protein signalling can improve plants' WUE_i and productivity due to higher g_m rates under drought conditions (Zait *et al.* 2021). More specifically, the canonical $G\alpha$ (RGA1) subunit gene of G protein regulated g_m in rice, which was reflected in improved photosynthetic capacity and overall WUE (Wang and Botella 2022). The optimization of g_m and therefore WUE is multifaceted and incorporates multiple organizational levels from cell biochemistry to whole leaf anatomy. There is also great intra-specific variability of g_m across crops (Tomás *et al.* 2014a; Chen *et al.* 2021) and trees (Momayyezi and Guy 2017; Peguero-Pina *et al.* 2017) and therefore, it is a reasonable target for breeding efforts which aim at maximizing WUE. Nevertheless, the practical performance of the population/individual's selection could be hindered by the low reliability of current g_m measurements (Pons *et al.* 2009; Lundgren and Fleming 2020). The development of more precise g_m measurement techniques (Márquez *et al.* 2023) could greatly improve the understanding of WUE constraint by g_m . Furthermore, the strong coupling of g_m with K_{leaf} (Flexas *et al.* 2013; Xiong *et al.* 2017) and g_s (Guiliani *et al.* 2013; Barbour and Kaiser 2016) might impede efforts to improve WUE_i through modification of g_m . As shown by Pathare *et al.* (2023) using rice cell wall mutants, modifying g_m indeed increases photosynthetic capacity but at the cost of simultaneously increasing g_s , resulting in no overall change in WUE_i .

Carboxylation rate

Another target to achieve improved photosynthesis is to improve the biochemical capacity for CO_2 assimilation, that is, improving the carboxylation efficiency of Rubisco for C_3 species (Gago *et al.* 2014; Flexas *et al.* 2016). Optimizing the efficiency of RuBP carboxylation by Rubisco has the potential of improving WUE by decreasing the concentration of CO_2 required to achieve high photosynthetic rates (Carmo-Silva *et al.* 2015). The maximum carboxylase activity of Rubisco (V_{cmax}) and the capacity for photosynthetic electron transport (J_{max}) can constrain the WUE from the carbon assimilation side. Maintenance of functional electron transport under drought stress led to higher WUE_i in *Magnolia grandiflora* (Vastag *et al.* 2020). Reduction of V_{cmax} under ozone treatment caused decoupling of photosynthesis and stomatal conductance, which led to lowered WUE_i in rice (Masutomi *et al.* 2019) and poplar clones (Xu *et al.* 2022). V_{cmax} and therefore photosynthetic capacity increases with leaf maturation, thus young spring foliage can experience reduced $\text{WUE}_{13\text{C}}$, which can be critical, especially during spring droughts (Cernusak 2020). Enhanced WUE_i of common bean genotypes under heat stress was linked to higher V_{cmax} (Suárez *et al.* 2021). Additionally, V_{cmax}/g_s ratio has been suggested as a useful trait to characterize WUE_i variability (positive correlation) across multiple plant species (Flexas *et al.* 2014). Acclimation

of WUE_i and WUE_{13C} was coupled to V_{cmax} and J_{max} across *Arabidopsis* genotypes in a study by Easlon *et al.* (2014). Moreover, the improvement of WUE_i in *Brassica juncea* was linked to higher carboxylation efficiency (A/C_i) under biochar treatment (Silva Gonzaga *et al.* 2019). Photosynthesis and therefore WUE_i can be limited by Rubisco and RuBP regeneration, especially under high irradiance conditions (Galmés *et al.* 2014). Plants with simultaneous stimulation of RuBP regeneration and electron transport can improve their WUE_i due to better photosynthetic capacity (López-Calcagno *et al.* 2020). Other alternatives to improve the WUE_{13C/bio} would be decreasing photorespiration by means of higher Rubisco efficiency for CO₂ (Whitney *et al.* 2011; Parry *et al.* 2013) or altering the photorespiratory CO₂ release by adjusting metabolic pathways in leaves (Peterhansel and Maurino 2011). Total leaf N content shows a significant positive impact on the carboxylation capacity of plants (Wright *et al.* 2003; Paillassa *et al.* 2020). The identification of specific amino acids affecting Rubisco kinetics (Orr *et al.* 2016) may provide suitable targets for improving CO₂ assimilation and consequently WUE_i (Nadal and Flexas 2019). Further exploration of optimization of Rubisco activity can positively influence the WUE of plants without any direct trade-off with growth capacity and yield of crops.

Respiration

Carbon loss through respiration is another process that decreases WUE_{bio} (Seibt *et al.* 2008; Gago *et al.* 2014; Tortosa *et al.* 2016). Plants with lower maintenance respiration rates can maintain higher WUE_{bio}. Moreover, respiration could be considered the main factor behind the gap between WUE_i and whole-plant WUE_{bio} (Medrano *et al.* 2017). High respiratory losses were linked to lower WUE_{bio} of C₄ *Miscanthus x giganteus* located in USA drylands (Maleski *et al.* 2019). Greater night-time respiration (i.e. high nocturnal transpiration) has been also recognized as one of the major factors behind the reduction of WUE_{bio} under magnesium deficiency of barley (Tränkner *et al.* 2016). High VPD fluxes led to larger reductions in photosynthesis in comparison to respiration, which decreased the overall productivity and WUE_{bio} of plants from a semi-arid ecosystem (Roby *et al.* 2020). The higher stability of mitochondria and susceptibility of chloroplasts, especially PSII, to abiotic stress can negatively influence the balance between carbon assimilation and respiration towards lower WUE_i (Dahal and Vanlerberghe 2017). Root respiration explained around 40 % of WUE_{bio} reduction in both well-irrigated and non-irrigated treatments of grapevine (Tomás *et al.* 2014b). Root respiration might be a major component of total plant respiration and thus an important target for further exploration for WUE_{bio} optimization (Escalona *et al.* 2012). Leaf development (maturation) connected with greater respiratory losses could be seen as an additional constraint to long-term WUE_{13C} (Zufferey 2016; Hernández-Montes *et al.* 2019). There is a still lack of precise quantification of day respiration or night-time respiration effect on whole-plant WUE_{bio} and further research is needed. Nevertheless, respiration is connected with plant growth and fruit ripening. Therefore, plant breeding or genetic manipulation efforts that would aim at reducing respiration rates would probably lead to a significant reduction of growth and/or yield. Higher respiratory losses could be also linked to the upregulation of antioxidant systems and artificial reduction of respiration

could be therefore defective. The inclusion of respiration for WUE calculation creates a more robust estimate, which improves the correlation with whole-plant WUE_{bio} (Cernusak *et al.* 2007; Zhang *et al.* 2019). For example, Senbayram *et al.* (2015) have shown that the 9.8–48.6 % beneficiary effect of nitrogen fertilization on daytime WUE_i was lost when nocturnal stomatal conductance and night-time respiration were taken into consideration. Therefore, the respiratory aspect of carbon balance should not be neglected for correct total plant WUE_{bio} estimates.

Leaf Anatomy and Plant Crown Architecture

Leaf anatomy can affect the mesophyll diffusion conductance to CO₂, carboxylation capacity and ultimately WUE in plants (Tomás *et al.* 2013; Carriquí *et al.* 2015). Increasing internal air volume might have positive effects on WUE_i (Mediavilla *et al.* 2001), probably due to enhanced internal CO₂ conductance to the site of carboxylation. Similarly, Guerfel *et al.* (2009) reported more efficient water use associated with thicker palisade parenchyma in olive trees. The leaves' architecture can influence the WUE_i due to variable mesophyll porosity and SD to intercellular airspace volume ratio in coniferous tree species (Trueba *et al.* 2022), and cell wall properties such as cell wall thickness (T_{cw}) might influence g_m and thus WUE_i (Flexas *et al.* 2021; Pathare *et al.* 2023). Mutant rice populations with higher leaf mass per area (LMA) showed improved whole-plant WUE_{bio} under both control and water-limited conditions (Reddy *et al.* 2020a). In the study by Horike *et al.* (2021), WUE_i of five shrub species covaried with LMA under drought stress. LMA differences explained WUE_{13C} variance across rice mutants through its influence on carbon gain (Reddy *et al.* 2020b). A study by Medrano *et al.* (2009) also reported a positive correlation between WUE_i and LMA in Mediterranean herbs and shrubs. Similarly, LMA was positively correlated with WUE_{13C} among trees (*Betula*, *Larix*, *Pinus*) in the boreal forest (Ge *et al.* 2022). A thicker leaf can be associated with a thicker boundary layer, which lowers transpiratory losses and ultimately improves WUE_{bio} (Bramley *et al.* 2013). The manipulation of leaf anatomy has been proposed as a potential theoretical target for improving photosynthetic capacity and WUE in plants (Tholen *et al.* 2012). The development of plants with thicker leaves and high internal air volume can theoretically improve their WUE.

Further macro-morphological constraint, which affects the whole-plant WUE_{bio}, is plant crown architecture (Christina *et al.* 2016; Medrano *et al.* 2017; McNeil *et al.* 2023). A more complex crown architecture creates shade for inner leaves, which can reduce evaporative demand and therefore improve WUE balance. A positive effect of shading treatment on leaf-level WUE_i has been observed for *Actinidia chinensis* (Chartzoulakis *et al.* 1993), *A. deliciosa* (Montanaro *et al.* 2009), *Citrus aurantium* (García-Sánchez *et al.* 2015), *C. sinensis* (Jifon *et al.* 2003; Syvertsen *et al.* 2003), *Coffea arabica* (Liu *et al.* 2018b) and *Fragaria xananassa* (Cordoba-Novoa *et al.* 2022). It is notable to say that shade leaves are optimized for low irradiance and if exposed to direct sunlight (crown damage) they can show decreased WUE_i (Dai *et al.* 2009). Moreover, the leaves of *Pinus taeda* in the lower parts of the crown showed significantly higher WUE_i in comparison to the upper part during the peak of the vegetation season (Blazier *et al.*

2004). The WUE_{13C} derived from wood in *Fagus crenata* and *Quercus crispula* showed a positive correlation with tree height, crown depth and crown width (Osada *et al.* 2004). Furthermore, Glenn *et al.* (2015) showed that the less complex pillar form of *Prunus persica* had lower WUE_i due to higher canopy transpiration in comparison to the common crown form. Leaf area index (LAI) as an indicator of crown density also shows a positive impact on WUE_{bio} across various terrestrial ecosystem types (Li *et al.* 2018; Luo *et al.* 2022). The raising WUE_{bio} of Alpine grasslands has been also linked to increasing LAI (Ma and Zhang 2022). Nevertheless, higher LAI and therefore greater total transpiration can be detrimental for arid regions where it can have a negative impact on WUE_{bio} (Malone *et al.* 2016). More complex crown architecture and higher LAI can enable plants to optimize and improve their whole-plant WUE due to the shading effect and probably also due to better microclimatic conditions within the crown.

Conclusion and Future Prospects

The WUE balance of plants is multifaceted and affected at multiple levels of organization from molecular to whole-plant level. The main constraining factors identified in this review were stomatal morphology and control, minimal and nocturnal conductance, mesophyll conductance, carboxylation efficiency, respiration rates, leaf anatomy and crown architecture. The traits are usually analysed in research papers separately or in specific combinations (e.g. stomatal morphology and gas exchange). We suggest that future research should include multi-trait analyses with the aim of WUE optimization, thereby deepening our understanding of the coupling and decoupling of carbon uptake and water-use traits. The technological progress of phenotyping platforms can lead to more robust experimental designs that could handle multi-trait analysis. The night-time transpiration and respiration seem to be under-developed major aspects of long-term WUE optimization, which could be further investigated. The effect of leaf hydraulic conductance and canopy structure on WUE is also not very well understood and can be improved. A better understanding of morpho-physiological constraints of WUE can help us to effectively develop more drought-resilient crop and tree species.

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Contributions by the Authors

PP conceived the paper idea. PP, APP and MM wrote the first draft. BS and LJL supervised the process and helped with the editing of the manuscript.

Conflict of Interest Statement

None declared.

Supporting Information

The following additional information is available in the online version of this article –

Data Availability

No original data was used in this commentary. The discussion and synthesis are based on already published studies.

References

- Ahmadi B, Ahmadi-pour A, Tootle G, Moradkhani H. 2019. Remote sensing of water use efficiency and terrestrial drought recovery across the contiguous United States. *Remote Sensing* 11:731.
- Amitrano C, Arena C, Roupheal Y, De Pascale S, De Micco V. 2019. Vapour pressure deficit: the hidden driver behind plant morphofunctional traits in controlled environments. *Annals of Applied Biology* 175:313–325.
- Amitrano C, Roupheal Y, Pannico A, De Pascale S, De Micco V. 2021. Reducing the evaporative demand improves photosynthesis and water use efficiency of indoor cultivated lettuce. *Agronomy* 11:1396.
- Andrade MT, Oliveira LA, Pereira TS, Cardoso AA, Batista-Silva W, DaMatta FM, Zsögön A, Martins SCV. 2022. Impaired auxin signaling increases vein and stomatal density but reduces hydraulic efficiency and ultimately net photosynthesis. *Journal of Experimental Botany* 73:4147–4156.
- Anfodillo T, Di Bisceglie DP, Urso T. 2002. Minimum cuticular conductance and cuticle features of *Picea abies* and *Pinus cembra* needles along an altitudinal gradient in the Dolomites (NE Italian Alps). *Tree Physiology* 22:479–487.
- Ávila-Lovera E, Haro R, Ezcurra E, Santiago LS. 2019. Costs and benefits of photosynthetic stems in desert species from southern California. *Functional Plant Biology* 46:175–186.
- Bacon MA. 2004. *Water use efficiency in plant biology*. Boca Raton, FL: Blackwell CRC Press.
- Baillie AL, Fleming AJ. 2020. The developmental relationship between stomata and mesophyll airspace. *New Phytologist* 225:1120–1126.
- Barbour MM, Kaiser BN. 2016. The response of mesophyll conductance to nitrogen and water availability differs between wheat genotypes. *Plant Science* 251:119–127.
- Barrera-Ayala D, Tapia G, Ferrio JP. 2023. Leaf carbon and water isotopes correlate with leaf hydraulic traits in three *Solanum* species (*S. peruvianum*, *S. lycopersicum* and *S. chilense*). *Agriculture* 13:525.
- Bertolino LT, Caine RS, Gray JE. 2019. Impact of stomatal density and morphology on water-use efficiency in a changing world. *Frontiers in Plant Science* 10:225.
- Bhaskara GB, Lasky JR, Razzaque S, Zhang L, Haque T, Bonnette JE, Civelek GZ, Verslues PE, Juenger TE. 2022. Natural variation identifies new effectors of water-use efficiency in *Arabidopsis*. *Proceedings of the National Academy of Sciences* 119:e2205305119.
- Blackman CJ, Li X, Choat B, Rymer PD, De Kauwe MG, Duursma RA, Tissue DT, Medlyn BE. 2019. Desiccation time during drought is highly predictable across species of *Eucalyptus* from contrasting climates. *New Phytologist* 224:632–643.
- Blazier MA, Hennessey TC, Lynch TB, Wittwer RE, Payton ME. 2004. Productivity, crown architecture, and gas exchange of North Carolina and Oklahoma/Arkansas loblolly pine families growing on a droughty site in southeastern Oklahoma. *Forest Ecology and Management* 194:83–94.
- Bogeat-Triboulot MB, Buré C, Gerardin T, Chuste PA, Le Thiec D, Hummel I, Durand M, Wildhagen H, Douthe C, Molins A, et al. 2019. Additive effects of high growth rate and low transpiration rate drive differences in whole plant transpiration efficiency among black poplar genotypes. *Environmental and Experimental Botany* 166:103784.
- Bramley H, Turner NC, Siddique KHM. 2013. Water use efficiency. In: Kole C, ed. *Genomics and breeding for climate-resilient crops*. Berlin, Heidelberg: Springer Berlin Heidelberg, 225–268.
- Brendel O. 2021. The relationship between plant growth and water consumption: a history from the classical four elements to modern stable isotopes. *Annals of Forest Science* 78:47.

- Brendel O, Epron D. 2022. Are differences among forest tree populations in carbon isotope composition an indication of adaptation to drought? *Tree Physiology* 42:26–31.
- Briggs LJ, Shantz HL. 1913. The water requirement of plants. In: *Bureau of plant industry bulletin*. Washington, DC: US Department of Agriculture; 282–285.
- Buckley TN. 2005. The control of stomata by water balance. *New Phytologist* 168:275–292.
- Buckley TN. 2019. How do stomata respond to water status? *New Phytologist* 224:21–36.
- Bueno A, Sancho-Knapik D, Gil-Pelegrín E, Leide J, Peguero-Pina JJ, Burghardt M, Riederer M. 2020. Cuticular wax coverage and its transpiration barrier properties in *Quercus coccifera* L. leaves: does the environment matter? *Tree Physiology* 40:827–840.
- Caine RS, Yin X, Sloan J, Harrison EL, Mohammed U, Fulton T, Biswal AK, Dionora J, Chater CC, Coe RA, et al. 2019. Rice with reduced stomatal density conserves water and has improved drought tolerance under future climate conditions. *New Phytologist* 221:371–384.
- Caird MA, Richards JH, Donovan LA. 2007. Nighttime stomatal conductance and transpiration in C₃ and C₄ plants. *Plant Physiology* 143:4–10.
- Carignano A, Vázquez-Piqué J, Tapias R, Ruiz F, Fernández M. 2019. Variability and plasticity in cuticular transpiration and leaf permeability allow differentiation of eucalyptus clones at an early age. *Forests* 11:9.
- Carmo-Silva E, Scales JC, Madgwick PJ, Parry MAJ. 2015. Optimizing Rubisco and its regulation for greater resource use efficiency. *Plant, Cell & Environment* 38:1817–1832.
- Carriqui M, Cabrera HM, Conesa MA, Coopman RE, Douthe C, Gago J, Gallé A, Galmés J, Ribas-Carbo M, Tomás M, et al. 2015. Diffusional limitations explain the lower photosynthetic capacity of ferns as compared with angiosperms in a common garden study: photosynthetic comparison in ferns and angiosperms. *Plant, Cell & Environment* 38:448–460.
- Cernusak LA. 2020. Gas exchange and water-use efficiency in plant canopies. *Plant Biology* 22:52–67.
- Cernusak LA, Aranda J, Marshall JD, Winter K. 2007. Large variation in whole-plant water-use efficiency among tropical tree species. *New Phytologist* 173:294–305.
- Chartzoulakis K, Therios I, Noitsakis B. 1993. Effects of shading on gas exchange, specific leaf weight and chlorophyll content in four kiwifruit cultivars under field conditions. *Journal of Horticultural Science* 68:605–611.
- Chen L, Luo W, Huang J, Peng S, Xiong D. 2021. Leaf photosynthetic plasticity does not predict biomass responses to growth irradiance in rice. *Physiologia Plantarum* 173:2155–2165.
- Chen JJ, Sun Y, Kopp K, Oki L, Jones SB, Hipps L. 2022. Effects of water availability on leaf trichome density and plant growth and development of *Shepherdia × utahensis*. *Frontiers in Plant Science* 13:855858.
- Christina M, Nouvellon Y, Laclau JP, Stape JL, Campoe OC, le Maire G. 2016. Sensitivity and uncertainty analysis of the carbon and water fluxes at the tree scale in *Eucalyptus* plantations using a metamodelling approach. *Canadian Journal of Forest Research* 46:297–309.
- Clarke JM, Richards RA, Condon AG. 1991. Effect of drought stress on residual transpiration and its relationship with water use of wheat. *Canadian Journal of Plant Science* 71:695–702.
- Clarke VC, Danila FR, von Caemmerer S. 2021. CO₂ diffusion in tobacco: a link between mesophyll conductance and leaf anatomy. *Interface Focus* 11:20200040.
- Clemente-Moreno MJ, Gago J, Díaz-Vivancos P, Bernal A, Miedes E, Bresta P, Liakopoulos G, Fernie AR, Hernández JA, Flexas J. 2019. The apoplastic antioxidant system and altered cell wall dynamics influence mesophyll conductance and the rate of photosynthesis. *The Plant Journal* 99:1031–1046.
- Condon AG, Richards RA, Rebetzke GJ, Farquhar GD. 2004. Breeding for high water-use efficiency. *Journal of Experimental Botany* 55:2447–2460.
- Corcuera L, Gil-Pelegrín E, Notivol E. 2012. Differences in hydraulic architecture between mesic and xeric *Pinus pinaster* populations at the seedling stage. *Tree Physiology* 32:1442–1457.
- Cordoba-Novoa HA, Pérez-Trujillo MM, Cruz Rincón BE, Flórez-Velasco N, Magnitskiy S, Moreno Fonseca LP. 2022. Shading reduces water deficits in strawberry (*Fragaria × Ananassa*) plants during vegetative growth. *International Journal of Fruit Science* 22:725–740.
- Coupeledru A, Lebon E, Christophe A, Gallo A, Gago P, Pantin F, Doligez A, Simonneau T. 2016. Reduced nighttime transpiration is a relevant breeding target for high water-use efficiency in grapevine. *Proceedings of the National Academy of Sciences* 113:8963–8968.
- Coupeledru A. 2021. Plant water-use efficiency. In: John Wiley & Sons, Ltd., ed. *eLS*. Wiley, 1–8.
- Dahal K, Vanlerberghe GC. 2017. Alternative oxidase respiration maintains both mitochondrial and chloroplast function during drought. *New Phytologist* 213:560–571.
- Dai Y, Shen Z, Liu Y, Wang L, Hannaway D, Lu H. 2009. Effects of shade treatments on the photosynthetic capacity, chlorophyll fluorescence, and chlorophyll content of *Tetrastigma hemsleyanum* Diels et Gilg. *Environmental and Experimental Botany* 65:177–182.
- Davies WJ, Wilkinson S, Loveys B. 2002. Stomatal control by chemical signalling and the exploitation of this mechanism to increase water use efficiency in agriculture. *New Phytologist* 153:449–460.
- Dawson TE, Burgess SSO, Tu KP, Oliveira RS, Santiago LS, Fisher JB, Simonin KA, Ambrose AR. 2007. Nighttime transpiration in woody plants from contrasting ecosystems. *Tree Physiology* 27:561–575.
- Dayer S, Herrera JC, Dai Z, Burrett R, Lamarque LJ, Delzon S, Bortolami G, Cochard H, Gambetta GA. 2020. The sequence and thresholds of leaf hydraulic traits underlying grapevine varietal differences in drought tolerance. *Journal of Experimental Botany* 71:4333–4344.
- Dayer S, Herrera JC, Dai Z, Burrett R, Lamarque LJ, Delzon S, Bortolami G, Cochard H, Gambetta GA. 2021. Nighttime transpiration represents a negligible part of water loss and does not increase the risk of water stress in grapevine. *Plant, Cell & Environment* 44:387–398.
- Des Marais DL, Auchincloss LC, Sukamtoh E, McKay JK, Logan T, Richards JH, Juenger TE. 2014. Variation in MPK12 affects water use efficiency in *Arabidopsis* and reveals a pleiotropic link between guard cell size and ABA response. *Proceedings of the National Academy of Sciences* 111:2836–2841.
- Dijkstra FA, Carrillo Y, Aspinwall MJ, Maier C, Canarini A, Tahaei H, Choat B, Tissue DT. 2016. Water, nitrogen and phosphorus use efficiencies of four tree species in response to variable water and nutrient supply. *Plant and Soil* 406:187–199.
- Dow GJ, Berry JA, Bergmann DC. 2017. Disruption of stomatal lineage signaling or transcriptional regulators has differential effects on mesophyll development, but maintains coordination of gas exchange. *New Phytologist* 216:69–75.
- Drake PL, Froend RH, Franks PJ. 2013. Smaller, faster stomata: scaling of stomatal size, rate of response, and stomatal conductance. *Journal of Experimental Botany* 64:495–505.
- Dubey R, Pandey BK, Sawant SV, Shirke PA. 2023. Drought stress inhibits stomatal development to improve water use efficiency in cotton. *Acta Physiologiae Plantarum* 45:30.
- Dunn J, Hunt L, Afsharinafar M, Meselmani MA, Mitchell A, Howells R, Wallington E, Fleming AJ, Gray JE. 2019. Reduced stomatal density in bread wheat leads to increased water-use efficiency. *Journal of Experimental Botany* 70:4737–4748.
- Durand M, Brendel O, Buré C, Le Thiec D. 2019. Altered stomatal dynamics induced by changes in irradiance and vapour-pressure deficit under drought: impacts on the whole-plant transpiration efficiency of poplar genotypes. *New Phytologist* 222:1789–1802.
- Duursma RA, Blackman CJ, Lopéz R, Martin-StPaul NK, Cochard H, Medlyn BE. 2019. On the minimum leaf conductance: its role in models of plant water use, and ecological and environmental controls. *New Phytologist* 221:693–705.

- Easlon HM, Nemali KS, Richards JH, Hanson DT, Juenger TE, McKay JK. 2014. The physiological basis for genetic variation in water use efficiency and carbon isotope composition in *Arabidopsis thaliana*. *Photosynthesis Research* 119:119–129.
- Ellsworth PV, Ellsworth PZ, Koteyeva NK, Cousins AB. 2018. Cell wall properties in *Oryza sativa* influence mesophyll CO₂ conductance. *New Phytologist* 219:66–76.
- Escalona JM, Tomàs M, Martorell S, Medrano H, Ribas-Carbo M, Flexas J. 2012. Carbon balance in grapevines under different soil water supply: importance of whole plant respiration. *Australian Journal of Grape and Wine Research* 18:308–318.
- Evans JR, Kaldenhoff R, Genty B, Terashima I. 2009. Resistances along the CO₂ diffusion pathway inside leaves. *Journal of Experimental Botany* 60:2235–2248.
- Even M, Sabo M, Meng D, Kreszies T, Schreiber L, Fricke W. 2019. Night-time transpiration in barley (*Hordeum vulgare*) facilitates respiratory carbon dioxide release and is regulated during salt stress. *Annals of Botany* 123:223–223.
- Farquhar GD, Hubick KT, Condon AG, Richards RA. 1989. Carbon isotope fractionation and plant water-use efficiency. In: Rundel PW, Ehleringer JR, Nagy KA, eds. *Ecological studies. Stable isotopes in ecological research*. New York, NY: Springer New York, 21–40.
- Fichot R, Laurans F, Monclus R, Moreau A, Pilate G, Brignolas F. 2009. Xylem anatomy correlates with gas exchange, water-use efficiency and growth performance under contrasting water regimes: evidence from *Populus deltoides* x *Populus nigra* hybrids. *Tree Physiology* 29:1537–1549.
- Flexas J, Ribas-Carbó M, Hanson DT, Bota J, Otto B, Cifre J, McDowell N, Medrano H, Kaldenhoff R. 2006. Tobacco aquaporin NtAQP1 is involved in mesophyll conductance to CO₂ *in vivo*. *The Plant Journal* 48:427–439.
- Flexas J, Niinemets U, Gallé A, Barbour MM, Centritto M, Diaz-Espejo A, Douthe C, Galmés J, Ribas-Carbo M, Rodriguez PL, et al. 2013a. Diffusional conductances to CO₂ as a target for increasing photosynthesis and photosynthetic water-use efficiency. *Photosynthesis Research* 117:45–59.
- Flexas J, Scoffoni C, Gago J, Sack L. 2013b. Leaf mesophyll conductance and leaf hydraulic conductance: an introduction to their measurement and coordination. *Journal of Experimental Botany* 64:3965–3981.
- Flexas J, Carriqui M, Coopman RE, Gago J, Galmés J, Martorell S, Morales F, Diaz-Espejo A. 2014. Stomatal and mesophyll conductances to CO₂ in different plant groups: underrated factors for predicting leaf photosynthesis responses to climate change? *Plant Science* 226:41–48.
- Flexas J, Díaz-Espejo A, Conesa MA, Coopman RE, Douthe C, Gago J, Gallé A, Galmés J, Medrano H, Ribas-Carbo M, et al. 2016. Mesophyll conductance to CO₂ and Rubisco as targets for improving intrinsic water use efficiency in C₃ plants. *Plant, Cell & Environment* 39:965–982.
- Flexas J, Clemente-Moreno MJ, Bota J, Brodrick TJ, Gago J, Mizokami Y, Nadal M, Perera-Castro AV, Roig-Oliver M, Sugiura D, et al. 2021. Cell wall thickness and composition are involved in photosynthetic limitation. *Journal of Experimental Botany* 72:3971–3986.
- Forster MA. 2014. How significant is nocturnal sap flow? *Tree Physiology* 34:757–765.
- Franco-Navarro JD, Rosales MA, Cubero-Font P, Calvo P, Álvarez R, Diaz-Espejo A, Colmenero-Flores JM. 2019. Chloride as macronutrient increases water use efficiency by anatomically-driven reduced stomatal conductance and increased mesophyll diffusion to CO₂. *The Plant Journal* 99:tpj.14423.
- Frank DC, Poulter B, Saurer M, Esper J, Huntingford C, Helle G, Treydte K, Zimmermann NE, Schleser GH, Ahlström A, et al. 2015. Water-use efficiency and transpiration across European forests during the Anthropocene. *Nature Climate Change* 5:579–583.
- Franks PJ, Beerling DJ. 2009. CO₂-forced evolution of plant gas exchange capacity and water-use efficiency over the Phanerozoic. *Geobiology* 7:227–236.
- Franks PJ, W. Doheny-Adams T, Britton-Harper ZJ, Gray JE. 2015. Increasing water-use efficiency directly through genetic manipulation of stomatal density. *New Phytologist* 207:188–195.
- Fullana-Pericàs M, Conesa MA, Soler S, Ribas-Carbó M, Granell A, Galmés J. 2017. Variations of leaf morphology, photosynthetic traits and water-use efficiency in Western-Mediterranean tomato landraces. *Photosynthetica* 55:121–133.
- Gago J, Douthe C, Florez-Sarasa I, Escalona JM, Galmes J, Fernie AR, Flexas J, Medrano H. 2014. Opportunities for improving leaf water use efficiency under climate change conditions. *Plant Science* 226:108–119.
- Galdon-Armero J, Fullana-Pericàs M, Mulet PA, Conesa MA, Martin C, Galmes J. 2018. The ratio of trichomes to stomata is associated with water use efficiency in *Solanum lycopersicum* (tomato). *The Plant Journal* 96:607–619.
- Galmés J, Conesa MA, Ochogavía JM, Perdomo JA, Francis DM, Ribas-Carbó M, Savé R, Flexas J, Medrano H, Cifre J. 2011. Physiological and morphological adaptations in relation to water use efficiency in Mediterranean accessions of *Solanum lycopersicum*. *Plant, Cell & Environment* 34:245–260.
- Galmés J, Conesa MA, Diaz-Espejo A, Mir A, Perdomo JA, Niinemets U, Flexas J. 2014. Rubisco catalytic properties optimized for present and future climatic conditions. *Plant Science* 226:61–70.
- García-Sánchez F, Simón I, Lidón V, Manera FJ, Simón-Grao S, Pérez-Pérez JG, Gimeno V. 2015. Shade screen increases the vegetative growth but not the production in ‘Fino 49’ lemon trees grafted on *Citrus macrophylla* and *Citrus aurantium* L. *Scientia Horticulturae* 194:175–180.
- Gašparović K, Živčák M, Brestič M, Hauptvogel P. 2021. Diversity of leaf cuticular transpiration and growth traits in field-grown wheat and egilops genetic resources. *Agronomy* 11:522.
- Ge Z, Man X, Cai T, Duan B, Xiao R, Xu Z. 2022. Environmental factors at different canopy heights had significant effects on leaf water-use efficiency in cold-temperate larch forest. *Sustainability* 14:5126.
- Gharun M, Klesse S, Tomlinson G, Waldner P, Stocker B, Rihm B, Siegwolf R, Buchmann N. 2021. Effect of nitrogen deposition on centennial forest water-use efficiency. *Environmental Research Letters* 16:114036.
- Gillon JS, Yakir D. 2000. Internal conductance to CO₂ Diffusion and C¹⁸O discrimination in C₃ leaves. *Plant Physiology* 123:201–214.
- Giuliani R, Koteyeva N, Voznesenskaya E, Evans MA, Cousins AB, Edwards GE. 2013. Coordination of leaf photosynthesis, transpiration, and structural traits in rice and wild relatives (Genus *Oryza*). *Plant Physiology* 162:1632–1651.
- Glenn DM, Bassett CB, Tworkoski T, Scorza R, Miller SS. 2015. Tree architecture of pillar and standard peach affect canopy transpiration and water use efficiency. *Scientia Horticulturae* 187:30–34.
- Glowacka K, Kromdijk J, Kucera K, Xie J, Cavanagh AP, Leonelli L, Leakey ADB, Ort DR, Niyogi KK, Long SP. 2018. Photosystem II Subunit S overexpression increases the efficiency of water use in a field-grown crop. *Nature Communications* 9:868.
- Gómez-Ocampo G, Ploschuk EL, Mantese A, Crocco CD, Botto JF. 2021. BBX21 reduces abscisic acid sensitivity, mesophyll conductance and chloroplast electron transport capacity to increase photosynthesis and water use efficiency in potato plants cultivated under moderated drought. *The Plant Journal* 108:1131–1144.
- Gosa SC, Lupo Y, Moshelion M. 2019. Quantitative and comparative analysis of whole-plant performance for functional physiological traits phenotyping: new tools to support pre-breeding and plant stress physiology studies. *Plant Science* 282:49–59.
- Grossiord C, Buckley TN, Cernusak LA, Novick KA, Poulter B, Siegwolf RTW, Sperry JS, McDowell NG. 2020. Plant responses to rising vapor pressure deficit. *New Phytologist* 226:1550–1566.
- Guerfel M, Baccouri O, Boujnah D, Chaibi W, Zarrouk M. 2009. Impacts of water stress on gas exchange, water relations, chlorophyll content and leaf structure in the two main Tunisian olive (*Olea europaea* L.) cultivars. *Scientia Horticulturae* 119:257–263.

- Guo X, Wang Y, Zhao P, Xu P, Yu G-H, Zhang L-Y, Xiong Y, Xiang C-B. 2019a. AtEDT1/HDG11 regulates stomatal density and water-use efficiency via *ERECTA* and *E2Fa*. *New Phytologist* 223:1478–1488.
- Guo T, Wang N, Xue Y, Guan Q, van Nocker S, Liu C, Ma F. 2019b. Overexpression of the RNA binding protein MhYTP1 in transgenic apple enhances drought tolerance and WUE by improving ABA level under drought condition. *Plant Science* 280:397–407.
- Hanba YT, Shibasaki M, Hayashi Y, Hayakawa T, Kasamo K, Terashima I, Katsuhara M. 2004. Overexpression of the barley aquaporin HvPIP2;1 increases internal CO₂ conductance and CO₂ assimilation in the leaves of transgenic rice plants. *Plant and Cell Physiology* 45:521–529.
- Hartmann H, Link RM, Schuldt B. 2021. A whole-plant perspective of isohydry: stem-level support for leaf-level plant water regulation. *Tree Physiology* 41:901–905.
- Hatfield JL, Dold C. 2019. Water-use efficiency: advances and challenges in a changing climate. *Frontiers in Plant Science* 10:103.
- Hatfield JL, Sauer TJ, Prueger JH. 2001. Managing soils to achieve greater water use efficiency: a review. *Agronomy Journal* 93:271–280.
- Hernández-Montes E, Tomás M, Escalona JM, Bota J, Medrano H. 2019. Leaf growth rate and nitrogen content determine respiratory costs during leaf expansion in grapevines. *Physiologia Plantarum* 165:746–754.
- Hetherington AM, Woodward FI. 2003. The role of stomata in sensing and driving environmental change. *Nature* 424:901–908.
- Horike H, Kinoshita T, Kume A, Hanba YT. 2021. Responses of leaf photosynthetic traits, water use efficiency, and water relations in five urban shrub tree species under drought stress and recovery. *Trees* 37:53–67.
- Husaini AM. 2022. High-value pleiotropic genes for developing multiple stress-tolerant biofortified crops for 21st-century challenges. *Heredity* 128:460–472.
- Isasa E, Link RM, Jansen S, Tezeh FR, Kaack L, Sarmiento Cabral J, Schuldt B. 2023. Addressing controversies in the xylem embolism resistance–vessel diameter relationship. *New Phytologist* 238:283–296.
- Jeffree CE. 2006. The fine structure of the plant cuticle. In: Riederer M, Mller C, eds. *Biology of the plant cuticle*. Oxford, UK: Blackwell Publishing Ltd.; 11–125.
- Jifon JL, Syvertsen JP. 2003. Moderate shade can increase net gas exchange and reduce photoinhibition in citrus leaves. *Tree Physiology* 23:119–127.
- Jin Y, Wang C, Zhou Z, Li Z. 2016. Co-ordinated performance of leaf hydraulics and economics in 10 Chinese temperate tree species. *Functional Plant Biology* 43:1082–1090.
- Kang J, Hao X, Zhou H, Ding R. 2021. An integrated strategy for improving water use efficiency by understanding physiological mechanisms of crops responding to water deficit: present and prospect. *Agricultural Water Management* 255:107008.
- Kardiman R, Ræbild A. 2018. Relationship between stomatal density, size and speed of opening in Sumatran rainforest species. *Tree Physiology* 38:696–705.
- Lamarque LJ, Delzon S, Troups H, Gravel A-I, Corso D, Badel E, Burtlett R, Charrier G, Cochard H, Jansen S, et al. 2020. Over-accumulation of abscisic acid in transgenic tomato plants increases the risk of hydraulic failure. *Plant, Cell & Environment* 43:548–562.
- Lawson T, Blatt MR. 2014. Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency. *Plant Physiology* 164:1556–1570.
- Lawson T, von Caemmerer S, Baroli I. 2010. Photosynthesis and stomatal behaviour. *Progress in Botany* 72. Berlin, Heidelberg: Springer Berlin Heidelberg, 265–304.
- Lawson T, Kramer DM, Raines CA. 2012. Improving yield by exploiting mechanisms underlying natural variation of photosynthesis. *Current Opinion in Biotechnology* 23:215–220.
- Leakey ADB, Ferguson JN, Pignon CP, Wu A, Jin Z, Hammer GL, Lobell DB. 2019. Water use efficiency as a constraint and target for improving the resilience and productivity of C₃ and C₄ crops. *Annual Review of Plant Biology* 70:781–808.
- Lei Z, He Y, Li X, He Z, Zhang Y, Zhang W, Liu F, Zhang Y. 2023. Domestication has reduced leaf water use efficiency associated with the anatomy of abaxial stomata in cotton. *Journal of Experimental Botany* 74:878–888.
- Li C, Yin C, Liu S. 2004. Different responses of two contrasting *Populus davidiana* populations to exogenous abscisic acid application. *Environmental and Experimental Botany* 51:237–246.
- Li Y, Li H, Li Y, Zhang S. 2017. Improving water-use efficiency by decreasing stomatal conductance and transpiration rate to maintain higher ear photosynthetic rate in drought-resistant wheat. *The Crop Journal* 5:231–239.
- Li Y, Shi H, Zhou L, Eamus D, Huete A, Li L, Cleverly J, Hu Z, Harahap M, Yu Q, et al. 2018. Disentangling climate and Lai effects on seasonal variability in water use efficiency across terrestrial ecosystems in China. *Journal of Geophysical Research, Biogeosciences* 123:2429–2443.
- Li S, Zhang J, Liu L, Wang Z, Li Y, Guo L, Li Y, Zhang X, Ren S, Zhao B, et al. 2020. SiTLFP8 reduces water loss to improve water-use efficiency by modulating cell size and stomatal density via endoreduplication. *Plant, Cell & Environment* 43:2666–2679.
- Li M, Dong H, Li J, Dai X, Lin J, Li S, Zhou C, Chiang VL, Li W. 2023. PtrVCS2 regulates drought resistance by changing vessel morphology and stomatal closure in *Populus trichocarpa*. *International Journal of Molecular Sciences* 24:4458.
- Lintunen A, Preisler Y, Oz I, Yakir D, Vesala T, Hölttä T. 2021. Bark transpiration rates can reach needle transpiration rates under dry conditions in a semi-arid forest. *Frontiers in Plant Science* 12:790684.
- Liu C, He N, Zhang J, Li Y, Wang Q, Sack L, Yu G. 2018a. Variation of stomatal traits from cold temperate to tropical forests and association with water use efficiency. *Functional Ecology* 32:20–28.
- Liu X, Qi Y, Li F, Yang Q, Yu L. 2018b. Impacts of regulated deficit irrigation on yield, quality and water use efficiency of Arabica coffee under different shading levels in dry and hot regions of southwest China. *Agricultural Water Management* 204:292–300.
- Liu Q, Wang Z, Yu S, Li W, Zhang M, Yang J, Li D, Yang J, Li C. 2021. Pu-miR172d regulates stomatal density and water-use efficiency via targeting *PuGTL1* in poplar. *Journal of Experimental Botany* 72:1370–1383.
- Liu H, Zhang C, Meng Y, Zhang F, Huang N, Wang J, Li Y. 2023. Hydraulic and economical traits in short- and long-shoot leaves of *Ginkgo biloba* males and females. *Forests* 14:535.
- López-Calcano PE, Brown KL, Simkin AJ, Fisk SJ, Violet-Chabrand S, Lawson T, Raines CA. 2020. Stimulating photosynthetic processes increases productivity and water-use efficiency in the field. *Nature Plants* 6:1054–1063.
- Lundgren MR, Fleming AJ. 2020. Cellular perspectives for improving mesophyll conductance. *The Plant Journal* 101:845–857.
- Luo H, Bie X, Yi G, Zhou X, Zhang T, Li J, Lai P. 2022. dominant impacting factors on water-use efficiency variation in inner Mongolia from 2001 to 2018: vegetation or climate? *Remote Sensing* 14:4541.
- Ma N, Zhang Y. 2022. Contrasting trends in water use efficiency of the alpine grassland in Tibetan plateau. *Journal of Geophysical Research: Atmospheres* 127:1–19.
- Ma WT, Yu YZ, Wang X, Gong XY. 2023. Estimation of intrinsic water-use efficiency from δ¹³C signature of C₃ leaves: assumptions and uncertainty. *Frontiers in Plant Science* 13:1037972.
- Machado R, Loram-Lourenço L, Farnese FS, Alves RDFB, de Sousa LF, Silva FG, Filho SCV, Torres-Ruiz JM, Cochard H, Menezes-Silva PE. 2021. Where do leaf water leaks come from? Trade-offs underlying the variability in minimum conductance across tropical savanna species with contrasting growth strategies. *New Phytologist* 229:1415–1430.
- Maleski JJ, Bosch DD, Anderson RG, Coffin AW, Anderson WF, Strickland TC. 2019. Evaluation of miscanthus productivity and

- water use efficiency in southeastern United States. *Science of the Total Environment* 692:1125–1134.
- Malone SL, Tulbure MG, Pérez-Luque AJ, Assal TJ, Bremer LL, Drucker DP, Hillis V, Varela S, Goulden ML. 2016. Drought resistance across California ecosystems: evaluating changes in carbon dynamics using satellite imagery. *Ecosphere* 7:1–19.
- Márquez DA, Stuart-Williams H, Cernusak LA, Farquhar GD. 2023. Assessing the CO₂ concentration at the surface of photosynthetic mesophyll cells. *New Phytologist* 238:1446–1460.
- Masutomi Y, Kinose Y, Takimoto T, Yonekura T, Oue H, Kobayashi K. 2019. Ozone changes the linear relationship between photosynthesis and stomatal conductance and decreases water use efficiency in rice. *Science of the Total Environment* 655:1009–1016.
- McAdam SAM, Brodribb TJ. 2016. Linking turgor with ABA biosynthesis: implications for stomatal responses to vapor pressure deficit across land plants. *Plant Physiology* 171:2008–2016.
- McAusland L, Davey PA, Kanwal N, Baker NR, Lawson T. 2013. A novel system for spatial and temporal imaging of intrinsic plant water use efficiency. *Journal of Experimental Botany* 64:4993–5007.
- McAusland L, Vialet-Chabrand S, Davey P, Baker NR, Brendel O, Lawson T. 2016. Effects of kinetics of light-induced stomatal responses on photosynthesis and water-use efficiency. *New Phytologist* 211:1209–1220.
- McNeil BE, Fahey RT, King CJ, Erazo DA, Heimerl TZ, Elmore AJ. 2023. Tree crown economics. *Frontiers in Ecology and the Environment* 21:40–48.
- Mediavilla S, Escudero A, Heilmeyer H. 2001. Internal leaf anatomy and photosynthetic resource-use efficiency: interspecific and intraspecific comparisons. *Tree Physiology* 21:251–259.
- Medrano H, Flexas J, Galmés J. 2009. Variability in water use efficiency at the leaf level among Mediterranean plants with different growth forms. *Plant and Soil* 317:17–29.
- Medrano H, Escalona JM, Flexas J, Martorell S, Tomás M. 2017. From leaf to plant water use efficiency: solving the gaps for a whole plant evaluation. *Acta Horticulturae* 1157:167–176.
- Messinger SM, Buckley TN, Mott KA. 2006. Evidence for involvement of photosynthetic processes in the stomatal response to CO₂. *Plant Physiology* 140:771–778.
- Mo Y, Yang R, Liu L, Gu X, Yang X, Wang Y, Zhang X, Li H. 2016. Growth, photosynthesis and adaptive responses of wild and domesticated watermelon genotypes to drought stress and subsequent re-watering. *Plant Growth Regulation* 79:229–241.
- Momayyezi M, Guy RD. 2017. Substantial role for carbonic anhydrase in latitudinal variation in mesophyll conductance of *Populus trichocarpa* Torr. & Gray: mesophyll conductance of cottonwood. *Plant, Cell & Environment* 40:138–149.
- Montanaro G, Dichio B, Xiloyannis C. 2009. Shade mitigates photoinhibition and enhances water use efficiency in kiwifruit under drought. *Photosynthetica* 47:363–371.
- Mukarram M, Choudhary S, Kurjak D, Petek A, Khan MMA. 2021. Drought: sensing, signalling, effects and tolerance in higher plants. *Physiologia Plantarum* 172:1291–1300.
- Nadal M, Flexas J. 2019. Variation in photosynthetic characteristics with growth form in a water-limited scenario: implications for assimilation rates and water use efficiency in crops. *Agricultural Water Management* 216:457–472.
- Negin B, Moshelion M. 2016. The evolution of the role of ABA in the regulation of water-use efficiency: from biochemical mechanisms to stomatal conductance. *Plant Science* 251:82–89.
- Ni Y, Guo YJ, Guo YJ, Han L, Tang H, Conyers M. 2012. Leaf cuticular waxes and physiological parameters in alfalfa leaves as influenced by drought. *Photosynthetica* 50:458–466.
- Nunes TDG, Slawinska MW, Lindner H, Raissig MT. 2022. Quantitative effects of environmental variation on stomatal anatomy and gas exchange in a grass model. *Quantitative Plant Biology* 3:e6.
- Orr D, Alcántara A, Kapralov MV, Andralojc J, Carmo-Silva E, Parry MAJ. 2016. Surveying Rubisco diversity and temperature response to improve crop photosynthetic efficiency. *Plant Physiology* 172:pp.00750.2016.
- Osada N, Tateno R, Hyodo F, Takeda H. 2004. Changes in crown architecture with tree height in two deciduous tree species: developmental constraints or plastic response to the competition for light? *Forest Ecology and Management* 188:337–347.
- Ouyang W, Struik PC, Yin X, Yang J. 2017. Stomatal conductance, mesophyll conductance, and transpiration efficiency in relation to leaf anatomy in rice and wheat genotypes under drought. *Journal of Experimental Botany* 68:5191–5205.
- Paillassa J, Wright IJ, Prentice IC, Pepin S, Smith NG, Ethier G, Westerband AC, Lamarque LJ, Wang H, Cornwell WK, et al. 2020. When and where soil is important to modify the carbon and water economy of leaves. *New Phytologist* 228:121–135.
- Papacek M, Christmann A, Grill E. 2019. Increased water use efficiency and water productivity of arabidopsis by abscisic acid receptors from *Populus canescens*. *Annals of Botany* 124:581–590.
- Papanatsiou M, Petersen J, Henderson L, Wang Y, Christie JM, Blatt MR. 2019. Optogenetic manipulation of stomatal kinetics improves carbon assimilation, water use, and growth. *Science* 363:1456–1459.
- Parry MAJ, Andralojc PJ, Scales JC, Salvucci ME, Carmo-Silva AE, Alonso H, Whitney SM. 2013. Rubisco activity and regulation as targets for crop improvement. *Journal of Experimental Botany* 64:717–730.
- Pathare VS, Panahabadi R, Sonawane BV, Apalla AJ, Koteyeva N, Bartley LE, Cousins AB. 2023. Altered cell wall hydroxycinnamate composition impacts leaf and canopy-level CO₂-uptake and water use in rice. *Plant Physiology*:kiad428. doi:10.1093/plphys/kiad428.
- Peguero-Pina JJ, Sisó S, Flexas J, Galmés J, García-Nogales A, Niinemets U, Sancho-Knapik D, Saz MA, Gil-Pelegrín E. 2017. Cell-level anatomical characteristics explain high mesophyll conductance and photosynthetic capacity in sclerophyllous Mediterranean oaks. *New Phytologist* 214:585–596.
- Pérez-Pérez JG, Dodd IC, Botía P. 2012. Partial rootzone drying increases water-use efficiency of lemon Fino 49 trees independently of root-to-shoot ABA signalling. *Functional Plant Biology* 39:366.
- Peterhansel C, Maurino VG. 2011. Photorespiration redesigned. *Plant Physiology* 155:49–55.
- Petrik P, Petek-Petrik A, Konôpková A, Fleischer P, Stojnic S, Zavadilova I, Kurjak D. 2022a. Seasonality of PSII thermostability and water use efficiency of in situ mountainous Norway spruce (*Picea abies*). *Journal of Forestry Research* 34:197–208.
- Petrik P, Petek-Petrik A, Kurjak D, Mukarram M, Klein T, Gömöry D, Štřelcová K, Frýdl J, Konôpková A. 2022b. Interannual adjustments in stomatal and leaf morphological traits of European beech (*Fagus sylvatica* L.) demonstrate its climate change acclimation potential. *Plant Biology* 24:1287–1296.
- Pitaloka MK, Caine RS, Hepworth C, Harrison EL, Sloan J, Chutteang C, Phunthong C, Nongngok R, Toojinda T, Ruengphayak S, et al. 2022. Induced genetic variations in stomatal density and size of rice strongly affects water use efficiency and responses to drought stresses. *Frontiers in Plant Science* 13:801706.
- Ponce-Campos GE, Moran MS, Huete A, Zhang Y, Bresloff C, Huxman TE, Eamus D, Bosch DD, Buda AR, Gunter SA, et al. 2013. Ecosystem resilience despite large-scale altered hydroclimatic conditions. *Nature* 494:349–352.
- Pons TL, Flexas J, von Caemmerer S, Evans JR, Genty B, Ribas-Carbo M, Brugnoli E. 2009. Estimating mesophyll conductance to CO₂: methodology, potential errors, and recommendations. *Journal of Experimental Botany* 60:2217–2234.
- Pospíšilová J, Batková P. 2004. Effects of pre-treatments with abscisic acid and/or benzyladenine on gas exchange of French bean, sugar beet, and maize leaves during water stress and after rehydration. *Biologia plantarum* 48:395–399.
- Price GD, von Caemmerer S, Evans JR, Yu J-W, Lloyd J, Oja V, Kell P, Harrison K, Gallagher A, Badger MR. 1994. Specific reduction of chloroplast carbonic anhydrase activity by antisense RNA in

- transgenic tobacco plants has a minor effect on photosynthetic CO₂ assimilation. *Planta* 193:331–340.
- Quan Q, Zhang F, Meng C, Ma F, Zhou Q, Sun F, Niu S. 2020. Shifting biomass allocation determines community water use efficiency under climate warming. *Environmental Research Letters* 15:094041.
- Rabarijaona A, Ponton S, Bert D, Ducousso A, Richard B, Levillain J, Brendel O. 2022. Provenance differences in water-use efficiency among sessile oak populations grown in a mesic common garden. *Frontiers in Forests and Global Change* 5:914199.
- Rao S, Tian Y, Zhang C, Qin Y, Liu M, Niu S, Li Y, Chen J. 2023. The JASMONATE ZIM-domain–OPEN STOMATA1 cascade integrates jasmonic acid and abscisic acid signaling to regulate drought tolerance by mediating stomatal closure in poplar. *Journal of Experimental Botany* 74:443–457.
- Reddy SH, Da Costa MVJ, Kambalimath SK, Rajanna Mavinahalli P, Muthurajan R, Chinnusamy V, Sevanthi AM, Neelamraju S, Gopala Krishnan S, Singh AK, et al. 2020a. Relative contribution of stomatal parameters in influencing WUE among rice mutants differing in leaf mass area. *Plant Physiology Reports* 25:483–495.
- Reddy SH, Singhal RK, DaCosta MVJ, Kambalimath SK, Rajanna MP, Muthurajan R, Sevanthi AM, Mohapatra T, Sarla N, Chinnusamy V, et al. 2020b. Leaf mass area determines water use efficiency through its influence on carbon gain in rice mutants. *Physiologia Plantarum* 169:194–213.
- Renninger HJ, Carlo NJ, Clark KL, Schäfer KVR. 2015. Resource use and efficiency, and stomatal responses to environmental drivers of oak and pine species in an Atlantic Coastal Plain forest. *Frontiers in Plant Science* 6:297.
- Resco de Dios V, Chowdhury FI, Granda E, Yao Y, Tissue DT. 2019. Assessing the potential functions of nocturnal stomatal conductance in C₃ and C₄ plants. *New Phytologist* 223:1696–1706.
- Riederer M, Schreiber L. 2001. Protecting against water loss: analysis of the barrier properties of plant cuticles. *Journal of Experimental Botany* 52:2023–2032.
- Roby MC, Scott RL, Moore DJP. 2020. High vapor pressure deficit decreases the productivity and water use efficiency of rain-induced pulses in semiarid ecosystems. *Journal of Geophysical Research, Biogeosciences* 125:1–14.
- Roig-Oliver M, Nadal M, Clemente-Moreno MJ, Bota J, Flexas J. 2020. Cell wall components regulate photosynthesis and leaf water relations of *Vitis vinifera* cv. Grenache acclimated to contrasting environmental conditions. *Journal of Plant Physiology* 244:153084.
- Roussel M, Le Thiec D, Montpied P, Ningre N, Guehl J-M, Brendel O. 2009. Diversity of water use efficiency among *Quercus robur* genotypes: contribution of related leaf traits. *Annals of Forest Science* 66:408–408.
- Sack L, Buckley TN. 2016. The developmental basis of stomatal density and flux. *Plant Physiology* 171:2358–2363.
- Santiago LS, Goldstein G, Meinzer FC, Fisher JB, Machado K, Woodruff D, Jones T. 2004. Leaf photosynthetic traits scale with hydraulic conductivity and wood density in Panamanian forest canopy trees. *Oecologia* 140:543–550.
- Schuldts B, Buras A, Arend M, Vitasse Y, Beierkuhnlein C, Damm A, Gharun M, Grams TEE, Hauck M, Hajek P, et al. 2020. A first assessment of the impact of the extreme 2018 summer drought on Central European forests. *Basic and Applied Ecology* 45:86–103.
- Schuster AC, Burghardt M, Riederer M. 2017. The ecophysiology of leaf cuticular transpiration: are cuticular water permeabilities adapted to ecological conditions? *Journal of Experimental Botany* 68:5271–5279.
- Seibt U, Rajabi A, Griffiths H, Berry JA. 2008. Carbon isotopes and water use efficiency: sense and sensitivity. *Oecologia* 155:441–454.
- Sellin A, Niglas A, Öunapuu E, Karusion A. 2013. Impact of phloem girdling on leaf gas exchange and hydraulic conductance in hybrid aspen. *Biologia plantarum* 57:531–539.
- Sellin A, Niglas A, Öunapuu-Pikas E, Kupper P. 2014. Rapid and long-term effects of water deficit on gas exchange and hydraulic conductance of silver birch trees grown under varying atmospheric humidity. *BMC Plant Biology* 14:72.
- Senbayram M, Tränkner M, Dittert K, Brück H. 2015. Daytime leaf water use efficiency does not explain the relationship between plant N status and biomass water-use efficiency of tobacco under non-limiting water supply. *Journal of Plant Nutrition and Soil Science* 178:682–692.
- Servato S. 2020. Why do plants have waxy leaves? Do we know after all? *Tree Physiology* 40:823–826.
- Shepherd T, Wynne Griffiths D. 2006. The effects of stress on plant cuticular waxes. *New Phytologist* 171:469–499.
- Silva Gonzaga MI, Oliveira da Silva PS, Carlos de Jesus Santos J, Ganassali de Oliveira Junior LF. 2019. Biochar increases plant water use efficiency and biomass production while reducing Cu concentration in *Brassica juncea* L. in a Cu-contaminated soil. *Ecotoxicology and Environmental Safety* 183:109557.
- Song W, Loik ME, Cui H, Fan M, Sun W. 2022. Effect of nitrogen addition on leaf photosynthesis and water use efficiency of the dominant species *Leymus chinensis* (Trin.) Tzvelev in a semi-arid meadow steppe. *Plant Growth Regulation* 98:91–102.
- Suárez JC, Urban MO, Contreras AT, Noriega JE, Deva C, Beebe SE, Polanía JA, Casanoves F, Rao IM. 2021. Water use, leaf cooling and carbon assimilation efficiency of heat resistant common beans evaluated in Western Amazonia. *Frontiers in Plant Science* 12:644010.
- Syvtertsen JP, Goni C, Otero A. 2003. Fruit load and canopy shading affect leaf characteristics and net gas exchange of ‘Spring’ navel orange trees. *Tree Physiology* 23:899–906.
- Szatniewska J, Zavadilova I, Nezval O, Krejza J, Petrik P, Čáter M, Stojanović M. 2022. Species-specific growth and transpiration response to changing environmental conditions in floodplain forest. *Forest Ecology and Management* 516:120248.
- Tai-Chung WU, Bai-Ling LIN, Wen-Yuan KAO. 2020. Active stomatal control of *Marsilea crenata*, an amphibious fern, in response to CO₂ and exogenous application of ABA. *TAIWANIA* 65:431–437.
- Tanaka Y, Sugano SS, Shimada T, Hara-Nishimura I. 2013. Enhancement of leaf photosynthetic capacity through increased stomatal density in Arabidopsis. *New Phytologist* 198:757–764.
- Tardieu F. 2022. Different avenues for progress apply to drought tolerance, water use efficiency and yield in dry areas. *Current Opinion in Biotechnology* 73:128–134.
- Terashima I, Hanba YT, Tholen D, Niinemets U. 2011. Leaf functional anatomy in relation to photosynthesis. *Plant Physiology* 155:108–116.
- Tholen D, Boom C, Noguchi K, Ueda S, Katase T, Terashima I. 2008. The chloroplast avoidance response decreases internal conductance to CO₂ diffusion in *Arabidopsis thaliana* leaves. *Plant, Cell & Environment* 31:1688–1700.
- Tholen D, Boom C, Zhu X-G. 2012. Opinion: prospects for improving photosynthesis by altering leaf anatomy. *Plant Science* 197:92–101.
- Thompson AJ, Andrews J, Mulholland BJ, McKee JMT, Hilton HW, Horridge JS, Farquhar GD, Smeeton RC, Smillie IRA, Black CR, et al. 2007. Overproduction of abscisic acid in tomato increases transpiration efficiency and root hydraulic conductivity and influences leaf expansion. *Plant Physiology* 143:1905–1917.
- Tomás M, Flexas J, Copolovici L, Galmés J, Hallik L, Medrano H, Ribas-Carbó M, Tosens T, Vislap V, Niinemets U. 2013. Importance of leaf anatomy in determining mesophyll diffusion conductance to CO₂ across species: quantitative limitations and scaling up by models. *Journal of Experimental Botany* 64:2269–2281.
- Tomás M, Medrano H, Brugnoli E, Escalona JM, Martorell S, Pou A, Ribas-Carbó M, Flexas J. 2014a. Variability of mesophyll conductance in grapevine cultivars under water stress conditions in relation to leaf anatomy and water use efficiency: genotypic variability of mesophyll conductance. *Australian Journal of Grape and Wine Research* 20:272–280.

- Tomás M, Medrano H, Escalona JM, Martorell S, Pou A, Ribas-Carbó M, Flexas J. 2014b. Variability of water use efficiency in grapevines. *Environmental and Experimental Botany* 103:148–157.
- Tortosa I, Escalona JM, Bota J, Tomás M, Hernández E, Escudero EG, Medrano H. 2016. Exploring the genetic variability in water use efficiency: evaluation of inter and intra cultivar genetic diversity in grapevines. *Plant Science* 251:35–43.
- Tränkner M, Jákli B, Tavakol E, Geilfus C-M, Cakmak I, Dittert K, Senbayram M. 2016. Magnesium deficiency decreases biomass water-use efficiency and increases leaf water-use efficiency and oxidative stress in barley plants. *Plant and Soil* 406:409–423.
- Trueba S, Thérault-Rancourt G, Earles JM, Buckley TN, Love D, Johnson DM, Brodersen C. 2022. The three-dimensional construction of leaves is coordinated with water use efficiency in conifers. *New Phytologist* 233:851–861.
- Uehlein N, Lovisolo C, Siefert F, Kaldenhoff R. 2003. The tobacco aquaporin NtAQP1 is a membrane CO₂ pore with physiological functions. *Nature* 425:734–737.
- Vadez V, Kholova J, Medina S, Kakker A, Anderberg H. 2014. Transpiration efficiency: new insights into an old story. *Journal of Experimental Botany* 65:6141–6153.
- Vadez V, Pilloni R, Grondin A, Hajjarpoor A, Belhouchette H, Brouziyne Y, Chehbouni G, Kharrou MH, Zitouna-Chebbi R, Mekki I, et al. 2023. Water use efficiency (WUE) across scales: from genes to landscape. *Journal of Experimental Botany*: erad052.
- Vandegheer RK, Zhao C, Cibils-Stewart X, et al. 2021. Silicon deposition on guard cells increases stomatal sensitivity as mediated by K⁺ efflux and consequently reduces stomatal conductance. *Physiologia Plantarum* 171:358–370.
- Vastag E, Orlović S, Konôpková A, Kurjak D, Cocozza C, Pšidová E, Lapin K, Kesić L, Stojnić S. 2020. *Magnolia grandiflora* L. shows better responses to drought than *Magnolia × soulangeana* in urban environment. *iForest - Biogeosciences and Forestry* 13:575–583.
- Viale-Chabrand S, Matthews JSA, Brendel O, Blatt MR, Wang Y, Hills A, Griffiths H, Rogers S, Lawson T. 2016. Modelling water use efficiency in a dynamic environment: an example using *Arabidopsis thaliana*. *Plant Science* 251:65–74.
- Wang Y, Botella JR. 2022. Heterotrimeric G protein signaling in abiotic stress. *Plants* 11:876.
- Wang Y, del Campo AD, Wei X, Winkler R, Liu W, Li Q. 2020. Responses of forest carbon and water coupling to thinning treatments from leaf to stand scales in a young montane pine forest. *Carbon Balance and Management* 15:24.
- Warren CR. 2007. Stand aside stomata, another actor deserves centre stage: the forgotten role of the internal conductance to CO₂ transfer. *Journal of Experimental Botany* 59:1475–1487.
- Wedegaertner K, Shekoofa A, Purdom S, Walters K, Duncan L, Raper TB. 2022. Cotton stomatal closure under varying temperature and vapor pressure deficit, correlation with the hydraulic conductance trait. *Journal of Cotton Research* 5:20–30.
- Whitney SM, Houtz RL, Alonso H. 2011. Advancing our understanding and capacity to engineer nature's CO₂-sequestering enzyme, Rubisco. *Plant Physiology* 155:27–35.
- Wright IJ, Reich PB, Westoby M. 2003. Least-cost input mixtures of water and nitrogen for photosynthesis. *The American Naturalist* 161:98–111.
- Wu T, Tissue DT, Li X, Liu S, Chu G, Zhou G, Li Y, Zheng M, Meng Z, Liu J. 2020. Long-term effects of 7-year warming experiment in the field on leaf hydraulic and economic traits of subtropical tree species. *Global Change Biology* 26:7144–7157.
- Xiong D, Flexas J. 2020. From one side to two sides: the effects of stomatal distribution on photosynthesis. *New Phytologist* 228:1754–1766.
- Xiong D, Flexas J, Yu T, Peng S, Huang J. 2017. Leaf anatomy mediates coordination of leaf hydraulic conductance and mesophyll conductance to CO₂ in *Oryza*. *New Phytologist* 213:572–583.
- Xiong D, Douthe C, Flexas J. 2018. Differential coordination of stomatal conductance, mesophyll conductance, and leaf hydraulic conductance in response to changing light across species: coordination of CO₂ diffusion and H₂O transport inside leaves. *Plant, Cell & Environment* 41:436–450.
- Xu Y, Feng Z, Peng J, Tarvainen L. 2022. Elevated ozone decreases the activity of Rubisco in poplar but not its activation under fluctuating light. *Tree Physiology* 42:1762–1775.
- Xylogiannis E, Sofo A, Dichio B, Montanaro G, Mininni AN. 2020. Root-to-shoot signaling and leaf water-use efficiency in peach trees under localized irrigation. *Agronomy* 10:437.
- Yang Z, Liu J, Tischer SV, Christmann A, Windisch W, Schnyder H, Grill E. 2016. Leveraging abscisic acid receptors for efficient water use in *Arabidopsis*. *Proceedings of the National Academy of Sciences* 113:6791–6796.
- Yang Z, Liu J, Poree F, Schaeufele R, Helmke H, Frackenpohl J, Lehr S, von Koskull-Döring P, Christmann A, Schnyder H, et al. 2019. Abscisic acid receptors and coreceptors modulate plant water use efficiency and water productivity. *Plant Physiology* 180:1066–1080.
- Yao G, Nie Z, Turner NC, Li F-M, Gao T-P, Fang X-W, Scoffoni C. 2021. Combined high leaf hydraulic safety and efficiency provides drought tolerance in *Caragana* species adapted to low mean annual precipitation. *New Phytologist* 229:230–244.
- Yi K, Maxwell JT, Wenzel MK, Roman DT, Sauer PE, Phillips RP, Novick KA. 2019. Linking variation in intrinsic water-use efficiency to isohydricity: a comparison at multiple spatiotemporal scales. *New Phytologist* 221:195–208.
- Yoo CY, Pence HE, Hasegawa PM, Mickelbart MV. 2009. Regulation of transpiration to improve crop water use. *Critical Reviews in Plant Sciences* 28:410–431.
- Zahoor SA, Ahmad S, Ahmad A, et al. 2019. Improving water use efficiency in agronomic crop production. In: Hasanuzzaman M, ed. *Agronomic crops*. Singapore: Springer Singapore, 13–29.
- Zait Y, Ferrero-Serrano A, Assmann SM. 2021. The α subunit of the heterotrimeric G protein regulates mesophyll CO₂ conductance and drought tolerance in rice. *New Phytologist* 232:2324–2338.
- Zhang X, Wollenweber B, Jiang D, Liu F, Zhao J. 2008. Water deficits and heat shock effects on photosynthesis of a transgenic *Arabidopsis thaliana* constitutively expressing ABP9, a bZIP transcription factor. *Journal of Experimental Botany* 59:839–848.
- Zhang FP, Sussmilch F, Nichols DS, Cardoso AA, Brodribb TJ, McAdam SAM. 2018. Leaves, not roots or floral tissue, are the main site of rapid, external pressure-induced ABA biosynthesis in angiosperms. *Journal of Experimental Botany* 69:1261–1267.
- Zhang Y, Yu X, Chen L, Jia G. 2019. Whole-plant instantaneous and short-term water-use efficiency in response to soil water content and CO₂ concentration. *Plant and Soil* 444:281–298.
- Zhao C, Chavan S, He X, Zhou M, Cazzonelli CI, Chen Z-H, Tissue DT, Ghannoum O. 2021a. Smart glass impacts stomatal sensitivity of greenhouse *Capsicum* through altered light. *Journal of Experimental Botany* 72:3235–3248.
- Zhao J, Feng H, Xu T, Xiao J, Guerrieri R, Liu S, Wu X, He X, He X. 2021b. Physiological and environmental control on ecosystem water use efficiency in response to drought across the northern hemisphere. *Science of the Total Environment* 758:143599.
- Zhu L, Li H, Thorpe MR, Hocart CH, Song X. 2021. Stomatal and mesophyll conductance are dominant limitations to photosynthesis in response to heat stress during severe drought in a temperate and a tropical tree species. *Trees* 35:1613–1626.
- Zsögön A, Alves Negrini AC, Peres LEP, Nguyen HT, Ball MC. 2015. A mutation that eliminates bundle sheath extensions reduces leaf hydraulic conductance, stomatal conductance and assimilation rates in tomato (*Solanum lycopersicum*). *New Phytologist* 205:618–626.
- Zufferey V. 2016. Leaf respiration in grapevine (*Vitis vinifera* 'Chasselas') in relation to environmental and plant factors. *VITIS - Journal of Grapevine Research* 55:65–72.