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Controlling stomatal aperture in semi-arid regions—The dilemma of saving water or being cool?

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ABSTRACT

Stomatal regulation of leaf gas exchange with the atmosphere is a key process in plant adaptation to the environment, particularly in semi-arid regions with high atmospheric evaporative demand. Development of stomata, integrating internal signaling and environmental cues sets the limit for maximum diffusive capacity of stomata, through size and density and is under a complex genetic control, thus providing multiple levels of regulation. Operational stomatal conductance to water vapor and CO₂ results from feed-back and/or feed-forward mechanisms and is the end-result of a plethora of signals originated in leaves and/or in roots at each moment. CO₂ assimilation *versus* water vapor loss, proposed to be the subject of optimal regulation, is species dependent and defines the water use efficiency (WUE). WUE has been a topic of intense research involving areas from genetics to physiology. In crop plants, especially in semi-arid regions, the question that arises is how the compromise of reducing transpiration to save water will impact on plant performance through leaf temperature. Indeed, plant transpiration by providing evaporative cooling, is a major component of the leaf energy balance. In this paper we discuss the dilemma of 'saving water or being cool' bringing about recent findings from molecular genetics, to development and physiology of stomata. The question of 'how relevant is screening for high/low WUE in crops for semi-arid regions, where drought and heat co-occur' is discussed.

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1. Introduction

Under semi-arid climates (receiving precipitation below potential evapotranspiration) plants are often subjected to periods of water deficits, with high impact on plant functioning and production. The effects depend on stress duration, intensity and rate of progression, as well as on genotype, developmental stage of plants and the interaction with other stresses [1–3]. In Mediterranean and some semi-arid sub-tropical climates, terminal drought (that occurs late in the crop cycle) is common, due to scarce seasonal rain-

fall or to limited pre-seasonal stored soil moisture. Often, terminal drought co-occurs with high air temperature and high air vapor pressure deficit (VPD) [4]. The great variability of the weather that characterizes semi-arid climates amplifies the potential of stress that plants may be subjected to. The situation is likely to be exacerbated by an enhanced frequency of extreme events induced by climate change, as is the case of heat waves [5].

Most crops cultivated today have been selected for optimal performance under the current climatic conditions and have progressed towards the yield potential with the green revolution [6]. Yield potential determines crop production in the absence of drought. However, with the increased risk of water shortage, sustainable production systems are being developed (selection of genotypes and agronomic management tools), where irrigation water and nutrients are used with parsimony [7]. Under these circumstances, the basic drought mechanisms – drought escape, avoidance or tolerance – become vital for crop production. Drought escape through changing phenology or sowing/planting date is desirable in the case of predictable drought. Avoidance and tolerance traits are important under both predictable and unpredictable drought scenarios.

Abbreviations: A_{max} , maximum net photosynthesis; A, net leaf carbon assimilation; VPD, air vapor pressure deficit; E, transpiration; E_{night} , Night-time transpiration; g_s , conductance for H₂O vapor and CO₂; g_{smax} , maximum leaf diffusive conductance; g_s , conductance for H₂O vapor and CO₂; g_{smax} , maximum leaf diffusive conductance; g_{snight} , Night-time conductance for H₂O vapor; RV, resident vegetation; S, pore size; SD, stomatal density; ST, soil tillage; WUE, water use efficiency; WUE_i, intrinsic water use efficiency; WUE_i, instantaneous water use efficiency; WUE_c, season-long crop water use efficiency; $\partial E/\partial A$, marginal unit water cost of plant carbon gain.

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Matching crop water demand with season supply of water will enable crops to escape terminal water stress [3]. Increased crop water use efficiency (WUE), defined as the ratio of leaf carbon assimilation (A) to transpiration (E), may be important to save water for the crucial periods of plant development [8], but has to be equated in terms of the balance between water savings and yield penalty [2]. Moreover, when drought and heat co-occur stomatal closure and decreased transpiration, associated with high WUE, may lead to a dramatic increase in leaf temperature (up to 7 °C above air temperature) [4]. If this situation stands for long periods leaf photo-damage and/or xylem embolism may occur, leading to severe defoliation and plant death.

Stomata play a central role in the pathways for both carbon uptake and water loss by plants. Regulation of stomatal aperture, estimated by stomatal conductance, is a complex process with operational limits set during leaf development, namely the pattern of stomata in epidermis that include distribution, size and density (number per unit area of leaf) [9]. Recent advances in molecular genetics of stomatal development together with the physical diffusion model of stomatal conductance revealed a direct relation between the physiology of stomata and the role of genes regulating stomatal pattern [10]. This regulation is dependent on several environmental stimuli [11], signaled via internal factors such as hormones and hydraulics [12–15]. Under high atmospheric evaporative demand, stomatal response to VPD is an important mechanism to save water, showing genotypic differences, even between closely related species [16] that can be explored by plant breeders. Similarly, the sensitivity of stomata to dehydrating soil is variable, explaining the isohydric or ‘pessimistic’ response as compared to the anisohydric or ‘optimistic’ (but riskier) response. Isohydric species are likely more susceptible to xylem cavitation and therefore tend to have stricter control of transpiration, whereas anisohydric tend to use available water in a less conservative way and have presumably a lower risk of xylem embolism [17].

In this paper we discuss how stomata are regulated under a variable environment, how the type of stomatal responses influences crop WUE and leaf/canopy temperature and ultimately how plant breeding and management can improve crop performance under hot and dry conditions to resolve the dilemma between saving water or being cool. We use examples from our own work in grapevine, a species with high genotypic diversity, including varieties exhibiting iso- and aniso-hydric stomatal control and which is cultivated under rainfed and irrigated conditions. This perennial crop is a recognized model to study plant water relations [18].

2. Stomatal regulation of water loss

2.1. The carbon compromise

Stomatal regulation of carbon uptake and water loss under a changing environment was a key step in the colonization of land by plants [19]. The evolutionary pathway of this regulation is still largely unknown [20], but may have been an incremental one, over more than 450 million years since stomata first evolved [21]. It implied a ‘carbon compromise’: to fix CO₂ from the atmosphere while avoiding lethal dehydration of the photosynthetic leaf tissues. An impermeable cuticle on leaf epidermis would prevent water vapor loss to the atmosphere, but would not allow CO₂ uptake as well. Stomata are the microscopic pores that provide a variable porosity in the epidermis. They were described for the first time over three centuries ago and begun to be studied with modern methods in the last years of the 19th century. Francis Darwin (Charles Darwin’s son) for example, recognized that stomata closed in response to plant water deficits [22]. Since then, there was vast progress in our understanding of stomatal functioning, from the

mechanics of opening and closing to the behavior of stomata in the field. In plants with adequate water supply stomata may regulate leaf temperature close to the optimum for metabolic processes, including photosynthesis [23] or prevent tissue heat damage under excessive radiation or temperature. A remarkable example of the cooling effect of leaf transpiration was shown by O.L. Lange [24] in the hot and dry environments of the Mauritania Sahara, where large leaved species (*Citrullus colocynthis*) were able to keep leaf temperature below the upper temperature limits for survival. Because large leaves have a low convective heat exchange with the air they would not survive without transpiration cooling. This apparently paradox phenomenon (existence of large-leaf species in deserts, instead of small-leaved ones that intercept less radiation) was also observed by Smith [25] in the Sonoran desert of southern California. He recorded leaf temperatures of large leaved desert perennials 20 °C below air temperature (40 °C or above). This was explained by very high rates of transpiration made possible by plants taking advantage of sporadic rainfall events.

Stomatal aperture, measured as the conductance (g_s) for CO₂ or water vapor, varies continuously with changes in the environment (light intensity, atmospheric CO₂ concentration, air temperature, air humidity, wind) as well as with time of day and plant water status [11]. Therefore, the term of reference will be the maximum leaf diffusive conductance (g_{smax}), which depends on pore size (S) and the number of stomatal pores per unit leaf area (stomatal density, SD). Other characteristics (mainly anatomical) such as the position of guard cells relative to the epidermal cells (e.g. sunken stomata as in *Pinus* and other conifers, or *Nerium* or deposition of waxes on the stomata) may reduce g_{smax} . There is a set of conditions that induce changes in stomatal size and density of stomata. For example, the increase in CO₂ concentration in the atmosphere stimulates the production of leaves with less number of stomata per unit leaf area and vice-versa in lower CO₂ environment [26]. The impact, however, may not be large as there is experimental evidence that density is negatively correlated with stomata size [27].

2.2. Water use efficiency

Plants differ in the amount of carbon assimilated per unit of mass of water lost, i.e. their *instantaneous water use efficiency* (WUE_i) that can be estimated as the ratio of leaf net carbon assimilation (A) to transpiration (E), i.e. A/E (in mmol CO₂ mol⁻¹ H₂O). The ratio A/E is highly variable with environmental conditions, namely vapor pressure deficit (VPD) that determines the transpiration rate. That means that comparison of plants under different climatic conditions cannot be done. In order to avoid ambiguity associated with the effects of VPD, we may use instead the ratio of carbon assimilation to stomatal conductance A/ g_s referred to as *intrinsic water use efficiency* (WUE_i) [28,29]. However, often we want to know the WUE encompassing a growing season of a crop. A *season-long crop water use efficiency* (WUE_c, g DM kg⁻¹ H₂O) can then be defined as the ratio of the net gain of plant biomass (dry matter) over a given period, by the water lost over the same time.

When comparing C₃ and C₄ species it is apparent that C₄ plants exhibit higher WUE due to higher A_{max} and lower g_{smax} . In warm regions, where C₄ species evolved, photorespiration was stimulated considerably, as well as transpiration demand [30]. By increasing CO₂ concentration nearby RuBisCO C₄ plants greatly enhanced carboxylation efficiency and were able to inhibit photorespiration. By producing smaller stomata (for a given stomatal density) or reducing stomatal aperture plants will function at low g_{smax} . Lower g_s will improve plant water status and mitigate hydraulic demands on the conducting pathway in the xylem, therefore preventing hydraulic failure [31].

In general, stomatal regulation of gas exchange at the leaf level operates in a way that maximizes carbon assimilated per water

transpired. Optimum WUE involves stomatal restriction of leaf transpiration to the periods when evaporative demand is highest. That is why stomata are more closed at midday and in the afternoon than in the morning [11]. In a daily basis, the lower the water available (or the higher the value of VPD), the lower the time allocated to carbon assimilation (stomata open). In stressed plants photosynthesis may be limited to the morning hours, before VPD is too high [23,32]. Another example of optimization in leaf A/E is the effect of wind. Increasing wind speed enhances carbon dioxide (CO₂) assimilation while reducing transpiration due to more efficient convective cooling (under high solar radiation loads), thus improving WUE [33].

In the attempt to reach a consensus theoretical model for stomatal conductance regulation an economic analogy was proposed [34] based on the hypothesis that *g_s* is regulated to minimize the amount of water spent to fix one unit of carbon, i.e. an optimality problem. In economics the marginal cost represents the total cost for producing one more unit. In this case, the marginal unit water cost of plant carbon gain ($\partial E/\partial A$) is constant over a specified time frame. In other words, the ratio of the sensitivities of *E* and *A* to changes in *g_s* [i.e. $(\partial E/\partial g_s)/(\partial A/\partial g_s)$] remain constant over the specified time frame [34], or to put it in a more colloquial statement, carbon gain is maximized for a given amount of water loss [35].

2.3. Night-time transpiration – significance and impact on WUE

Even though the optimization models seem to apply to many situations [35] there are apparent inefficiencies. One example is the existence of night transpiration, due to incomplete stomatal closure during the night, which involves the loss of water in the dark without carbon assimilation. Night-time transpiration (*E_{night}*) can result in a significant water loss and in a major reduction of WUE, as reported for both crop species [36–38] and model plants [39–41]. Rates of *E_{night}* are documented across a wide phylogenetic and ecological number of species, (e.g. genus *Rosa*) and are generally low, within the range of 5–15% of day transpiration [36,38,42] but they may reach higher values (up to 25% to 30%) in dry environments [36,43]. Konarska et al. [44] found that *E_{night}* was observed in several forest species, and amounted to 7 and 20% of midday *E* of sunlit and shaded leaves, respectively. Other studies describe variation on *g_{snight}* between different genotypes [45–47] and also between closely related species [40,48]. Also important is the fact that *E_{night}* may reduce WUE at the landscape level and be relevant for the closing of water balance, locally and globally [49]. In general, after stomatal closure in the beginning of the night, leaf gas conductance start to increase but the maximum of *E_{night}* occurs in the hours just before dawn [50]. The time sequence of nocturnal stomatal closure suggests an endogenous regulation, possibly as part of circadian rhythms rather than a direct response to changes in VPD or in temperature. Night-time stomatal opening may be adaptive for the plant with the circadian clock providing an anticipation of sunrise, when carbon assimilation will start. High values of predawn stomatal conductance mean that leaves are ready to take full advantage of the first morning sun light to fix carbon while VPD and temperature are still low. Later in the day, conditions are less favorable and WUE is reduced. This is of great importance in semi-arid environments.

The mechanisms regulating *E_{night}* in plants remain unclear [49]. It is known that *g_{snight}* responds to similar internal and external factors as daytime transpiration, e.g. ABA, CO₂ concentration, wind speed, drought stress and VPD [36,37,51]. Also, in *Arabidopsis thaliana* mutants with abnormal stomatal closure in darkness (*open all night long – opal*) stomata close normally in response to ABA and atmospheric CO₂ [41]. This suggests that the response of stomata to light to dark transition can be partly decoupled from ABA or CO₂ signaling pathways. It is possible that selection for high *g_{snight}* and

related water loss may have occurred in habitats characterized by abundant water resources but low nutrient availability. This may indicate that *E_{night}* would be more related to nutrition than to a “cooling function” in plants.

2.4. Regulation of stomata movements at the molecular level

Regulation of stomatal movement is one of the most studied model systems for cellular signaling transduction and involves many proteins responsible for controlling stomatal responses to the environment [52,53]. This regulation is exerted either via stomata density and pattern or via stomatal aperture. Among the genes known to affect stomata functioning is ERECTA gene family. ERECTA are putative leucine-rich repeat receptor-like kinases that have been related to the perception of drought stress signals across cell membrane in the mutant of *Arabidopsis* with improved transpiration efficiency [54]. ERECTA are known to affect stomata patterning [55], which will have implications on CO₂ diffusion through the mesophyll, with impact on photosynthesis [54]. ERECTA genes are considered to improve WUE [56] [45] and increase drought tolerance [57]. But they also exert pleiotropic effects, independently from the effects on plant water status, which includes a role in the circadian clock and in thermotolerance improvement [55,58].

On the other hand, several proteins located in the plasma membrane and tonoplast of guard cells, including channels and carriers are known to be involved in the control of stomata movements [41,53,59–61]. The activity of these proteins is regulated via post-translational modifications, particularly via phosphorylation [62]. Several of these proteins play signal transduction roles during plant adaptation to stress, with an involvement ranging from stress signal perception to stress-responsive gene expression [63]. The expression of these responsive genes is known to be regulated by ABA-dependent and ABA-independent pathways [64]. Although several studies report the convergence between both pathways [65] the knowledge on how the two signaling pathways regulate each other is limited [63].

ABA induced stomata closure is related with protein modification via PP2C (type 2C protein phosphatases), which affects the activity of several channels and carriers. The ABA perception by the guard cells is undertaken by several ABA receptors involved in the network of ABA responses, namely RCAR/PYR1/PYL-PP2C complexes. When the complex binds ABA [58], inhibition of the PP2C activity occurs and targets are phosphorylated. ABA signaling transduction regulating stomatal response involves several genes [66] and evidence showed that plant membrane transport systems play a significant role in this response, either passively through channels and carriers or actively by primary and secondary transporters using ATP hydrolysis and ions gradients to drive solutes across membranes [67]. ABA is imported into guard cells by ABCG40 transporter, activating the S-type (slow-activating sustained) anion channels and the R-type (rapid-transient) anion channels, which facilitates the efflux of anions such as malate²⁻, Cl⁻, and NO³⁻ see review [60].

An elevation of cytoplasmic Ca²⁺ concentration due to Ca²⁺-permeable channels located in the tonoplast and the plasma membrane is also observed during ABA dependent stomatal closure [68,69]. These proteins are encoded by genes belonging to the TPC1 (two-pore channel 1), CNGC (cyclic nucleotide-gated channel), and GLR (glutamate receptor-like) families [66]. Simultaneously, H⁺-ATPase and inward K⁺ channels (KAT1/KAT2) are inhibited, inducing a depolarization of the plasma membrane [70] and the activation of the efflux of potassium, which is facilitated by GORK (guard cell outward rectifying K⁺) channels [71–73]. The inhibition of GORK activity in guard cells results in defects in K⁺ efflux and lower stomatal closure [72]. More recently, a stress-responsive K⁺ uptake permeases (KUPs), sharing redundant

functions with GORK channels were identified [74]. KUP6 protein, an *A. thaliana* K⁺/H⁺ symporter, localized to the plasma membrane of guard cells, is highly up-regulated in response to the ABA treatment and water deficit. However, Becker and colleagues [71] observed an up-regulation of GORK transcription upon onset of drought in guard cells to be ABA insensitive. These data shows that plants have tools to adjust stomatal movement in an ABA independent way. ABA independent pathways have been elusive but there are evidences supporting the role of another hormone (salicylic acid) and gas molecules as NO, CO and H₂S in these pathways [75,76].

Aquaporins are also considered to have a role on the regulation of the opening and closure of stomata, namely by providing plants with the means to rapidly and reversibly modify water permeability [77,78]. A recent report [79] shows the direct role of a specific Plasma membrane Intrinsic Protein (PIP2;1) in stomatal movement. It is suggested that the ABA-triggered stomatal closure requires an increase in guard cell permeability to water, through OST1-dependent phosphorylation of PIP2;1.

In addition to the regulation of protein activity, evidence shows that transcriptional factors are also involved in membrane transport in guard cell signaling, therefore controlling stomatal movement. At guard cells, several TFs regulate stomatal opening in an ABA dependent way [80]. Different R2R3MYB family members controlling either dark-light or ABA pathways and involved in the opening or the closure of stomata were identified. Particularly *AtMyb60* is involved in stomata opening in the light, as well as in the ABA and desiccation signaling perception. A functional ortholog to *AtMyb60*, from grapevine *VvMyb60*, was shown to be ABA sensitive [81]. Several other transcription factors families were identified acting as repressor or positive regulators of drought resistance [53,80].

The last decades provided the community with a large body of data not only of highly scientific relevance but also with breeding potential [61]. There are still some gaps to be closed but the genes that code for the proteins involved in stomata movement and regulation are excellent candidates for breeding programs.

3. Leaf and canopy temperature

3.1. Regulation of leaf temperature

Leaves only absorb a small amount of the incident radiation and from this absorbed energy most is dissipated as sensible or as latent heat (transpiration). In well-watered plants, transpiration represents the most effective way of leaf cooling. Under water deficits, with stomata closed, transpiration only occurs via cuticle. Furthermore, cuticular transpiration increases exponentially with rising temperature due to the increase in the water permeability of cuticle and in VPD [17].

Regulation of leaf temperature is strongly dependent on leaf morphology, i.e., leaf size and shape [82]. These traits are highly variable and likely to be influenced by different selection pressure factors. They are determinant in plant's growth performance in terms of the response to heat, water and light stress [11,82–84]. Plants in drier climates tend to present smaller leaves, whereas larger leaves are more common in humid climates [84]. Indeed, energy balance models predict that leaf temperature is higher in large canopy leaves due to thicker boundary layers for a given radiation and wind speed.

The relevance of stomata in leaf cooling is especially recognized in tall canopies like fruit and forest trees. However, even in dwarf canopies the higher stomatal density and $g_{s,max}$ than it would be predicted on the basis of regulating transpirational water loss suggests the need for transpirational cooling [85].

As a result of a combination of large influx of absorbable energy with insufficient loss of heat, leaf overheating may take place, leading to a down regulation of photosynthesis or photoinhibition. In general, high temperatures only last a few hours of the day, so in most cases plants can overcome it without major negative effects. However, if the condition prolongs and 'lethal limits' are crossed, permanent injuries such as leaf necroses may occur. Thresholds for heat damage are characteristic of the species but vary with organs and tissues. These threshold temperatures can vary from 45 to 50 °C in temperate zone species to 50–65 °C in sub-tropical C₄ grasses and woody plants as well as in grasses of the steppes [83]. Increased temperature is known to accelerate leaf aging and senescence as a result of high ABA and reduced cytokinin content produced in wilting leaves [17].

3.2. Optimal temperature for leaf functioning. Thresholds for leaf survival

The interval of leaf temperatures for attaining maximum photosynthesis vary with species, ranging from 20 to 35 °C in temperate zone C₃ crops to over 35 °C for C₄ species, whereas the optimum for growth is somewhat lower [11]. The higher optimal temperature for carbon assimilation in C₄ plants results from the inhibition of photorespiration induced by the CO₂ concentrating mechanisms in the bundle-sheaths they acquired during evolution.

Optimal temperatures for carbon assimilation are also dependent on the environmental conditions during the growth period that may induce plant's acclimation to the particular weather conditions of the season [23,53].

Biochemically, high temperatures pose several challenges to cells, namely to the photosynthetic-related enzymes. Cells have to ensure organelle stability, deal with unfavorable O₂ to CO₂ ratios and unfavorable oxidative status that decrease both the rate of CO₂ fixation and the activity of the enzymes of the Calvin cycle. Photosynthesis occurs in different sub-compartments of the chloroplast, which are metabolically distinct. Organelle stability (membrane integrity and fluidity) is essential in the control of the biochemical environment and therefore protein activity. High temperature is typically associated with a higher potential for O₂ production (via high light). Furthermore, the relative solubility of O₂ to CO₂ increases with temperature, leading to a higher competition for RuBisCO catalytic site and lower CO₂ fixation. Higher O₂ concentration also has the potential to disturb the oxi-reduction homeostasis in the cell, further contributing to lower carbon assimilation since several enzymes of the Calvin cycle are only active when reduced [59].

Temperature also influences the rate of enzymatic reactions, exerting a positive effect until a denaturation threshold is achieved, which causes enzyme inactivation. Super-optimal temperatures (above such threshold) lead to enzyme inactivation. For most enzymes, the temperature denaturation threshold is unknown. Koning [86] describes that most enzymes are denatured above 50 °C and that the optimum temperature for typical enzymes is comprised between 40 and 50 °C. As an exercise, the optimum temperature of several photosynthetic-related enzymes was searched in the enzyme database BRENDA (version 2015.2) [87]. We also searched for ferredoxin and ferredoxin-thioredoxin reductase, key players in keeping Calvin cycle enzymes reduced and therefore active. Considering those enzymes with more than four representatives (Table 1), it was found that the range of optimum temperatures is wide. The 35–40 °C range is a cornerstone for protein activity and denaturation, with strong impact on the carbon assimilation and redistribution within the plant, in both C₃ and C₄ species.

The optimum temperature is calculated using purified enzymes and may not reflect the *in vivo* inactivation threshold. For exam-

Table 1

Range of optimum temperatures and median optimum temperatures for some photosynthetic-related enzymes in higher plants available at the BRENDA (BRaunschweig ENzyme Database), database, using the tool “functional enzyme parameters” (www.brendaenzymes.org/statistics.php?valueSelect=Temperature.Optimum&taxTerm=&secNumber=). We searched for several enzymes (see table footnote) but for this table we considered only the enzymes with more than four entries at the database: RuBisCO – C₃ & C₄; Phosphoglycerate kinase – C₃ & C₄; PEP carboxylase – C₄ & CAM; Ferredoxin – regulatory protein which activates several photosynthetic enzymes via protein reduction. The activity of Ferredoxin depends on the photochemical reactions.

Enzyme	EC number	N° entries in BRENDA	Range of optimum temperatures	Median optimum temperatures
RuBisCO	4.1.1.39	11	25–40 °C	25 °C
Phosphoglycerate kinase	2.7.2.3	5	22–30 °C	25 °C
PEP carboxylase	4.1.1.31	15	25–67 °C	30 °C
Ferredoxin	1.8.7.1	4	22–37 °C	33.5 °C

Footnote: Enzymes considered: Calvin cycle: phosphoribulokinase [EC:2.7.1.19], ribulose-bisphosphate carboxylase-oxygenase [EC:4.1.1.39], phosphoglycerate kinase [EC:2.7.2.3], glyceraldehyde-3-phosphate dehydrogenase [EC:1.2.1.13], fructose-bisphosphate aldolase [EC:4.1.2.13], fructose-1,6-bisphosphatase [EC:3.1.3.11], transketolase [EC:2.2.1.1], sedoheptulose-bisphosphatase [EC:3.1.3.37], ribose 5-phosphate isomerase [EC:5.3.1.6]; C₄ enzymes: phosphoenolpyruvate carboxylase [EC: 4.1.1.31], carbonic anhydrase [EC:4.2.1.1]; cellular redox homeostasis dependent of the photochemical reactions: ferredoxin [EC:1.8.7.1]; ferredoxin-thioredoxin reductase [EC:1.8.7.2].

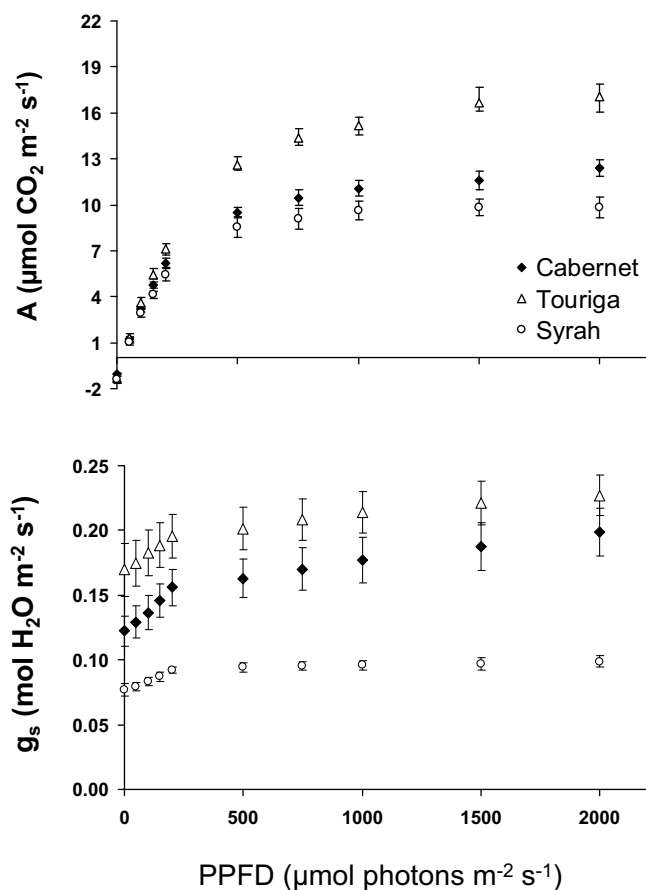


Fig. 1. Light response curves for net photosynthesis (A) and stomatal conductance to water vapor (g_s) measured for three *Vitis vinifera L* varieties: ‘Touriga Nacional’, ‘Syrah’ and ‘Cabernet Sauvignon’ subjected to deficit irrigation in the field, in Alentejo. Vines were 6–7 years old and were grafted on the rootstock 1103–Paulsen. Gas exchange measurements were done with a portable infrared gas analyzer (Li-cor 6400, Li-Cor Inc.) with a light chamber 6400–02 B (Li-Cor Inc.) equipped with a LED red/blue light source. The light curves were obtained by measuring A and g_s at steady state under different PPFs (2000–0 $\mu\text{mol m}^{-2} \text{s}^{-1}$), at constant air CO_2 (360 mL L^{-1}), a T block set at 25 °C and an air flow rate set at 500 mmol s^{-1} . Measurements were done between 9.00 and 13.00 h in August 2006, on 4–6 plants per variety, one leaf per plant. Under the moderate water stress conditions of the trial (ψ_{pd} around –0.5 MPa), ‘Touriga Nacional’ had higher values of stomatal conductance to water vapor and lower T_{leaf} than the ‘Syrah’ and ‘Cabernet Sauvignon’, with 33.9 ± 1.0 °C for ‘Touriga Nacional’, 36.9 ± 0.2 °C for ‘C. Sauvignon’ and 35.6 ± 0.2 °C for ‘Syrah’. Leaf gas exchange and temperature values are means \pm SE ($n=4-6$ plants). Adapted from Costa and co-authors [129].

ple, it was shown that the RuBisCO deactivation state is caused by reduced activity of RuBisCO activase, which is more sensitive to temperature than RuBisCO itself [88]. In the last decades a large body of knowledge has been generated illustrating the several lev-

els of protein activity regulation [59]. The events taking place at protein level will determine protein activity and pathway fitness as a single enzyme can block the full pathway (i.e. the protein with lowest temperature threshold). Such information, when combined with kinetic studies, is highly relevant to the understanding of crop productivity under challenging temperature. However, the whole regulatory network needs to be deciphered, in order to identify temperature QTLs and their potential impact. Such information can be used in breeding strategies for more efficient photosynthesis under temperature constraints.

4. Genotypes – a role for breeding?

Given the expected water restrictions, in particular for Mediterranean environments and some semiarid subtropical climates, genotypes that are able to compromise between high WUE and leaf cooling capacity are required. To avoid thermal damage, the best adapted genotypes must have the ability to maintain stomata open and transpire when optimum temperature is exceeded, therefore benefiting from leaf evaporative cooling [89]. Evaporative cooling can be promoted under irrigation. In rainfed crops a strict control of stomatal aperture to prevent xylem cavitation may lead to supra-optimal leaf temperatures, therefore requiring resilient metabolism towards heat stress.

There is evidence for genetic variability in traits that control stomatal response to VPD, with impact on performance under heat and drought conditions. For example, in some soybean genotypes the response of leaf transpiration to VPD was found to be linear at low VPD but slowing down until a plateau was reached for a given VPD threshold. This allows soil water conservation during periods of low rainfall [8]. Synergism between soil water deficits and VPD on reducing stomatal aperture was also reported, for example, in trees of the Mediterranean (*Quercus ilex* and *Quercus suber*) in which stomatal response to VPD was more pronounced during summer drought than under pre-drought conditions [16].

The temperature regime during canopy development can also influence the upper limit of canopy conductance via stomata density and size. Long-term effects of a higher thermal regime during the growing season were studied in grapevines, showing an enhanced g_s as a result of longer and wider open stomata, as compared to plants grown at a lower temperature regime [90].

4.1. Iso- and aniso-hydric responses

Differences in stomatal sensitivity to water deficits between species or cultivars may serve to compensate for differences in the vulnerability of xylem to cavitation [91]. According to the type of stomatal regulation in place, plants have been classified as presenting an isohydric or ‘pessimistic’ stomatal behavior, in contrast with the anisohydric or ‘optimistic’ behavior, where a more fluc-



Fig. 2. View of a grapevine canopy where two red grape varieties ('Touriga Nacional' and 'Cabernet Sauvignon'), grafted on the same rootstock (SO4) are grown side by side in a non-irrigated vineyard located at the Lisbon winegrowing region (Torres Vedras), in Portugal. Image taken at the ripening period after a dry season with heat waves, showing a higher senescence and/or leaf burn on the variety 'Touriga Nacional' (characterized by a higher stomatal conductance to water vapor relatively to 'Cabernet Sauvignon').

tuating water potential in response to dehydration is allowed [92]. This classification does not reflect a strict genotypic character since the growing conditions (e.g. field *versus* greenhouse, potted vs soil grown) and the degree of imposed stress also modulate the response [93].

The anisohydric response involves the use of soil water resources by the plant until lower water potentials are attained than in the isohydric case. This is due to higher stomatal conductance/leaf transpiration, with plants of the anisohydric group presenting cooler leaves and higher photosynthetic rates than isohydric ones (Fig. 1). However, a negative side of it can occur – these plants may suffer a rapid dehydration under hot spells due to high transpiration rates not compensated by a fast enough soil water uptake, resulting in the senescence and dry out of basal leaves of the canopy (Fig. 2). On the other hand, a more conservative response *vis-à-vis* the decrease in soil water is exhibited by isohydric plants, with stomata showing a quick and sensitive reaction to dehydration. This enables fluctuations in plant water potential in response to soil water deficit to be minimized and maintained above (less negative) the critical value for xylem cavitation for longer periods. This protective response will have costs in terms of lower CO₂ assimilation rates and reduced growth [93] and under heat-wave situations leaf temperatures are likely to attain critical thresholds for leaf damage.

The different response to water scarcity is also associated with different root/shoot ratios, with anisohydric stomatal behavior being often associated with larger root systems and high capacity for osmoregulation that support water uptake until low soil water content [4]. Osmoregulation requires a dehydration signal to be developed, more likely to take place in aniso- than in isohydric plants. The anisohydric strategy will allow a closer match between water availability and consumption, with a positive impact on seasonal growth.

Differences in the signals responsible for stomatal control between iso and anisohydric plants have been identified [17,92]. It was shown that in addition to its direct action in stomata, ABA may induce closure of aquaporins in bundle sheath cells, decreasing water flow to the mesophyll cells and therefore reinforcing the

effect on stomata with a hydraulic component [91]. This multiple effect of ABA might explain the more sensitive response to water deficits in isohydric plants as compared to anisohydric ones [94,95]. This is very important in plants with greater susceptibility to xylem cavitation.

4.2. Roots and rootstocks

Differences in plant responses to water stress have been associated to differential adjustments in fine root hydraulic physiology and suberization [96]. Roots which are able to maintain high root hydraulic conductivity under water stress conditions are also able to maintain greater water supply to the shoot [97]. Indeed, long-distance water transport from roots to leaves is highly dependent on maintenance of xylem functionality.

Recently, it was reported that ABA might exert a control on leaf hydraulic conductance, with an indirect effect on stomatal conductance [94]. Nonetheless, ABA cannot fully explain the control of stomatal conductance [98] and recent reports demonstrate the role of aquaporins in root-specific hydraulics [99]. Gambetta and colleagues [100] observed an up-regulation of several aquaporins both under well-watered and drought conditions, associated with high root hydraulic conductance. Some aquaporins were also reported to have a significant link with stomatal conductance and leaf hydraulic conductivity under mild water deficits [101,102]. It was further observed that plant senses the first stages of the stress by increasing root hydraulic conductance, stimulating root-to-shoot chemical signaling, increasing xylem sap pH and modulating aquaporin expression [102].

Perennial fruit crops are often grafted onto rootstocks that provide better resistance to biotic and abiotic stresses as well as maintain the desirable qualities of harvested fruits. As in own roots, rootstock effects on scion are mediated by either chemical or hydraulic signaling [103,104]. Scion transpiration rate and its acclimation to water deficit are controlled by the rootstock through different and complex genetic architecture [104]. Nonetheless, at early stages of development, the scion appears to be the main driver of shoot growth and biomass allocation in grafted plants [105]. Grafting the same variety in different rootstocks significantly alter overall scion physiological performance, as shown for example in grapevine [104,106] and in other woody species [107,108].

The adaptation of rootstocks to drought and its influence on the scion is a complex trait under the control of several physiological and molecular processes. Although there are evidences for the contribution of rootstock to the genetic variability of WUE [109], differences among rootstocks are not always confirmed in field experiments [110]. Until now, rootstocks are classified qualitatively. Nonetheless, the single report on the genetic control of drought tolerance conferred to scion by the rootstock [104] identify several quantitative loci for scion transpiration and WUE. This identification of rootstock QTL related to improved WUE would facilitate breeding for improved drought tolerant rootstocks. Unfortunately, the identification of these genes is being retarded by the low mapping resolution of such analyses and the number of distinct genotypes in the population used. Further research is still needed, to improve the understanding of rootstock contribution to water stress adaptation, in particular in the context of the ongoing climate change [109].

4.3. Is there an ideotype for semi-arid zones?

Under semi-arid regions an 'ideal' crop should be able to combine some of the following strategies: to capture soil water efficiently, to move more of the available water through the crop (rather than being wasted as soil evaporation or drainage), to exert an efficient stomatal regulation in response to soil or atmospheric

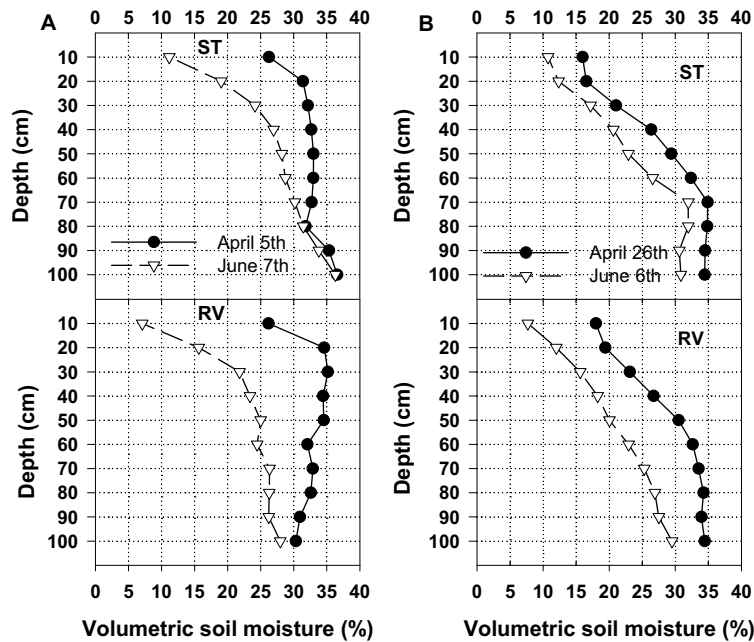


Fig. 3. Effect of floor management practices on soil water depletion curves measured *in situ* during Spring, on two sites, at the third year after experiment setup of the trial. (A) Grapevine variety 'Cabernet Sauvignon', grown in Alenquer, a coastal non-irrigated winegrowing region of Portugal, 2004; (B) Grapevine variety 'Aragonez', grown in Estremoz, Alentejo, an irrigated hot and dry winegrowing region in South Portugal, 2006. Each point is the mean of measurements made on 12 (A) or 16 (B) access tubes installed in the row between two contiguous vines. ST – soil tillage; RV – resident vegetation. Adapted from Monteiro and Lopes (2007) [92] (A) and Lopes et al. (2011) [99] (B).

water deficits and/or have an improved crop transpiration efficiency [4,111].

Irrigation changes substantially crop responses to the environment. Under irrigation, genotypes should benefit from being closer to the anisohydric type (as defined above), using the available water in order to have open stomata and maintain higher carbon assimilation rates until lower soil water content. These genotypes are in general resilient to xylem cavitation and need a large and vigorous root system. In the case of using deficit irrigation strategies, where water supply may be well below total evapotranspiration of the crop, close monitoring of canopy temperature is desirable, especially in periods when heat waves can occur and crops were not acclimated to high temperatures.

It is interesting to refer the case of cotton and bread wheat that were bred for higher yields at supra-optimal temperatures under irrigation. Successive commercial releases of both crops showed genetic-controlled increases in stomatal conductance that were accompanied by yield increases [112,113]. It was demonstrated that stomatal response to temperature (not to light or VPD) was the responsible factor for separating low and high-yielding cotton lines. Those changes in g_s were independent of changes in photosynthetic rates and rather associated with leaf cooling, thus providing a valuable avoidance of supra-optimal temperatures [113].

On the other hand, isohydric behavior is adapted to rainfed situations due to highly sensitive stomata to VPD and/or soil water deficits. However, these genotypes will require thermal resilience at the mesophyll level, to sustain periods of supra-optimal temperature without major leaf damage [4]. A conservative pattern of water use, rather than deep or profuse rooting as in anisohydric crops, is critical for the terminal drought tolerance of these crops. For example, drought tolerant genotypes of chickpea showed a lower water uptake and a lower stomatal conductance at the vegetative stage than sensitive ones, while tolerant genotypes extracted more water than sensitive genotypes after flowering [114]. In other words, higher seed yield corresponded to genotypes that were able to save water early in the season and use it in late season.

5. Management for coping with drought and heat stress

The sustainability of modern irrigated agriculture and increased competition for water resources with other economic sectors is forcing agronomists to use water more efficiently [115,116]. Higher air temperatures and less precipitation, together with increasing limitations in the available water resources in semi-arid agricultural areas, led to the adoption of alternative irrigation strategies such as deficit irrigation (irrigation below full crop evapotranspiration losses) as means to save water via increased transpiration efficiency [93]. Indeed, it is known that mild to moderate water deficits may lead to an increase in WUE due to the maintenance of carbon assimilation while transpiration is reduced by partial closure of stomata [93].

Other strategies to improve water productivity under irrigation involve optimization of the timing and duration of irrigation events to reduce evaporation and percolation, together with the selection of the most suitable crops/genotypes for specific climate conditions, optimal sowing and harvesting times or precise irrigation to prevent water deficits, taking into account the weather conditions and crop growth stage [116,117]. Selection of the most adequate crops and daily and seasonal irrigation strategies strongly depend on the eco-physiological and phenological characteristics of the species and also of the variety. In the case of deficit irrigation, water can be reduced or withheld at specific phenological stages as it happens with regulated deficit irrigation (RDI). RDI creates water deficits during specific periods of the season to save water while minimizing or eliminating negative impacts on crop revenue [118]. Imposition of a mild water stress to crops enables a better control of canopy development, avoiding dense canopies, therefore reducing water consumption and improving WUE [119]. However, in warm regions implementation of deficit irrigation must be done judiciously. When exposed to heat waves, mildly stressed vines become more vulnerable to leaf burn (Fig. 2). Under such conditions deficit irrigation should be replaced by full irrigation in order to increase evaporative cooling [120].

Together with irrigation, precise plant monitoring is an absolute need to cope with the increase in frequency of extreme events (drought and heat waves) [116]. Imaging techniques, such as thermography, provides a remote, non-intrusive approach to monitor crop performance and water status at different scales of time and space. Thermography has permitted to overcome limitations posed by temperature point measurements and to complement the assessment of soil and plant water status. It is being applied in modern precision agriculture [121].

Cover cropping is a cultural practice widely used with perennial crops in many areas of the world, being recommended to promote environmental sustainability [116,122]. The benefits of cover crops are many, ranging from environmental protection (e.g. control of soil erosion, enhancement of soil structure and biodiversity, sequestering carbon) to crop management, including control of crop vigor and improved fruit composition, as described for example in grapevine [122]. Despite those potential benefits, the adoption of cover crops in Mediterranean non-irrigated fruit crops has been limited by the concern of excessive water competition between the swards and the crop [123,124]. However, in Mediterranean and semi-arid regions water competition by the swards is effective only during spring [119,125,126], when favorable temperatures combined with high soil water availability can induce high vegetative growth rates and transpiration of the sward species. During the summer, sward vegetation dries out, becoming dead mulch that will reduce soil evaporation [123]. Interestingly, the development of deeper roots in the case of vines was observed after several years of competition with swards, therefore increasing the capacity for water extraction by the roots in deeper soil layers and the potential for sustained transpiration under heat stress [127,128]. Indeed, a higher water extraction from deep soil layers was observed in the treatment with permanent resident vegetation (RV) as compared with the soil tillage treatment (ST) where much lower or almost no water was extracted from deep layers (between 0.80 and 1.0 m deep), (Fig. 3). These results can be explained by the likely lower soil evaporation caused by the mulching effect of cover cropping residues during summer [123], by the smaller total crop leaf area in the RV treatment due to competition with swards and the increased water uptake by crop roots from deeper soil layers, as a result of a compensatory growth of the root system. Summarizing, the use of cover crops is a management practice that can have a positive influence on crops water use by preventing excessive vigor, in case water is fully available in spring, or by maximizing the volume of soil explored by roots through the enhancement of the exploitation of soil water reserves into deeper layers.

6. Concluding remarks and future perspectives

Basic science has already generated great advances in our understanding of plant stress tolerance. The increase in drought and high temperature predicted in climate change scenarios for the Mediterranean and semi-arid zones still poses great challenges to crop breeding and management in the near future. To deal with these scenarios information is a most wanted resource, being necessary to gather and integrate information at many levels.

The inextricable nature of ‘carbon compromise and leaf temperature’ dilemma involves a series of interactions between plants and the environment and has to be framed into the particular agricultural system being pursued (rainfed or irrigated). In general, genotypes desirable for semi-arid areas should be able to (i) maximize the extraction of available soil water (e.g. vigorous roots/rootstocks); (ii) minimize water loss per unit of fixed carbon by stomatal regulation (high WUE) and optimize its use according to the phase of the growing cycle; (iii) adjust canopy size to available water; (iv) show heat-avoidance traits (as e.g. paraheliotropic

movements) or heat-resistance at the leaf mesophyll level and/or (v) maintain cool canopies when irrigated, in order to cope with periods of enhanced temperatures.

The operational combination of different traits will vary according to the drought/heat scenarios that we wish to target. Crop management tools are required to complement the use of well-adapted genotypes, such as irrigation and floor management strategies, accompanied by close plant monitoring during the susceptible phases of crop development.

Areas of research that should be reinforced include the study of species thresholds for high temperature and for xylem cavitation to guide us in preventing irreversible damages. These types of studies when performed under controlled conditions should be followed by field experiments to enable the full assessment of the genotypes resilience and acclimation mechanisms. Indeed, leaves may reach temperatures higher than the threshold defined for a decrease in maximum photochemical efficiency and still no photodamage be observed [8]. The combination of the intensity and duration of stress will define the final conditions that dictate plant survival or failure.

Future research should also provide a molecular basis for an improved understanding of the different strategies that plants use to regulate their water status and temperature. Molecular markers identified for their association with physiological traits (or their proxies), such as water uptake (roots), water use efficiency (stomata and photosynthesis), water status (aquaporins and osmoregulation) or canopy temperature, as important drivers of plant development under stress conditions, can play a major role in supporting crop breeding for drought-prone environments.

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References

- [1] M.M. Chaves, J.P. Maroco, J.S. Pereira, Understanding plant responses to drought—from genes to the whole plant, *Funct. Plant Biol.* 30 (2003) 239–264.
- [2] A. Blum, Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive? *Austr. J. Agric. Res* 56 (2005) 1159–1168.
- [3] V. Vadez, J. Kholova, M. Zaman-Allah, N. Belko, Water: the most important molecular component of water stress tolerance research, *Funct. Plant Biol.* 40 (2013) 1310–1322.
- [4] A. Blum, Towards a conceptual ABA ideotype in plant breeding for water limited environments, *Funct. Plant Biol.* 42 (2015) 502–513.
- [5] IPCC, Climate change: impacts, adaptation, and vulnerability. Part A: global and sectoral aspects, in: C.B. Field, D.J. Barros, K.J. Dokken, M.D. Mach, T.E. Mastrandrea, M. Bilir, K.L. Chatterjee, Y.O. Ebi, R.C. Estrada, B. Genova, E.S. Girma, A.N. Kissel, S. Levy, P.R. Mastrandrea, L.L. White (Eds.), *Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, Cambridge University Press Cambridge, United Kingdom and New York, NY, USA, 2014, p. 2014.
- [6] R.E. Evenson, D. Gollin, Assessing the impact of the Green Revolution, 1960–2000, *Science* 300 (2003) 758–762.
- [7] M. Reynolds, R. Tuberosa, Translational research impacting on crop productivity in drought-prone environments, *Curr. Opin. Plant Biol.* 11 (2008) 171–179.
- [8] V. Medina, M.E. Gilbert, Physiological trade-offs of stomatal closure under high evaporative gradients in field grown soybean, *Funct. Plant Biol.* 43 (2016) 40–51.
- [9] P.J. Franks, S. Casson, Connecting stomatal development and physiology, *New Phytol.* 201 (2014) 1079–1082.
- [10] G.J. Dow, J.A. Berry, D.C. Bergmann, The physiological importance of developmental mechanisms that enforce proper stomatal spacing in *Arabidopsis thaliana*, *New Phytol.* 201 (2014) 1205–1217.

- [11] H.G. Jones, Plants and Microclimate: a Quantitative Approach to Environmental Plant Physiology, 3rd ed., Cambridge University Press, London, 2014.
- [12] W.J. Davies, J. Zhang, Root signals and the regulation of growth and development of plants in drying soil, *Ann. Rev. Plant Biol.* 42 (1991) 55–76.
- [13] A. Christmann, E.W. Weiler, E. Steudle, E. Grill, A hydraulic signal in root-to-shoot signalling of water shortage, *Plant J.* 52 (2007) 167–174.
- [14] S.A.M. McAdam, F.C. Sussmilch, T.J. Brodribb, Stomatal responses to vapour pressure deficit are regulated by high speed gene expression in angiosperms, *Plant Cell Environ.* 39 (2016) 485–491.
- [15] C.M. Rodríguez-Dominguez, T.N. Buckley, G. Egea, A. d. Cires, V. Hernandez-Santana, S. Martorell, A. Diaz-Espejo, Most stomatal closure in woody species under moderate drought can be explained by stomatal responses to leaf turgor, *Plant Cell Environ.* (2016), <http://dx.doi.org/10.1111/pce.12774>.
- [16] T.S. David, M.O. Henriques, C. Kurz-Besson, J. Nunes, F. Valente, M. Vaz, J.S. Pereira, R. Siegwolf, M.M. Chaves, L.C. Gazarini, J.S. David, Water-use strategies in two co-occurring Mediterranean evergreen oaks: surviving the summer drought, *Tree Physiol.* 27 (2007) 793–803.
- [17] M. Keller, The Science of Grapevines: Anatomy and Physiology, Academic Press, 2015.
- [18] S.Y. Rogiers, D.H. Greer, J.M. Hatfield, R.J. Hutton, S.J. Clarke, P.A. Hutchinson, A. Somers, Stomatal response of an anisohydric grapevine cultivar to evaporative demand, available soil moisture and abscisic acid, *Tree Physiol.* 32 (2012) 249–261.
- [19] P.J. Franks, Z.J. Britton-Harper, No evidence of general CO₂ insensitivity in ferns: one stomatal control mechanism for all land plants? *New Phytol.* (2016), <http://dx.doi.org/10.1111/nph.14020>.
- [20] T.J. Brodribb, S.A.M. McAdam, G.J. Jordan, T.S. Field, Evolution of stomatal responsiveness to CO₂ and optimization of water-use efficiency among land plants, *New Phytol.* 183 (2009) 839–847.
- [21] J.A. Raven, The evolution of vascular land plants in relation to supracellular transport processes, *Adv. Bot. Res.* 5 (1977) 153–219.
- [22] D.J. Beerling, P.J. Franks, Evolution of stomatal function in 'lower' land plants, *New Phytol.* 183 (2009) 921–925.
- [23] E.D. Schulze, Carbon dioxide and water vapour exchange in response to drought in the atmosphere and in the soil, *Annu. Rev. Plant Physiol.* 37 (1986) 247–274.
- [24] O. Lange, Untersuchungen über den Wärmehaushalt und hitzeresistenz mauretanischer Wüsten unter savannenpflanzen, *Flora* 147 (1959) 595–651.
- [25] W. Smith, Temperature of desert plants: another perspective on the adaptability of leaf size, *Science* 201 (1978) 614–616.
- [26] P.J. Franks, D.J. Beerling, Maximum leaf conductance driven by CO₂ effects on stomatal size and density over geologic time, *PNAS* 106 (2009) 10343–10347.
- [27] T. Lawson, M.R. Blatt, Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency, *Plant Physiol.* 164 (2014) 1556–1570.
- [28] H. Medrano, J. Gulías, M.M. Chaves, J. Galmés, J. Flexas, Photosynthetic water use efficiency, in: J. Flexas, F. Loreto, H. Medrano (Eds.), *Terrestrial Photosynthesis in a Changing Environment: A Molecular, Physiological and Ecological Approach*, Cambridge University Press, Cambridge, UK, 2009, pp. 523–548.
- [29] M.M. Chaves, J. Osório, J.S. Pereira, Water use efficiency and photosynthesis, in: M.A. Bacon (Ed.), *Water Use Efficiency in Plant Biology*, Blackwell Publishing/Oxford, 2004, pp. 42–74.
- [30] R.F. Sage, The evolution of C₄ photosynthesis, *New Phytol.* 161 (2004) 341–370.
- [31] H. Griffiths, G. Weller, L.F.M. Toy, R.J. Dennis, You're so vein: bundle sheath physiology, phylogeny and evolution in C₃ and C₄ plants, *Plant Cell Environ.* 36 (2013) 249–261.
- [32] T. Faria, D. Silvério, E. Breia, R. Cabral, A. Abadia, J. Abadia, J.S. Pereira, M.M. Chaves, Differences in the response of carbon assimilation to summer stress (water deficits, high light and temperature) in four Mediterranean tree species, *Physiol. Plant.* 102 (1998) 419–428.
- [33] S.J. Schymanski, D. Or, Wind increases leaf water use efficiency, *Plant Cell Environ.* 39 (2016) 1448–1459, <http://dx.doi.org/10.1111/pce.12700>, n/a–n/a.
- [34] I.R. Cowan, G.D. Farquhar, Stomatal function in relation to leaf metabolism and environment, *Symposia of the Society for Experimental Botany* (1977) 471–505.
- [35] S.A. McAdam, T.J. Brodribb, The evolution of mechanisms driving the stomatal response to vapor pressure deficit, *Plant Physiol.* 167 (2015) 833–843.
- [36] M.A. Caird, J.H. Richards, L.A. Donovan, Nighttime stomatal conductance and transpiration in C₃ and C₄ Plants, *Plant Physiol.* 143 (2007) 4–10.
- [37] H. Easlon, J.H. Richards, Drought response in self-compatible species of tomato (*Solanaceae*), *Am. J. Bot.* 96 (2009) 605–611.
- [38] B. McNellis, A.R. Howard, Phylogenetic and ecological patterns in nighttime transpiration among five members of the genus *Rubus* co-occurring in western Oregon, *Ecol. Evol.* 5 (2015) 3557–3569.
- [39] M.M. Barbour, T.N. Buckley, The stomatal response to evaporative demand persists at night in *Ricinus communis* plants with high nocturnal conductance, *Plant Cell Environ.* 30 (2007) 711–721.
- [40] A.R. Howard, L.A. Donovan, Helianthus nighttime conductance and transpiration respond to soil water not nutrient availability, *Plant Physiol.* 143 (2007) 145–155.
- [41] J.M. Costa, F. Monnet, D. Jannaud, N. Leonhardt, B. Ksas, I.M. Reiter, F. Pantin, B. Genty, OPEN ALL NIGHT LONG: the dark side of stomatal control, *Plant Physiol.* 167 (2015) 289–294.
- [42] T.N. Buckley, T.L. Turnbull, S. Pfautsch, M.A. Adams, Nocturnal water loss in mature subalpine *Eucalyptus delegatensis* tall open forests and adjacent *E. pauciflora* woodlands, *Ecol. Evol.* 1 (2011) 435–450.
- [43] V. Resco-de-Dios, M. Loik, R.R. Smith, M. Aspinwall, D. Tissue, Genetic variation in circadian regulation of nocturnal stomatal conductance enhances carbon assimilation and growth, *Plant Cell Environ.* 38 (2015) 1752–1764.
- [44] J. Konarska, J. Uddling, B. Holmer, M. Lutz, F. Lindberg, H. Pleijel, S. Thorsson, Transpiration of urban trees and its cooling effect in a high latitude city, *Int. J. Biometeorol.* 60 (2015) 159–172.
- [45] J.M. Escalona, S. Fuentes, M. Tomás, S. Martorell, J. Flexas, H. Medrano, Responses of leaf night transpiration to drought stress in *Vitis vinifera* L., *Agric. Water Manag.* 118 (2013) 50–58.
- [46] R. Schoppach, E. Claverie, W. Sadok, Genotype-dependent influence of night-time vapour pressure deficit on night-time transpiration and daytime gas exchange in wheat, *Funct. Plant Biol.* 41 (2014) 963–971.
- [47] S. Rogiers, S. Clarke, Nocturnal and daytime stomatal conductance respond to root-zone temperature in 'Shiraz' grapevines, *Ann. Bot.* 111 (2013) 433–444.
- [48] N.G. Phillips, J.D. Lewis, B. Logan, D.T. Tissue, Inter- and intra-specific variation in nocturnal water transport in *Eucalyptus*, *Tree Physiol.* 30 (2010) 586–596.
- [49] M. Zeppel, J. Lewis, N. Phillips, D. Tissue, Consequences of nocturnal water loss: a synthesis of regulating factors and implications for capacitance, embolism and use in models, *Tree Physiol.* 34 (2014) 1047–1055.
- [50] V. Resco-de-Dios, J. Roy, J.P. Ferrio, J.G. Alday, D. Landais, A. Milcu, A. Gessler, Processes driving nocturnal transpiration and implications for estimating land evapotranspiration, *Sci. Rep.* 5 (2015) 10975.
- [51] M. Zeppel, J. Lewis, B. Chaszar, R. Smith, B. Medlyn, T. Huxman, D. Tissue, Nocturnal stomatal conductance responses to rising [CO₂], temperature and drought, *New Phytol.* 193 (2012) 929–938.
- [52] A.M. Hetherington, F.I. Woodward, The role of stomata in sensing and driving environmental change, *Nature* 424 (2003) 901–908.
- [53] M.M. Chaves, J.M. Costa, N.J.M. Saibo, Recent advances in photosynthesis under drought and salinity plant Responses to Drought and Salinity Stress: Developments in a Post-genomic Era, 57, *Advances in Botanical Research*, 2011, pp. 49–104.
- [54] J. Masle, S.R. Gilmore, G.D. Farquhar, The ERECTA gene regulates plant transpiration efficiency in *Arabidopsis*, *Nature* 436 (2005) 866–870.
- [55] M.v. Zanten, L.B. Snoek, M.C.G. Proveniers, A.J.M. Peeters, The many functions of ERECTA, *Trends Plant Sci.* 14 (2009) 214–218.
- [56] H.T. Xing, P. Guo, X.L. Xia, W.L. Yin, PdERECTA a leucine-rich repeat receptor-like kinase of poplar, confers enhanced water use efficiency in *Arabidopsis*, *Plant* 234 (2011) 229–241.
- [57] M.W. Blair, A.J. Cortes, D. This, Identification of an ERECTA gene and its drought adaptation association with wild and cultivated common bean, *Plant Sci.* 242 (2016) 250–259.
- [58] H. Shen, X. Zhong, F. Zhao, Y. Wang, B. Yan, Q. Li, G. Chen, B. Mao, J. Wang, Y. Li, G. Xiao, Y. He, H. Xiao, J. Li, Z. He, Overexpression of receptor-like kinase ERECTA improves thermotolerance in rice and tomato, *Nat. Biotechnol.* 33 (2015) 996–1003.
- [59] B.B. Buchanan, W. Gruissem, K. Vickers, R.L. Jones, *Biochemistry and Molecular Biology of Plants*, 2nd ed., John Wiley & Sons, 2015.
- [60] V. Cotelle, N. Leonhardt, 14–3–3 Proteins in guard cell signaling, *Front. Plant Sci.* 6 (2015) 1210.
- [61] H. Kolliet, M. Nuhkat, M.R. Roelfsema, Closing gaps: linking elements that control stomatal movement, *New Phytol.* 203 (2014) 44–62.
- [62] T. Zhang, S. Chen, A.C. Harmon, Protein phosphorylation in stomatal movement, *Plant Signal. Behav.* 9 (2014) e972845.
- [63] T. Yoshida, J. Mogami, K. Yamaguchi-Shinozaki, ABA-dependent and ABA-independent signaling in response to osmotic stress in plants, *Curr. Opin. Plant Biol.* 21 (2014) 133–139.
- [64] K. Yamaguchi-Shinozaki, K. Shinozaki, Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses, *Annu. Rev. Plant Biol.* 57 (2006) 781–803.
- [65] X. Xie, Y. Wang, L. Williamson, G.H. Holroyd, C. Tagliavia, E. Murchie, J. Theobald, M.R. Knight, W.J. Davies, H.M. Leyer, A.M. Hetherington, The identification of genes involved in the stomatal response to reduced atmospheric relative humidity, *Curr. Biol.* 16 (2006) 882–887.
- [66] K.M. Jarzaniak, M. Jasinski, Membrane transporters and drought resistance—a complex issue, *Front. Plant Sci.* 5 (2014) 687.
- [67] B. Schulz, Functional classification of plant plasma membrane transporters, in: A.S. Murphy, B. Schulz, W. Peer (Eds.), *The Plant Plasma Membrane*, Springer/Berlin, 2011, pp. 131–176.
- [68] J.I. Schroeder, S. Hagiwara, Cytosolic calcium regulates ion channels in the plasma membrane of *Vicia faba* guard cells, *Nature* 338 (1998) 427–430.
- [69] J.M. Ward, J.I. Schroeder, Calcium-activated K⁺ channels and calcium-induced calcium release by slow vacuolar ion channels in guard cell vacuoles implicated in the control of stomatal closure, *Plant Cell* 6 (1994) 669–683.

- [70] Y. Osakabe, K. Yamaguchi-Shinozaki, K. Shinozaki, L.-S.P. Tran, ABA control of plant macroelement membrane transport systems in response to water deficit and high salinity, *New Phytol.* 202 (2014) 35–49.
- [71] D. Becker, S. Hoth, P. Ache, S. Wenkel, M.R. Roelfsema, O. Meyerhoff, W. Hartung, R. Hedrich, Regulation of the ABA-sensitive potassium channel gene GORK in response to water stress, *FEBS Lett.* 554 (2003) 119–126.
- [72] E. Hosal, A. Vasseur, K. Moulène, I. Dreyer, F. Gaymard, F. Poree, J. Boucherez, A. Lebaudy, D. Bouchez, A.A. Verry, T. Simonneau, J.B. Thibaud, H. Sentenac, The *Arabidopsis* outward K⁺ channel GORK is involved in regulation of stomatal movements and plant transpiration, *Proc. Natl. Acad. Sci. U. S. A.* 100 (2003) 5549–5554.
- [73] C. Eisenach, M. Papanatsiou, E.K. Hillert, M.R. Blatt, Clustering of the K⁺ channel GORK of *Arabidopsis* parallels its gating by extracellular K⁺, *Plant J.* 78 (2014) 203–214.
- [74] Y. Osakabe, N. Arinaga, T. Umezawa, S. Katsura, K. Nagamachi, H. Tanaka, H. Ohiraki, K. Yamada, S.U. Seo, M. Abo, E. Yoshimura, K. Shinozaki, K. Yamaguchi-Shinozaki, Osmotic stress responses and plant growth controlled by potassium transporters in *Arabidopsis*, *Plant Cell* 25 (2013) 609–624.
- [75] M. Issak, E. Okuma, S. Munemasa, Y. Nakamura, I.C. Mori, Y. Murata, Neither endogenous abscisic acid nor endogenous jasmonate is involved in salicylic acid-, yeast elicitor-, or chitosan-induced stomatal closure in *Arabidopsis thaliana*, *Biosci. Biotechnol. Biochem.* 77 (2013) 1111–1113.
- [76] C. Garcia-Mata, L. Lamattina, Gasotransmitters are emerging as new guard cell signaling molecules and regulators of leaf gas exchange, *Plant Sci.* 201–202 (2013) 66–73.
- [77] F. Chaumont, S.D. Tyerman, Aquaporins: highly regulated channels controlling plant water relations, *Plant Physiol.* 164 (2014) 1600–1618.
- [78] R.B. Heinen, Q. Ye, F. Chaumont, Role of aquaporins in leaf physiology, *J. Exp. Bot.* 60 (2009) 2971–2985.
- [79] A. Grondin, O. Rodrigues, L. Verdoucq, S. Merlot, N. Leonhardt, C. Maurel, Aquaporins contribute to ABA-triggered stomatal closure through OST1-mediated phosphorylation, *Plant Cell* 27 (2015) 1945–1954.
- [80] E. Cominelli, M. Galbiati, C. Tonelli, Transcription factors controlling stomatal movements and drought tolerance, *Transcription* 1 (2010) 41–45.
- [81] M. Galbiati, J.T. Matus, P. Francia, F. Rusconi, P. Cañón, C. Medina, L. Conti, E. Cominelli, C. Tonelli, P. Arce-Johnson, The grapevine guard cell-related VvMYB60 transcription factor is involved in the regulation of stomatal activity and is differentially expressed in response to ABA and osmotic stress, *BMC Plant Biol.* 11 (2011) 142.
- [82] B.E. Campitelli, J.R. Stinchcombe, Natural selection maintains a single-locus leaf shape cline in Ivy leaf morning glory, *Ipomoea hederacea*, *Mol. Ecol.* 22 (2013) 552–564.
- [83] A. Nicotra, M. Cosgrove, A. Cowling, C. Schlichting, C. Jones, Leaf shape linked to photosynthetic rates and temperature optima in South African *Pelargonium* species, *Oecologia* 154 (2008) 625–635.
- [84] D.J. Peppe, D.L. Royer, B. Cariglino, S.Y. Oliver, S. Newman, E. Leight, G. Enikolopov, M. Fernandez-Burgos, F. Herrera, J.M. Adams, E. Correa, E.D. Curran, J.M. Erickson, L.F. Hinojosa, J.W. Hoganson, A. Iglesias, C.A. Jaramillo, K.R. Johnson, G.J. Jordan, N.J.B. Kraft, E.C. Lovelock, C.H. Lusk, U. Niinemets, J. Peñuelas, G. Rapson, S.L. Wing, Sensitivity of leaf size and shape to climate: global patterns and paleoclimatic applications, *New Phytol.* 190 (2011) 724–739.
- [85] F.I. Woodward, Do plants really need stomata? *J. Exp. Bot.* 49 (1998) 471–480.
- [86] R.S. Koning, Enzyme Kinetics, in: *Plant physiology information website* (1994).
- [87] I. Schomburg, A. Chang, C. Ebeling, M. Gremse, C. Heldt, G. Huhn, D. Schomburg, BRENDA, the enzyme database: updates and major new developments, *Nucleic Acids Res.* 32 (2004) D431–D433.
- [88] S.J. Crafts-Brandner, M.E. Salvucci, Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO₂, *Proc. Natl. Acad. Sci.* 97 (2000) 13430–13435.
- [89] G.D. Farquhar, T.D. Sharkey, Stomatal conductance and photosynthesis, *Annu. Rev. Plant Physiol.* 33 (1982) 317–345.
- [90] V.O. Sadras, A. Montoro, M.A. Moran, P.J. Aphalo, Elevated temperature altered the reaction norms of stomatal conductance in field-grown grapevine, *Agric. For. Meteorol.* 165 (2012) 35–42.
- [91] A. Shatil-Cohen, Z. Attia, M. Moshelion, Bundle-sheath cell regulation of xylem-mesophyll water transport via aquaporins under drought stress: a target of xylem-borne ABA? *Plant J.* 67 (2011) 72–80.
- [92] F. Tardieu, T. Simonneau, Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours, *J. Exp. Bot.* 49 (1998) 419–432.
- [93] M.M. Chaves, O. Zarrouk, R. Francisco, J.M. Costa, T. Santos, A.P. Regalado, M.L. Rodrigues, C.M. Lopes, Grapevine under deficit irrigation: hints from physiological and molecular data, *Ann. Bot.* 105 (2010) 661–676.
- [94] F. Pantin, F. Monnet, D. Jannaud, J.M. Costa, J. Renaud, B. Muller, T. Simonneau, B. Genty, The dual effect of abscisic acid on stomata, *New Phytol.* 197 (2013) 65–72.
- [95] M. Moshelion, O. Halperin, R. Wallach, R. Oren, D.A. Way, Role of aquaporins in determining transpiration and photosynthesis in water-stressed plants: crop water-use efficiency, growth and yield, *Plant Cell Environ.* 38 (2015) 1785–1793.
- [96] F.H. Barrios-Masias, T. Knipfer, A.J. McElrone, Differential responses of grapevine rootstocks to water stress are associated with adjustments in fine root hydraulic physiology and suberization, *J. Exp. Bot.* 66 (2015) 6069–6078.
- [97] E. Steudle, Water uptake by plant roots: an integration of views, *Plant Soil* 226 (2000) 45–56.
- [98] S. Martorell, A. Diaz-Espejo, M. Tomàs, A. Pou, H.E. Aou-ouad, J.M. Escalona, J. Vadell, M. Ribas-Carbó, J. Flexas, H. Medrano, Differences in water-use-efficiency between two *Vitis vinifera* cultivars (Grenache and Tempranillo) explained by the combined response of stomata to hydraulic and chemical signals during water stress, *Agric. Water Manage.* 156 (2015) 1–9.
- [99] C. Lovisolo, F. Secchi, A. Nardini, S. Salleo, R. Buffa, A. Schubert, Expression of PIP1 and PIP2 aquaporins is enhanced in olive dwarf genotypes and is related to root and leaf hydraulic conductance, *Physiol. Plant.* 130 (2007) 543–551.
- [100] G.A. Gambetta, C.M. Manuck, S.T. Drucker, T. Shaghasi, K. Fort, M.A. Matthews, M.A. Walker, A.J. McElrone, The relationship between root hydraulics and scion vigour across *Vitis* rootstocks: what role do root aquaporins play? *J. Exp. Bot.* 63 (2012) 6445–6455.
- [101] A. Pou, J. Flexas, M.D. Alsina, J. Bota, C. Carambula, F. de Herralde, J. Galmes, C. Lovisolo, M. Jimenez, M. Ribas-Carbo, D. Rusjan, F. Secchi, M. Tomas, Z. Zsofi, H. Medrano, Adjustments of water use efficiency by stomatal regulation during drought and recovery in the drought-adapted *Vitis* hybrid Richter-110 (*V. berlandieri* x *V. rupestris*), *Physiol. Plant.* 134 (2008) 313–323.
- [102] O. Zarrouk, I. Garcia-Tejero, C. Pinto, T. Genebra, F. Sabir, C. Prista, T.S. David, M.C. Loureiro-Dias, M.M. Chaves, Aquaporins isoforms in cv. Touriga Nacional grapevine under water stress and recovery—regulation of expression in leaves and roots, *Agric. Water Manage.* 164 (2016) 167–175.
- [103] C.J. Soar, P.R. Dry, B. Loveys, Scion photosynthesis and leaf gas exchange in *Vitis vinifera* L. cv. Shiraz: mediation of rootstock effects via xylem sap ABA, *Aust. J. Grape Wine Res.* 12 (2006) 82–96.
- [104] E. Marguerit, O. Brendel, E. Lebon, C.V. Leeuwen, N. Ollat, Rootstock control of scion transpiration and its acclimation to water deficit are controlled by different genes, *New Phytol.* 194 (2012) 416–429.
- [105] J.P. Tandonnet, S. Cookson, P. Vivin, N. Ollat, Scion genotype controls biomass allocation and root development in grafted grapevine, *Aust. J. Grape Wine Res.* 16 (2010) 290–300.
- [106] S. Tramontini, M. Vitali, L. Centioni, A. Schubert, C. Lovisolo, Rootstock control of scion response to water stress in grapevine, *Environ. Exp. Bot.* 93 (2013) 20–26.
- [107] S. Jiménez, J. Dridi, D. Gutiérrez, D. Moret, J.J. Irigoyen, M.A. Moreno, Y. Gogorcena, Physiological, biochemical and molecular responses in four *Prunus* rootstocks submitted to drought stress, *Tree Physiol.* 33 (2013) 1061–1075.
- [108] X. Sun, H. Yan, X. Kang, F. Ma, Growth, gas exchange, and water-use efficiency response of two young apple cultivars to drought stress in two scion-one rootstock grafting system, *Photosynthetica* 51 (2013) 404–410.
- [109] N. Ollat, A. Peccoux, D. Papura, D. Esmenjaud, E. Marguerit, J. Tandonnet, L. Bordenave, S. Cookson, F. Barrieu, L. Rossettsch, Rootstocks as a component of adaptation to environment, in: H. Gerós, M.M. Chaves, H.M. Gil, S. Delrot (Eds.), *Grapevine in a Changing Environment A Molecular and Ecophysiological Perspective*, Wiley-Blackwell, West Sussex, UK, 2016, pp. 68–108.
- [110] R.M. Stevens, J.M. Pech, M.R. Gibberd, R.R. Walker, P.R. Nicholas, Reduced irrigation and rootstock effects on vegetative growth, yield and its components, and leaf physiological responses of Shiraz, *Aust. J. Grape Wine Res.* 16 (2010) 413–425.
- [111] A.G. Condon, R.A. Richards, G.J. Rebetzke, G.D. Farquhar, Breeding for high water-use efficiency, *J. Exp. Bot.* 55 (2004) 2447–2460.
- [112] J.W. Radin, Z. Lu, R.G. Percy, E. Zeiger, Genetic variability for stomatal conductance in Pima cotton and its relation to improvements of heat adaptation, *Proc. Natl. Acad. Sci. U. S. A.* 91 (1994) 7217–7221.
- [113] Z. Lu, R.G. Percy, C.O. Qualset, E. Zeiger, Stomatal conductance predicts yield in irrigated pima cotton and bread wheat grown at high temperatures, *J. Exp. Bot.* 49 (1998) 453–460.
- [114] M. Zaman-Allah, D.M. Jenkinson, V. Vadez, A conservative pattern of water use rather than deep or profuse rooting, is critical for the terminal drought tolerance of chickpea, *J. Exp. Bot.* 62 (2011) 4239–4252.
- [115] H. Medrano, M. Tomás, S. Martorell, J. Flexas, E. Hernández, J. Rosselló, A. Pou, J.M. Escalona, J. Bota, From leaf to whole-plant water use efficiency (WUE) in complex canopies: limitations of leaf WUE as a selection target, *Crop J.* 3 (2015) 220–228.
- [116] J.M. Costa, M. Vaz, J. Escalona, R. Egipto, C. Lopes, H. Medrano, M.M. Chaves, Modern viticulture in southern Europe: vulnerability and strategies for adaptation to water scarcity, *Agric. Water Manage.* 164 (2016) 5–18.
- [117] FAO, <http://www.fao.org/nr/nr-home/en>, (2016).
- [118] A.D. Goldhamer, M. Viveros, Effects of preharvest irrigation cutoff durations and postharvest water deprivation on almond tree performance, *Irrig. Sci.* 19 (2006) 125–131.
- [119] A. Monteiro, C.M. Lopes, Influence of cover crop on water use and performance of vineyard in Mediterranean Portugal, *Agric. Ecosyst. Environ.* 121 (2007) 336–342.
- [120] C.M. Lopes, J.M. Costa, A. Monteiro, R. Egipto, I. Tejero, M.M. Chaves, Varietal behaviour under water and heat stress, in: *Congress of OENOVITI International Network*, Germany, 2014, pp. 20–56.

- [121] J.M. Costa, O.M. Grant, M.M. Chaves, Thermography to explore plant–environment interactions, *J. Exp. Bot.* 64 (2013) 3937–3949.
- [122] C.M. Lopes, A. Monteiro, J.P. Machado, N. Fernandes, A. Araújo, Cover cropping in a sloping non-irrigated vineyard: II—effects on vegetative growth, yield, berry and wine quality of ‘Cabernet Sauvignon’ grapevines, *Ciência Téc Vitiv.* 23 (2008) 37–43.
- [123] T.L. Prichard, Water use and infiltration, in: C.A. Ingels, R.L. Bugg, G.T. McGourty, L.P. Christensen (Eds.), *Cover Cropping in Vineyards. A Grower's Handbook*, University of California Oakland, USA, 1998, pp. 86–90.
- [124] C. Lopes, A. Monteiro, F.E. Ruckert, B. Gruber, B. Steinberg, H.R. Schultz, Transpiration of grapevines and co-habiting cover crop and weed species in a vineyard. A snapshot at diurnal trends, *Vitis* 43 (2004) 111–117.
- [125] A. Pellegrino, E.L.M. Voltz, J. Wery, Relationships between plant and soil water status in vine (*Vitis vinifera* L.), *Plant and Soil* 266 (2004) 129–142.
- [126] C.M. Lopes, T.P. Santos, A. Monteiro, M.L. Rodrigues, J.M. Costa, M.M. Chaves, Combining cover cropping with deficit irrigation in a Mediterranean low vigor vineyard, *Sci. Hortic.* 129 (2011) 603–612.
- [127] F. Celette, J. Wery, E. Chantelot, J. Celette, C. Gary, Belowground interactions in a vine (*Vitis vinifera* L.)—tall fescue (*Festuca arundinacea* Shreb.) intercropping system: water relations and growth, *Plant Soil* 276 (2005) 205–217.
- [128] O. Zarrouk, J.M. Costa, R. Francisco, C. Lopes, M.M. Chaves, Drought and water management in Mediterranean vineyards, in: S. Delrot, M.M. Chaves, H. Gerós, H. Medrano (Eds.), *Grapevine in a Changing Environment A Molecular and Ecophysiological Perspective*, Wiley-Blackwell, UK, 2016, pp. 38–67.
- [129] J.M. Costa, M.F. Ortuno, C.M. Lopes, M.M. Chaves, Grapevine varieties exhibiting differences in stomatal response to water deficits, *Funct. Plant Biol.* 39 (2012) 179–189.