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## Review

# Role of aquaporins in determining transpiration and photosynthesis in water-stressed plants: crop water-use efficiency, growth and yield

Menachem Moshelion[1], Ofer Halperin[2], Rony Wallach[2], Ram Oren[3,4] & Danielle A. Way[3,5]

*[1]The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture and [2]Department of Soil and Water Sciences, The Robert H. Smith Faculty of Agriculture, Food and Environment, Hebrew University of Jerusalem, Rehovot 76100, Israel, [3]Nicholas School of the Environment, Duke University, Durham, NC 27708, USA, [4]Department of Forest Ecology and Management, Swedish University of Agricultural Sciences (SLU), SE-901 83, Umeå, Sweden and [5]Department of Biology, Western University, London, ON, Canada N6A5B7*

## ABSTRACT

**The global shortage of fresh water is one of our most severe agricultural problems, leading to dry and saline lands that reduce plant growth and crop yield. Here we review recent work highlighting the molecular mechanisms allowing some plant species and genotypes to maintain productivity under water stress conditions, and suggest molecular modifications to equip plants for greater production in water-limited environments. Aquaporins (AQPs) are thought to be the main transporters of water, small and uncharged solutes, and $CO_2$ through plant cell membranes, thus linking leaf $CO_2$ uptake from the intercellular airspaces to the chloroplast with water loss pathways. AQPs appear to play a role in regulating dynamic changes of root, stem and leaf hydraulic conductivity, especially in response to environmental changes, opening the door to using AQP expression to regulate plant water-use efficiency. We highlight the role of vascular AQPs in regulating leaf hydraulic conductivity and raise questions regarding their role (as well as tonoplast AQPs) in determining the plant isohydric threshold, growth rate, fruit yield production and harvest index. The tissue- or cell-specific expression of AQPs is discussed as a tool to increase yield relative to control plants under both normal and water-stressed conditions.**

*Key-words*: anisohydric; drought stress; isohydric; risk-taking.

## INTRODUCTION

The ability of plants to convert solar energy, $CO_2$ and water into organic matter and oxygen through photosynthesis positions them at the very base of the food chain. Because the water potential of the mesophyll is an order of magnitude higher than that of the atmosphere, plants transpire most of the water they absorb from the soil in exchange for the $CO_2$ they obtain from the atmosphere, making water availability a major limiting factor for the growth and productivity of terrestrial plants, including the crops humans rely on for food.

Vascular plants have evolved complex roots and hydraulic systems to absorb water and minerals from the soil and transport them to the transpiring leaf in a coordinated fashion to prevent desiccation. The arrangement and redundancy of major veins in the leaf play an essential role in distributing water equitably across the lamina and could buffer the impacts of hydraulic damage; nevertheless, hydraulic conductance is highly dynamic (Sack & Holbrook 2006). This dynamic regulation of water homeostasis is based on the counterbalancing of two systems: (1) stomatal gas conductance ($g_s$), which controls the rate at which water vapour is lost from leaves during transpiration ($E$) and (2) the radial permeability or hydraulic conductivity of the plant's vascular system, which controls the rate at which water enters the roots (known as $L_{Pr}$; reviewed by Maurel *et al*. 2010) and the radial water outflux through the leaf towards the evaporation sites on the mesophyll cell walls (known as leaf hydraulic conductance, $K_{leaf}$; reviewed by Sack & Holbrook 2006).

The status of the leaf water balance is determined by the ratio between the movement of water into the leaf and the movement of water out of the leaf, and is described in terms of leaf relative water content (RWC) and/or leaf water potential ($\Psi_{leaf}$; Levin *et al*. 2007; Ache *et al*. 2010; Nardini *et al*. 2010; Shatil-Cohen *et al*. 2011). Periods of declining soil moisture are commonly associated with increased evaporative demand [e.g. increased vapour pressure deficit, VPD (Oishi *et al*. 2010)]. Thus, maintaining a sufficient supply of water to the leaves is challenging because the mechanism driving water flux (cohesion tension) during transpiration places the xylem under tension, making it vulnerable to cavitation-induced embolism (Zimmermann 1983). Stomata sense the declining $\Psi_{leaf}$ and respond by reducing $E$, thereby limiting further variation in RWC and $\Psi_{leaf}$ (Brodribb & Jordan 2008). Thus, for a leaf to sustain RWC and $\Psi_{leaf}$ at levels high enough to keep its stomata open, the $K_{leaf}$, stem hydraulic conductance and $Lp_r$ must all be sufficiently high (Sack & Holbrook 2006). Nevertheless, when the evaporative demand exceeds the supply of water to the transpiration

*Correspondence: M. Moshelion. Fax: 972-8-9489899; e-mail: moshelio@agri.huji.ac.il*

stream (i.e. under unfavourable water conditions), $g_s$ decreases, protecting the plant from severe dehydration and its hydraulic system from cavitation. It was recently reported that guard cell-autonomous abscisic acid (ABA) synthesis is involved in this response (Bauer *et al*. 2013); however, the mechanism by which guard cells sense the minimum critical RWC and/or $\Psi_{leaf}$ values is different in different plants and is not well understood. $L_{Pr}$ and $K_{leaf}$ are dynamic, as well, and sensitive to many soil and atmospheric factors, including drought, salinity, light intensity and relative humidity (Steudle 2000; Cochard *et al*. 2007; Levin *et al*. 2007; Shatil-Cohen *et al*. 2011).

There is a well-established positive correlation between whole plant $g_s$ and yield (DeWitt 1958; Sinclair *et al*. 1984; Kemanian *et al.* 2005). Taken together, the facts that $K_{leaf}$ in crop plants are much higher than those observed in other functional types (Sack & Holbrook 2006), and that commercial breeding has led to linear increases in yield, but exponential decreases in midday $\Psi_{leaf}$ (Boyer 1982), reveals the dramatic side effect of breeding for yield on plants' physiological parameters. This also suggests that studying the cellular mechanisms controlling water balance in the whole plant and re-synchronizing its parameters may lead to novel strategies for developing drought-resistant crops.

In this review, we discuss the role of molecular and whole-plant physiological parameters that control water-balance regulation in anisohydric and isohydric plants. We will focus on the relations between AQP activity in the mesophyll, tonoplast and the cells enveloping the vascular system [root endodermis and shoot bundle sheath (BS)] and their roles in controlling leaf water status and, consequently, isohydric or anisohydric stomatal behaviour.

## ROLE OF VASCULAR AQUAPORINS (AQPS) IN CONTROLLING RADIAL HYDRAULIC CONDUCTANCE

The dynamic and controlled behaviour measured as $K_{leaf}$ (Cochard *et al*. 2007; Levin *et al*. 2007) and $L_{Pr}$ (Maggio & Joly 1995; Carvajal *et al*. 1996; Clarkson *et al*. 2000; Tournaire-Roux *et al*. 2003; Gorska *et al*. 2008; Bramley *et al.* 2010) is strongly dependent on and responsive to ambient environmental signals. Recently, it has been suggested that the leaf BS and root endodermis cells (as well as other parenchymal cells surrounding the xylem) may act as 'hydraulic control centre' tissues in the regulation of $K_{leaf}$ and $L_{Pr}$, respectively, making these tissues attractive targets for improving water-use efficiency in crop species (Sack & Holbrook 2006; Shatil-Cohen *et al*. 2011).

The BS is made up of cells that tightly encase the dead xylem conduits. These cells may play a role in regulating the radial transport activity of the xylem system (Kinsman & Pyke 1998; Shatil-Cohen *et al*. 2011). Anatomical studies have demonstrated that the BS is hydraulically isolated (Sack & Holbrook 2006; Nardini *et al*. 2010; Shatil-Cohen *et al*. 2011), and there is physiological evidence for its mineral selectivity capabilities involving silica (Yamaji & Ma 2009), $K^+$ and $Na^+$ (Shapira *et al*. 2009), with only negligible apoplast pathways bypassing the BS (Shatil-Cohen *et al*. 2011). It was recently shown that $K_{leaf}$ is dynamically controlled by the permeability of the BS cell membranes to water, with the osmotic permeability coefficient ($P_f$) likely reflecting the regulated activity of AQPs in the BS cells (Shatil-Cohen *et al*. 2011, 2011; Prado *et al*. 2013). A putative role for tonoplast intrinsic proteins (TIP) and plasma membrane-intrinsic proteins (PIP) AQPs in dynamics of leaf hydraulic and stomatal conductance in grapevine was recently presented (Pou *et al*. 2013). As $K_{leaf}$ is linked to $g_s$, and $g_s$ is related to yield, a greater understanding of the regulation of BS function may open up novel avenues for improving crop yield under variable water supplies.

At the other end of the plant hydraulic system lies the endodermis, a layer of root inner cortex cells that tightly encases the stele of vascular plants. As with the BS, the endodermis acts as a regulatory checkpoint, as its hydrophobic Casparian strip, which separates the stele from passive apoplastic diffusion (Moon *et al*. 1986; Alassimone *et al*. 2010), has a major effect on the radial transport of water and ions (Ranathunge & Schreiber 2011). Thus, the endodermis (together with other xylem-surrounding cells) represents the most critical boundary along the apoplastic route, controlling plant radial water movement (Alassimone *et al*. 2010) most likely via AQP activity (Tournaire-Roux *et al*. 2003; Maurel *et al*. 2008, 2009).

Plant water channels known as AQPs are considered to be the main transmembrane pathway for water, $CO_2$ and some other small uncharged molecules (Uehlein *et al*. 2003; Maurel *et al*. 2008; Sade *et al*. 2013). The total number of AQPs found in plants is considerably higher than that found in any other kingdom [e.g. 35 in *Arabidopsis thaliana* (Boursiac *et al*. 2005) and 37 in tomato (*Solanum lycopersicum*) as compared with 3 and 13 in yeast and humans, respectively], suggesting their unique role in regulating plant water balance under uncertain environmental conditions (Tyerman *et al*. 2002; Maurel *et al*. 2009). Plant AQPs have a broad localization pattern in organs, tissues and subcellular compartments. Most of the documented *Arabidopsis* PIP isoforms have been found in vascular tissues with different cellular patterns (Maurel *et al*. 2008). Relatively strong RNA expression of several AQPs was detected in the vascular cells of *Arabidopsis* root (Birnbaum *et al*. 2003). In *Brassica napus* (Frangne *et al*. 2001), a relative of *Arabidopsis*, a greater abundance of TIPs was noted in the BS cells as compared with the adjacent mesophyll and parenchyma cells. However, to date, no study has described BS-specific AQPs or their expression pattern(s).

The level of the stress phytohormone ABA increases progressively in the xylem sap ($ABA_{xyl}$) of many plants exposed to drought (Tardieu & Simonneau 1998; Holbrook *et al*. 2002; Christmann *et al*. 2007), very likely because of increased secretion from its site of production in the vascular parenchyma tissue (Endo *et al*. 2008; Galvez-Valdivieso *et al*. 2009). The transcript level of most AQPs decreases in response to drought, as well as various other abiotic stresses (Jang *et al*. 2004; Alexandersson *et al*. 2005). While some studies report that ABA treatment decreases conductance of the root system (Markhart *et al*. 1979), there have been several reports of an increase in the expression of certain root AQPs in response to ABA (Mahdieh & Mostajeran 2009; Parent *et al*. 2009). These reports, as well as others (e.g. Hose *et al*. 2000; Quintero *et al*. 1999), demonstrate the transient increase in $L_{Pr}$ that can occur in response to increases in [ABA]. Interestingly, a similar ABA treatment decreased $K_{leaf}$, most likely because of modification of AQP expression and activation in the BS cells (Shatil-Cohen *et al*. 2011), suggesting a role for BS cells in converting [$ABA_{xyl}$] signal to a $\Psi_{leaf}$ signal. This lower $\Psi_{leaf}$ leads to stomatal closure. In this new role as a vascular control centre, the BS response to drought stress signals from the root might temporarily block the vasculature–mesophyll water pathway and control the hydraulic regulation system that balances plant water status by minimizing water loss and maximizing water uptake. This indirect hydraulic effect has been confirmed by Pantin *et al*. (2013), who found that xylem-fed ABA reduced $K_{leaf}$ and thereby could induce stomatal closure in ABA insensitive *Arabidopsis* mutants. The opposing effects of ABA on hydraulics seen in leaves and roots might be related to the absolute concentration of ABA at the target tissues, as leaf ABA levels increase earlier, and to much higher levels, than in the roots in response to water stress. This leaf ABA is assumed to be transported, later, to the roots via the phloem system (Christmann *et al*. 2005). The contrast in root and leaf responses to ABA may also be caused by differential sensitivity of specific AQPs to ABA (Parent *et al*. 2009).

AQP activity can also be regulated by post-translational mechanisms, such as divalent cations and pH in the cytosol, trafficking or redistribution of AQPs and heteromerization (Chaumont *et al*. 2005; Kaldenhoff *et al*. 2007; Yaneff *et al*. 2013). A novel form of post-transcriptional regulation of AQPs by drought-induced microRNA (miRNA) has recently been demonstrated in cotton plants (Zhang *et al*. 2007) and in mice cells exposed to hyperosmolality (Huebert *et al*. 2011). Thus, the use of an artificial microRNA approach to silence AQPs in an organ, or even specifically in target tissues (e.g. the BS), might be an effective way to modify plant water stress responses.

## ISOHYDRISM VERSUS ANISOHYDRISM

Depending on their genetically dictated molecular and physiological attributes, plants budget their water in very different ways, with important consequences for their survival, growth and yield. Natural strategies that have evolved in plants to help them cope with water stress range along a continuum from the 'leaf water overdraft' or 'risk-taking' behaviour displayed by anisohydric plants to the water-conserving behaviour displayed by isohydric plants. Isohydric plants maintain a constant minimum daily $\Psi_{leaf}$ and RWC by reducing $g_s$ and $E$ when faced with water stress; anisohydric plants allow $\Psi_{leaf}$ to decrease with rising evaporative demand, reaching a lower $\Psi_{leaf}$ and RWC under drought conditions relative to situations in which they are well-watered (Fig. 1) (Tardieu & Simonneau 1998; McDowell *et al*. 2008; Sade *et al*. 2010). But there is limited knowledge on the molecular and cellular criteria differentiating these two types of plants, which constrains our ability to manipulate the stomatal behaviour of crop species to improve either water-use efficiency or drought tolerance.

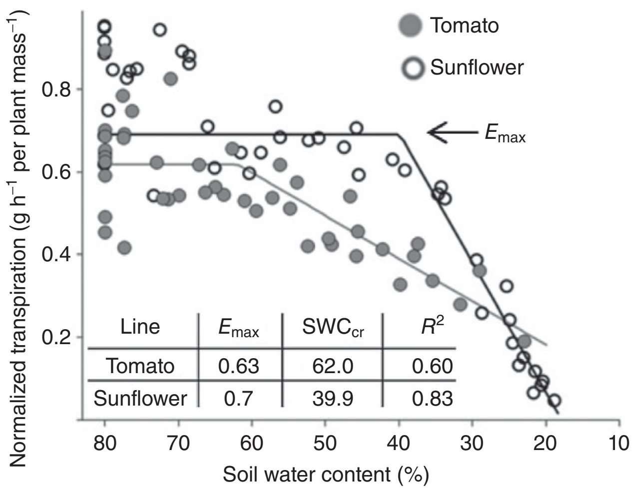


**Figure 1.** Midday whole plant normalized transpiration versus soil water content (SWC) of isohydric tomato plants (Sade *et al*. 2009) and anisohydric sunflower plants (Tardieu *et al*. 1988). Both species maintained a constant transpiration level ($E_{max}$) at the given ambient conditions and a sharp decrease in $E$ at the critical SWC value ($SWC_{cr}$), with $E$ declining linearly with decreasing water availability. Under ample water supply, anisohydric $E_{max}$ was consistently higher than that of isohydric plants across the range of examined SWC levels. The isohydric plants reached $SWC_{cr}$ at a higher SWC level. Measurements were conducted for 12 d during the summer of 2011 in a semi-commercial greenhouse (natural light conditions and vents and/or cooled moist air were used to ensure that maximum temperatures in the greenhouse did not exceed 35 °C) in Rehovot, Israel. Temperature and relative humidity during the experiment were between 18 and –34 °C and 48 and 92%, respectively. The experimental setup included 10 temperature-compensated load cells (1042 C4; Vishay Intertechnology, PA, USA) mounted with 4 L pots; $n = 5$ for each cultivar, *Lycopersicon esculentum* Mill. cv. M82 and *Helianthus annuus* L. cv. OPAL, randomly arranged in the greenhouse and measured simultaneously. Each pot contained one plant and soil moisture sensor (5TE; Decagon Devices, WA, USA). Pots were filled with a commercial potting medium and a commercial fertilizer solution (Super Grow 6-6-6+3; Hortical, Kadima, Israel) was applied daily at 0.2% (v/v) with the irrigation water. All sensors were connected a to CR1000 datalogger through AM16/32B multiplexers (Campbell Scientific, UT, USA). Readings of the weighing lysimeters and the environmental sensors were taken every 15 s and averages for each 3 min period were stored in a datalogger for further analysis. Soil moisture was measured every 3 min. For more technical details please see Sade *et al*. (2010) and Wallach *et al*. (2010).

The existence of both isohydric and anisohydric behaviour raises the question of the costs of the risk for a species that operates with lower water content under drought conditions. Anisohydric plants 'take a risk' by sustaining longer periods of substantial $E$ in return for longer periods of continued net $CO_2$ assimilation ($A_{net}$) and associated growth, even in the presence of a certain amount of stress. Under conditions characterized by adequate irrigation and mild to moderate abiotic stress, this strategy proves advantageous, and

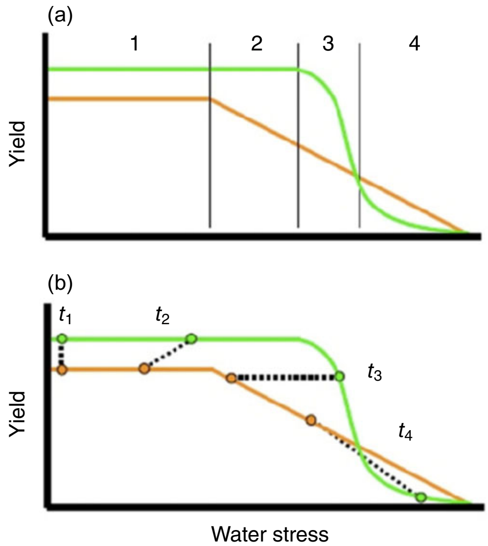


**Figure 2.** Idealized responses of crop yield to water stress for isohydric (orange line) and anisohydric plants (green line). (a) Under ample water supply (region 1), anisohydric plants have higher $g_s$ than isohydric plants, obtaining higher $A_{net}$ and yield. As mild water stress develops (region 2), isohydric plants reduce $g_s$ linearly with decreasing water availability, limiting $A_{net}$ and yield, but anisohydric plants maintain high $g_s$ by allowing leaf water potential to decline, thus maintaining high $A_{net}$ and yield potential. As water stress increases further (region 3), $g_s$, $A_{net}$ and yield in isohydric plants continue to decline linearly; in anisohydric plants, $g_s$ declines precipitously as hydraulic failure necessitates stomatal closure, limiting $A_{net}$ and yield. Lastly, when water stress is severe (region 4), isohydric plants maintain some photosynthesis and yield because of their intact hydraulic system, while anisohydric plants die from drought. (b) However, because anisohydric plants have higher $g_s$, they move along the response curve at a faster rate. Thus, before water stress develops (time 1, $t_1$), anisohydric plants will have greater yield than isohydric plants. After some length of drought (time 2, $t_2$), this may still be the case, but as the length of the drought increases (time 3, $t_3$), yield in both groups is equivalent. By time 4 ($t_4$), isohydric plants are still alive, while anisohydric plants have died from drought stress. The evaluation of recovery from drought is an important (fifth) step in the evaluation of the plant's resilience. This step reveals the plant's desiccation (embolism) resistance and ability to recover its pre-stress productivity, reflecting the extent of the damage caused by severe drought, such as cavitation or leaf/root loss. Our model for an ideal drought-resistant crop would be anisohydric with high desiccation resistance and quick recovery following any drought-induced injury.

anisohydric plants may outperform isohydric plants in terms of growth and yield (Lin *et al.* 2007; Peng *et al.* 2007; McDowell *et al.* 2008; Sade *et al.* 2009). However, the risk of the anisohydric strategy becomes apparent when levels of severe water stress are reached quickly and this risk partially offsets the inherent physiological advantage of the anisohydric strategy (Fig. 2). However, some plants have been reported to switch from one behaviour to the other as water stress develops. For example, grapevines showed isohydric-like behaviour when soil water content was low, but switched to an anisohydric-like behaviour with increasing levels of soil water content (Zhang *et al.* 2011) and isohydric olive trees switched to anisohydric-like behaviour when fruit load was high (Naor *et al.* 2013). The ability to switch strategies raises the possibility that we can manipulate isohydry in crop species to achieve both drought tolerance under severe drought and high productivity under low water stress, if we can determine the mechanistic basis to these two strategies.

## INVOLVEMENT OF AQPS IN ANISOHYDRIC PLANT BEHAVIOUR

The greater the difference between leaf water demand and the ability of the roots to supply enough water to meet this demand, the greater the potential stress for the plant. Plants regulate this disparity between demand and supply – that is their water balance – using phytohormones and hydraulic signals transported via the vascular system, and AQPs have been suggested to regulate water transport across roots such that transpirational demand is matched by root water transport capacity (Vandeleur *et al.* 2008). Isohydric behaviour has been linked to an interaction between hydraulic and chemical (e.g. ABA) information, whereas anisohydric behaviour has not been associated with any such interaction (Tardieu & Simonneau 1998; Gallé *et al.* 2013). We propose that AQPs play a key role in the transduction of chemical signals into hydraulic signals. In this way, they are instrumental for differentiating between the isohydric and anisohydric strategies and the switch between those behaviours.

Generalizations about the relative responses of isohydric and anisohydric species to drought are complicated by the differences inherent in measuring traits across species. However, the overexpression of a tomato tonoplast AQP (SlTIP2;2) caused changes in the regulation of water balance in the isohydric tomato cultivar M82. The constitutive expression of SlTIP2;2 increased the $P_f$ of the cell and extended the capacity of the vacuole for osmotic buffering of the cytoplasm under stress conditions (Sade *et al.* 2009). Under conditions of mild to moderate drought, the 'converted' M82 plants transpired more and for longer periods than the control plants and reached a lower RWC. These plants showed significant increases in fruit yield, harvest index and plant mass relative to the controls under both normal and drought conditions (Sade *et al.* 2009). Thus, the transformed isohydric plants were made to act in an anisohydric fashion (Sade *et al.* 2009) that improved productivity under water stress. A similar effect was seen in *Arabidopsis* with another TIP (Lin *et al.* 2007; Peng *et al.* 2007). These observations raise the question of whether anisohydric behaviour should be viewed as a valuable agronomic trait (Sade *et al.* 2012). Interestingly, the expression of a plasma membrane AQP, NtAQP1, in both isohydric tomato and *Arabidopsis* led to similar drought resistance via a different mechanism. In this second situation, the plants maintained their isohydric behaviour (i.e. a constant leaf RWC under declining soil water) while displaying improved hydraulic conductivity (Sade *et al.* 2010).

In contrast to what has been mentioned earlier, overexpression of another PIP AQP (*Arabidopsis* PIP1;2) in tobacco (*Nicotiana tabacum*), resulted in the opposite behaviour of plant sensitivity to abiotic stress (Aharon *et al*. 2003), whereby improved growth rates and transpiration were seen only under well-irrigated conditions and the genetic modification imparted higher sensitivity to water stress in the plants. Unfortunately, the identity of the key tissue(s) controlling the water-balance system could not be conclusively determined, but this demonstrates that the role of AQPs is likely to be specific to the AQP being studied and highlights the need to investigate individual AQPs for their potential in modifying crop water-use efficiency, drought tolerance and productivity under a range of water supplies.

## ROLE OF AQPS IN ROOT-TO-SHOOT AND SHOOT-TO-ROOT LONG-DISTANCE SIGNALS AND THEIR IMPACT ON ROOT ARCHITECTURE

The manipulation of AQPs to alter hydraulic conductivity and the isohydricity of a species of interest is likely to have downstream effects on other aspects of plant–water relations and physiology, rather than being localized to the target AQP. For example, artificial down-regulation of AQPs usually results in compensatory increases in root size, $P_f$ and $L_{Pr}$ (Kaldenhoff *et al*. 1998; Martre *et al*. 2002; Siefritz *et al*. 2002), suggesting the existence of a feedback mechanism connecting AQPs, $L_{Pr}$ and root size (Vandeleur *et al*. 2014). The morphology and distribution of the root system (or, the root architecture) can give one plant a significant advantage over another individual, particularly in the face of certain types of stress. Root architecture is guided by genetics (e.g. taproot or fibrous root systems), yet, is largely determined by environmental factors (Schiefelbein & Benfey 1991; López-Bucio *et al*. 2002; Hodge 2004). This phenotypic plasticity provides a wide range of advantages to the plant, allowing it to collect signals and information from its environment and incorporate them into the 'decision-making' process regarding growth and development (Malamy 2005). An example of this phenotypic plasticity in water-use traits was demonstrated for loblolly pine (*Pinus taeda* L.) grown in sites with varying soil porosity, soil water-holding capacity, and therefore, water availability to roots. The root-to-leaf-area ratio was five times greater in sand versus loam, compensating for the reduction in water availability in the sandy environment. As a result, plants grown in soils of lower water availability (i.e. sand) required less negative water potentials to exhaust their water supply than plants grown in loam, and maintained close to constant midday water potentials on days of high evaporative demand (Hacke *et al*. 2000).

During normal growth and development, and in response to environmental signals, hormones modulate the architecture of the root system (Aloni 2006). Several phytohormones play roles in the formation of lateral roots. For example, the accumulation of auxin in root pericycle cells is sufficient to trigger the acquisition of pericycle founder cell (FC) identity, which gives rise to the formation of lateral roots (Casimiro *et al*. 2003; De Smet *et al*. 2007). The initiation of lateral roots depends on a shoot apical source of auxin, suggesting coordination and balance between leaf development and the emergence of lateral roots (Reed *et al*. 1988). ABA was also reported to reduce the elongation of lateral roots, a fact that points to its general regulatory role in lateral root development (Xiong *et al*. 2006; De Smet *et al*. 2007).

Rapid ABA biosynthesis may also facilitate isohydric behaviour: roots of an isohydric wheat variety (*Triticum aestivum* cv. Kobomugi) rapidly up-regulate ABA production upon sensing water stress, thereby increasing shoot ABA pools and inducing stomatal closure, while the anisohydric variety *T. aestivum* cv. GK Othalom shows a weaker root ABA induction that fails to substantially increase leaf ABA concentrations or alter stomatal behaviour without much greater levels of water stress (Gallé *et al*. 2013). A similar strong reliance on ABA for mediating stomatal closure under water stress was seen in the isohydric species *Pinus radiata* when compared with the anisohydric conifer *Callitris rhomboidea* (Brodribb & McAdam 2013), hinting that this difference in hormonal control may be widespread in differentiating between these two strategies.

## ROLE OF AQPS IN PHOTOSYNTHETIC $CO_2$ FIXATION

While AQPs are critical for determining water flux rates in plants, which alters $CO_2$ assimilation rates via stomatal control, they also affect photosynthetic rates through their effect on mesophyll conductance ($g_m$; Sade *et al*. 2013). Mesophyll conductance, defined as the capacity for $CO_2$ diffusion from the intercellular airspace to the site of carboxylation in the chloroplasts, has been linked with AQP function in numerous species (Terashima & Ono 2002; Kawase *et al*. 2013; Perez-Martin *et al*. 2014). Transgenic studies in well-watered tobacco, rice and *Arabidopsis* have demonstrated that when AQP levels are reduced, $A_{net}$ is suppressed, while $A_{net}$ is enhanced in AQP overexpressing lines (Uehlein *et al*. 2003, 2008; Hanba *et al*. 2004; Flexas *et al*. 2006; Heckwolf *et al*. 2011; Kawase *et al*. 2013). While there has been considerable research on stomatal limitations to photosynthesis during drought, the role of $g_m$ in leaf drought responses has only been appreciated in recent years. It is now well-accepted that $g_m$ can be as much, or more, of a limitation for photosynthesis as $g_s$ (Yamori 2006; Flexas *et al*. 2012; Perez-Martin *et al*. 2014), and that $g_m$ is dynamically regulated during drought stress, with a decrease in $g_m$ commonly reported in water-stressed plants (Monti *et al*. 2006; Warren 2008; Perez-Martin *et al*. 2014). While leaf anatomical traits (such as thicker leaves and cell walls) can cause a low $g_m$ in leaves adapted to, or developed under, dry conditions, changes in $g_m$ in a fully developed leaf that occur during a drought must rely on non-structural means. AQPs are therefore likely to have strong impacts on crop photosynthetic performance under drought both directly (through their effects on $CO_2$ transport and $g_m$) and indirectly (via their effects on water transport and therefore on $g_s$).

Thus, while stomatal closure caused by water stress increases the resistance to $CO_2$ diffusion into the leaf intercellular airspace, declines in $g_m$ during drought can further restrict $CO_2$ diffusion to Rubisco (ribulose-1,5-bisphosphate

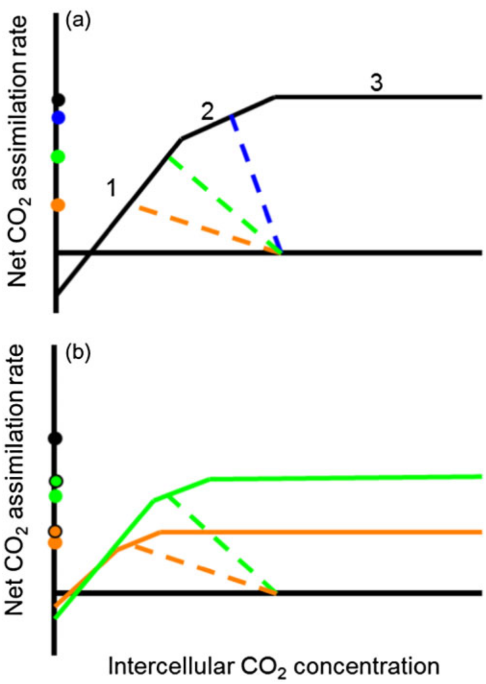


**Figure 3.** (a) The response of net $CO_2$ assimilation ($A_{net}$) to intercellular $CO_2$ concentrations (Ci) (A/Ci curve). The black line (biochemical demand curve) indicates how $A_{net}$: (1) increases steeply at low Ci where Rubisco carboxylation is limiting (indicating the maximum rate of Rubisco carboxylation, $V_{cmax}$); (2) increases less steeply where it is co-limited by Rubisco and electron transport capacity; and (3) shows little response to $CO_2$ at high Ci where electron transport is limiting (indicating maximum electron transport rates, $J_{max}$). Dashed lines indicate the effect of stomatal conductance ($g_s$) on Ci and $A_{net}$ (supply curve). When $g_s$ is high, as in well-watered leaves (blue line), Ci and $A_{net}$ (blue circle) are relatively high, and $A_{net}$ is co-limited (region 2). Under drought, as $g_s$ declines, so do Ci and $A_{net}$. Isohydric leaves (orange line and circle) reduce $g_s$, and therefore $A_{net}$, more than anisohydric leaves (green line and circle). Under short-term water stress, only the supply curve changes, such that leaves can return to well-watered conditions by increasing $g_s$; maximum $A_{net}$ (black circle) does not change. (b) Under long-term drought, the biochemical demand curve may acclimate until $A_{net}$ is co-limited at the prevailing Ci. Anisohydric leaves that operate at a higher $g_s$ may have greater maximum $A_{net}$ (black-lined green circle) than isohydric leaves (black-lined orange circle), but neither leaf can return to the original pre-drought maximum $A_{net}$ (black circle) by changing the supply curve (i.e. increasing $g_s$).

carboxylase/oxygenase). In well-watered plants, the intercellular $CO_2$ concentration (Ci) is normally around 0.7–0.8 of ambient $CO_2$ concentrations, but as water stress develops, Ci often falls to 0.6 of ambient $CO_2$ (Lawlor & Tezara 2009). As Ci declines, photosynthesis also drops, as the biochemical demand curve of photosynthesis is linearly related to Ci and Cc (the chloroplastic $CO_2$ concentration) below current ambient $CO_2$ concentrations (region 1 in Fig. 3a). While isohydric leaves will maintain a constant RWC of ~80% by decreasing $g_s$ (Peng *et al*. 2007; Sade *et al*. 2009), in anisohydric C3 plants, a drop in $\Psi_{leaf}$ from 0 to −1 MPa reduces $g_s$ and $A_{net}$ by 30–50%, while full stomatal closure and an absence of photosynthetic carbon fixation occur when leaf RWC reaches around 60%. As many crop plants show a linear and positive correlation between $E$ and yield (DeWitt 1958; Sinclair *et al*. 1984; Kemanian *et al.* 2005), stomatal closure under water shortage conditions will reduce carbon gain and could lead to significant decreases in plant growth and yield.

The photosynthetic biochemical machinery is usually not down-regulated by short-term drought, as increasing ambient $CO_2$ levels (which increases Ci and Cc) restores pre-drought photosynthetic rates (Cornic *et al*. 1989) and measurements of $V_{cmax}$ show little change over a transient drought stress (Monti *et al*. 2006; Cano *et al*. 2013). However, optimality theory predicts that plants regulate their production of photosynthetic proteins such that carbon fixation at the prevailing Cc is co-limited by Rubisco and electron transport capacities. Under long-term drought, if Cc stays low, because of stomatal closure and/or decreases in $g_m$, the photosynthetic apparatus may thus acclimate to reduce the production and maintenance of underutilized photosynthetic proteins, such as electron transport enzymes (Fig. 3). This type of response, where biochemical demand is only reduced in comparison with well-watered plants after extensive drought stress, was seen in beet (*Beta vulgaris*), where photosynthetic capacity measured on a Cc basis declined in droughted plants, but only after 50 days of water stress (Monti *et al*. 2006) and in cedar seedlings (*Cedrus* spp.) (Epron 1997). While this photosynthetic down-regulation theoretically frees up nitrogen and energy for allocation to other uses, such as increased root growth to enhance access to water, it also limits the ability of the leaf to respond to better conditions if the drought stress ends (Fig. 3b). If $g_s$ increases in Fig. 3b when water supplies are restored, the leaves will operate at a higher Ci and Cc, but because the biochemical demand curve has acclimated, this will not lead to significant increases in photosynthesis. Thus, the duration of the drought may significantly alter the plant growth and yield responses, with longer droughts limiting the later growth of plants, even when water supplies are restored.

While in most cases water stress reduces $g_m$, under longer droughts, $g_m$ may recover while $g_s$ remains low (Galle *et al*. 2009). Thus, while $g_s$ and $g_m$ are usually well correlated, they can be decoupled by water stress. An increase in the ratio of $g_m$ to $g_s$, as seen when comparing natural populations from dry regions with those from wetter conditions, results in greater water-use efficiency (defined as the ratio of $A_{net}/E$) (Duan *et al*. 2009), as predicted by earlier work (Parry *et al*. 2005). The decoupling of $g_s$ and $g_m$ during extended drought or in dry climates might also help prevent or minimize the scenario laid out in Fig. 3. If $g_s$ and $g_m$ are both reduced equivalently under water stress, Cc will be substantially lower than the already low Ci, increasing the risk of photosynthetic acclimation if the drought is prolonged. But if $g_m$ increases when $g_s$ remains low, the difference between Ci and Cc will be minimized, and the photosynthetic machinery will be exposed to a higher $CO_2$ concentration, which may not lead to down-regulation and longer-lasting photosynthetic effects.

To our knowledge, there are no studies determining whether isohydric and anisohydric species differ in their regulation of AQPs during drought in ways that affect $g_m$ and $CO_2$ transport, and thereby affect photosynthesis. However, as isohydric species close their stomata earlier during a water stress, they might be expected to have a higher ratio of $g_m$ to $g_s$ as a strategy for maximizing $A_{net}$ during more frequent bouts of low $g_s$.

## CONCLUSIONS

Because some AQPs appear to either be related to $H_2O$ or $CO_2$ transport (Otto *et al*. 2010; Flexas *et al*. 2012), we may be able to manipulate AQPs in ways that maximize $CO_2$ diffusion rates while not affecting, or even minimizing, $E$ during periods of water limitation. Plants where AQPs are overexpressed can have not only higher photosynthetic rates than wild-type controls, but also faster growth, higher biomass and greater yield (Sade *et al*. 2009; Kawase *et al*. 2013).

Despite the large number of attempts to improve the abiotic stress tolerance of commercial crop plants, no major progress has been made, emphasizing the complexity of the different traits involved. Future research will provide a molecular basis for understanding the different strategies that plants use to regulate their water balance and water-use efficiency with a new focus for further exploration of the vasculature-stomata axis. The identification of specific AQP genes with defined roles in the plant's water budgeting will enhance our understanding of stomatal regulation and provide novel molecular tools for improving plant resistance to many other types of abiotic (and perhaps even biotic) stress, thereby contributing to our future food security. Further research will examine the effects of desiccation in combination with the effects of higher temperatures – key for the development of a new generation of high-yield crops with improved water-use efficiency capable of thriving in the face of the impending climatic challenges. Anisohydric plants 'take a risk' in the face of drought conditions by sustaining longer periods of transpiration and $CO_2$ assimilation and may outperform isohydric plants under conditions of mild to moderate drought. Ultimately, a 'calculated-risk-taking' trait could be identified as we increase our understanding of this molecular mechanism. This would enable us to eventually generate plants with dynamic anisohydric–isohydric behaviour regulated by environmental conditions and the plant's developmental stage.

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