VvPIP2;4N aquaporin involvement in controlling leaf hydraulic capacitance and resistance in grapevine

VvPIP2;4N aquaporin involvement in controlling leaf hydraulic capacitance and resistance in grapevine

Article *in* Physiologia Plantarum · April 2016

Impact Factor: 3.14 · DOI: 10.1111/ppl.12463

6 authors, including:

Marco Vitali

Università degli Studi di Torino

17 PUBLICATIONS **118** CITATIONS

SEE PROFILE

Hervé Cochard

French National Institute for Agricultural Res…

294 PUBLICATIONS **8,196** CITATIONS

SEE PROFILE

Irene Perrone

Umeå University

16 PUBLICATIONS **521** CITATIONS

SEE PROFILE

Claudio Lovisolo

Università degli Studi di Torino

60 PUBLICATIONS **1,886** CITATIONS

SEE PROFILE

VvPIP2;4N aquaporin involvement in controlling leaf hydraulic capacitance and resistance in grapevine

Marco Vitali[a,*], Hervé Cochard[b,c], Giorgio Gambino[d], Alexandre Ponomarenko[b], Irene Perrone[d] and Claudio Lovisolo[a,b]

[a]Department of Agricultural, Forest and Food Sciences (DISAFA), University of Turin, Largo Paolo Braccini 2, 10095 Grugliasco, TO, Italy

[b]INRA, UMR 547 PIAF, F-63100 Clermont-Ferrand, France

[c]University Blaise Pascal, UMR 547 PIAF, F-63177 Aubière, France

[d]Institute for Sustainable Plant Protection, National Research Council (IPSP-CNR), Grugliasco unit, Largo Paolo Braccini, 10095 Grugliasco, TO, Italy

*****Corresponding author, e-mail: marco.vitali@unito.it

Hydraulic capacitance in a plant tissue (C) buffers the xylem tension, storing and releasing water and has been highlighted in recent years as an important factor that affects water relations such as drought tolerance and embolism formation. Aquaporins are well known to control leaf hydraulic resistance (Rh) but their role in the control of C is unknown. Here, we assess Rh and C on detached grapevines leaves (cv. Brachetto) wild type (WT) and over-expressing the aquaporin gene *VvPIP2;4N* (OE). For this purpose, we developed a new method inspired from the pressure-volume curve technique and the rehydration-kinetic-method, which allowed us to monitor the dynamics of dehydration and rehydration in the same leaf. The recovery after dehydration was measured in dark, light non-transpirative conditions, light-transpirative conditions and light-transpirative condition adding abscisic acid. Pressurizing to dehydrate leaves in the OE line, the recorded Rh and C were respectively lower and higher than those in the WT. The same results were obtained in the dark recovery by rehydration treatment. In the presence of light, either when leaves transpired or not (by depressing vapor pressure deficit), the described effects disappeared. The change in Rh and C did not affect the kinetics of desiccation of detached leaves in dark in air, in OE plants compared to WT ones. Our study highlighted that both Rh and C were influenced by the constitutive over-expression of *VvPIP2;4N*. The effect of aquaporins on C is reported here for the first time and may involve a modulation of cell reflection coefficient.

Abbreviations – +0.5, dehydration treatment with pressure applied of 0,5 MPa; +1, dehydration

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ppl.12463

treatment with pressure applied of 1 MPa; Ψ, leaf water potential; AQPs, aquaporins; C, hydraulic capacitance; dark, rehydration treatment in dark condition; light transp, rehydration treatment in light and transpirative condition; light transp ABA, rehydration treatment in light transpirative condition supplying ABA in the rehydrating water; light VPD ≈ 0, rehydration treatment in light condition without transpiration; OE, over-expressing; Rh, hydraulic resistance; RWC, relative water content; WT, wild type.

Introduction

Water is the most limiting resource for plant life and yield (Lange et al. 1982). Although the majority of a plant's fresh weight consists of water, the amount of water retained by the plant in the biomass is less than one percent of the total water transpired via stomata. Consequently, a huge quantity of water is required to enable photosynthesis and plant growth. Therefore, water uptake from the soil, its transport, storage and usage are mediated through a system that has evolved to fully exploit the chemical and physical properties of water.

The cohesion-tension theory, formulated by Dixon (1914), explains the transport of water in the soil-plant-atmosphere continuum. In this system, water moves from high to low water potential (Ψ), and thus, towards transpiring leaves. Transpiration itself drives the rise of the xylem sap and submits water to a considerable tension. Such tension is balanced by the hydrogen bonds among water molecules, which prevent the breaking of the water column. However, under different conditions (e.g., water shortage, freezing, high evaporative demand), this tension can increase and cavitation can occur by air seeding mechanisms (Angeles et al. 2004).

In Dixon's theory, water transport in plants behaves like an electrical circuit, and follows Ohm's law as described by van den Honert (1948); hence, the flow is due to the water potential gradient and is hindered by the hydraulic resistance (Rh). Moreover, the pathway can be split into the water transport of individual organs (root, stem, leaf), each with its own Rh that affects the water flux (Tyree and Ewers 1991, Sperry et al. 1998). However, in this equation, the hydraulic capacitance (C) has to be considered as an important variable that affects the output. Analogously to an electrical circuit, C has the function of a capacitor (or condenser) used to store charge temporarily, thus buffering a power surge. Therefore, in plants, C represents the ability to store water and to buffer the system reducing the degree of tension in the xylem in transient water status conditions. Capacitance corresponds to the ratio between the change in water content and the change in water potential ($C=\Delta RWC/\Delta\Psi$; Tyree and Ewers 1991, Sperry et al. 2008); its effect is to make the amount of water entering a region different from the amount of water leaving it, whenever $\Delta\Psi$ changes.

Leaves are the final component of the water transport system and via their stomata; they balance carbon nutrition and water loss by transpiration, thereby playing a key role in the regulation of the water status and the strategy of responses to drought stress. To prevent deleterious dehydration, stomatal conductance is controlled by a complex regulation of guard cells, involving chemical and

hydraulic signals (Comstock 2002). The relevance of leaf C was recently investigated in relation to various physiological traits, such as leaf thickness (Sack et al. 2003), leaf water content per unit dry weight, leaf mass per unit area and lignin content (Blackman and Brodribb 2011). In addition, the latter study described the 'dynamic C' (computed as the volume of flowing water measured by a flowmeter) to be highly coordinated with leaf hydraulic conductance (Blackman and Brodribb 2011).

Aquaporins (AQPs) exercise a strategic function in the leaf water pathway by controlling symplastic water movements (Kaldenhoff et al. 2008), and being the main link between the symplastic and apoplastic pathways, e.g., bundle sheath cells. These water channels, without changing the flux direction, can enormously increase the water movement across membranes, and therefore, decrease the Rh. Aquaporins can be modulated at several levels, via transcription, translation, trafficking and gating (opening and closing of the pore) and by environmental and developmental factors (Chaumont and Tyerman 2014), such as: irradiation (Prado et al. 2013, Lopez et al. 2013), transpiration (Sakurai-Ishikawa et al. 2011, Laur and Hacke 2013), circadian rhythms (Hachez et al. 2008), abscisic acid (ABA) feeding (Shatil-Cohen et al. 2011, Pantin et al. 2013), auxin feeding (Péret et al. 2012) and shoot wounding (Sakurai-Ishikawa et al. 2011, Vandeleur et al. 2014).

Several experiments using transgenic plants overexpressing or silencing AQP genes have been performed (reviewed by Martínez-Ballesta and Carvajal 2014) and have demonstrated that the transcriptional modulation of AQPs generally modifies the Rh, however, to date, no results exist concerning the effects of AQPs on C.

In this study, grapevine plants over-expressing *VvPIP2;4N* (an aquaporin previously described by Perrone et al. 2012, extremely efficient in facilitating cell-to-cell water pathways), were used to assess the role of this AQP isoform on leaf Rh and C during leaf dehydration and recovery. The hydraulic parameters were evaluated by a new method derived from the pressure-volume curve (Tyree and Hammel 1972) and the rehydration kinetic technique explained by Blackman and Brodribb (2011).

Materials and methods

Plant material

The experiments were performed on leaves of potted 'Brachetto' grapevines; 10 wild-type (WT) and 10 transgenic plants from line 16, which overexpressed *VvPIP2;4N* (OE), previously described by Perrone et al. (2012). The 4-year-old plants (two buds pruned with bud-break in March, non-grafted) were grown in a greenhouse on a mixture of peat–loam, under natural light and CO_2 concentration conditions. Plants were irrigated regularly according to their needs. In this experiment, fully expanded, mature leaves were used.

Assessment of Rh and C in the dehydration and rehydration processes

To assess leaf Rh and C during dehydration, a method similar to the pressure-volume curve technique was used (PV curve, Tyree and Hammel 1972), whereas for the rehydration phase, a modified

rehydration kinetic method (see C_{dyn} measurements, Blackman and Brodribb 2011) was applied.

The new method proposed required the use of a high-precision balance (Mettler Toledo AT261 deltarange, Greifensee, CH) and a modified Scholander pressure bomb, inverted over the balance, and controlled by an external manometer (Bourdon, FR; class 0.1). The cut surface of the petiole, which passed through the sealing system of the pressure chamber, was immersed in a cylinder (50 ml, filled with deionized water, placed on the balance plate (Fig. 1). The balance plate was isolated from the laboratory atmosphere and the relative humidity inside the balance chamber was kept close to 100% using wet paper. By applying and releasing the pressure in the chamber, the flow out/in of the leaf was measured by an increase or decrease in weight measured by the balance, as explained below.

Dehydration phase (Fig. 1A):

Leaves were collected at 18.00. The petiole extremity, cut under water, was submerged in deionised water in non-transpirative conditions (dark, sealed bag) overnight, to allow full leaf hydration. During the following day (at 9.00; 12.00; 15.00), the leaves were removed from water and were immediately placed in the pressure chamber. After measurement of the native water potential (Ψ_{leaf}), the pressure chamber was upturned on the balance, placing the petiole in the 50-mL cylinder. Starting from this steady state, the pressure was increased to a value of +0.5 (noted +0.5 thereafter) and +1.0 MPa (+1) and was kept constant; the pressure rose by 0.05 MPa per second regulated by a needle valve. The mean native Ψ_{leaf}, after one night hydration in water, was –0.01 MPa for both WT and OE leaves.

This kind of measurement was also possible with water-stressed leaves, taking care to maintain the pressure inside the chamber slightly lower than that balancing the leaf water potential. This was necessary to avoid water uptake by the leaf and thus, to maintain a stable weight on the balance to begin measurements.

Rehydration phase (Fig. 1B):

The pressure was applied to the dehydrated leaves until Ψ_{leaf} = –1 MPa (dehydration, +1). After reaching this level of dehydration, the pressure was released and the chamber was removed. The petioles remained suspended by the lid of the pressure chamber and immersed in the 50-mL cylinder. The water uptake by the leaves began immediately after depressurization and was monitored for 1 h in different conditions:

1) dark and non-transpirative condition (Dark, leaf in a dark glass bell);

2) light and low transpirative condition (Light VPD ≈ 0, a 2 L glass baker was placed over the leaf with artificial light set at 500 µmol photons m^{-2} s^{-1}, wet paper was previously used to reduce the VPD to 0 with formation of condensed water on the glass surface);

3) light and transpirative condition (Light transp, artificial light set at 500 µmol photons m^{-2} s^{-1}, leaf in laboratory atmosphere);

4) light, transpirative condition and ABA (Light transp ABA, conditions as in 3), and ABA solution

(100 µmol final concentration) supplied in the cylinder.

In the transpiring treatments, the air temperature was between 19°C and 24°C and the relative humidity was between 45 and 60% (max VPD 10 Pa/KPa).

The Rh and capacitance C were obtained from the equation describing the pressure-volume curves and was computed using the formula:

$f(x) = \mathrm{P} * \mathrm{C} * 1^{(-\mathrm{x}/(\mathrm{Rh}*\mathrm{C}))}$ (Tyree and Hammel 1972)

where: x is the cumulative water in/out of the leaf and P is the pressure applied. Using this equation Rh correspond to the slope of the first part of the curve and C to the *plateau* phase, as highlighted in Fig. 2.

To avoid errors in the calculation due to the transpiration, in the rehydration phase, C was computed subtracting mathematically the transpiration rate from the water uptake weight measurements above 600 seconds. Until this time, transpiration marginally affected the water uptake (see Fig. 4). Data were normalized to the dry weight (70°C, 12 h) and the leaf area (measured by the area-meter Li3000, Lincoln NE, USA) of the single leaves. SigmaPlot 12.3 software (Systat Software, San Jose CA, USA) was used for data elaboration and statistical analysis by *t*-test and one-way ANOVA (after passed Shapiro-Wilk test). To perform ANOVA analysis, data were normalized when the homogeneity of variance test (Bartlett's test) failed.

Aquaporin expression profile

Leaves for the aquaporin expression analysis were collected in light and dark conditions following the same time-course and sampling protocol as for leaves used in the physiological tests (collected at 18.00 and left rehydrated overnight in a dark, sealed bag). Leaves in the dark treatment were harvested in liquid nitrogen at 9.00, whereas leaves in the light treatment were submitted to artificial irradiation for 1 h before harvesting. The real-time RT-PCR (qRT-PCR) quantification of transgenic *VvPIP2;4N*, endogenous *PIP2* genes and *PIP1*-type aquaporins were carried out as previously reported (Perrone et al. 2012) on two biological replicates (three technical replicates each).

Leaf dehydration dynamics in dark conditions

For each line, 22 leaves were sampled at 18.00; petioles were cut under water to avoid embolism formation. Leaves were left free to rehydrate through the petiole in deionised water overnight, as above. The following day at 8.00, water was removed and leaves were left dehydrated on the bench in the dark in the laboratory atmosphere. The fresh weight of fully hydrated leaves was measured with a balance (Denver Instruments Company TR603D; Arvada CO, USA), and then for each line, the weight and Ψ_{leaf} were measured every hour during dehydration. The dry weight was recorded after drying the leaves at 70°C for 12 h as above, and was used to calculate the relative water content

(RWC). The C was newly computed according to Koide et al. (2000), as:

$$C = \frac{RWC/\Psi leaf}{\text{dry weight}}$$

Results

Dehydration phase

During dehydration, water was forced to exit through the petioles, and a higher flow out through the petiole of the OE leaves was observed in comparison to the WT, as shown in Fig. 2, when leaves were pressurized to +0.5 MPa (+0.5). These differences were also observed if the data were normalized by the leaf dry weight or leaf area (Fig. 2).

By collecting the data during three different daily time-points (Fig. S1) – morning, noon and afternoon – we observed that for both lines, the amount of water that exited from the petioles followed an increasing trend and reached a maximum at noon and a minimum in the morning.

The mean Rh and C, obtained by pressurizing leaves (dehydration phase), are shown in Fig. 3 (and Fig. S3). When leaves were pressurized to +0.5 MPa (+0.5), Rh was very low in both lines with no significant differences, whereas at +1 MPa (+1), the Rh increased drastically and a significant difference was observed in the OE line ($P < 0.01$), where the Rh was lower than in WT (Fig. 3A). Conversely, C was significantly higher (+38%, $P < 0.05$) in the OE line in the first treatment (+0.5), but this difference disappeared when the pressure was increased to +1 MPa (+1) (Fig. 3B).

Rehydration phase

Subsequent to dehydration to $\Psi_{leaf} = -1.0$ MPa (+1 treatment), leaves were left to rehydrate for 1 h (rehydration phase) by subjecting them to different stimuli. The total time course of the amount of water flowing into the petiole after the pressure release is shown in Fig. 4. Moreover, the first 600 s of the experiment, which was used to compute Rh and C, are highlighted in Fig. 4B. These figures illustrate that for all lines and conditions, the recovery from stress occurred via a slow rise in the volume of water absorbed, and that transpiration began at about 600 s following depressurization, as suggested by the divergence among treatments with (light transp, light transp ABA) or without transpiration (dark, light VPD ≈ 0). Finally, although the standard errors overlapped for all treatments, the leaves of the WT line in dark conditions appeared to behave differently from those in the other treatments, which took up more water.

The recovery behavior following dehydration can be analyzed by Rh, C (calculated from the dynamics shown in Fig. 5) and the Ψ_{leaf} reached after 1 h of rehydration (Table 1). Major differences in Rh between the two lines were observed in dark conditions (Fig. 5A), where in WT leaves, the Rh was significantly higher than in OE leaves ($P < 0.01$). This result agrees with the difference observed in the +1 treatment (where dark conditions were ensured by the pressure chamber) between WT and OE,

although at a higher order of magnitude. The switch from dark to light VPD ≈ 0 conditions decreased the Rh in WT to levels similar to those in the OE line, whereas Rh was not affected by the transition between dark and light in OE leaves. Transpiration ('light transp' treatment) did not have any effect on the Rh in WT lines, whereas a slight increase was observed in the transgenic line. Finally, after the addition of ABA to the solution absorbed through the petiole (light transp ABA), the Rh in WT leaves increased, but not significantly, compared to other rehydration conditions in the light and from the transgenic line. However, in general, we observed a reduction in Rh in WT following the transfer from the dark to the light conditions adopted in the rehydration experiments, whereas the Rh in the OE line tended to increase with increased transpiration.

The C computed from the same dataset did not differ between WT and transgenic leaves; only the dark condition strongly affected this parameter, causing it to be significantly lower ($P < 0.001$) in the WT than in all other treatments.

The Ψ_{leaf} recorded in the pressurization experiment and its recovery are reported in Table 1. During dehydration, there were no differences in the native Ψ_{leaf} and consequently in the final Ψ_{leaf} reached. However, in contrast, during rehydration, the leaves of the two lines revealed a different ability to recover the Ψ_{leaf} within 1 h after de-pressurization. In particular, both lines in dark conditions showed a higher recovery rate, reaching a Ψ_{leaf} close to 0. On the contrary, Ψ_{leaf} decreased when leaves were subjected to artificial light and transpiration, whereas ABA treatment facilitated the recovery of Ψ_{leaf}. The statistical analysis showed differences in the Ψ_{leaf} between the two lines in the 'light VPD≈0' and 'light transp' treatments.

Based on these observations, the expression levels of transgenic *VvPIP2;4N*, together with those of other known *PIP2* genes and a PIP1-type aquaporin were quantified by qRT-PCR in dark and light-transpiration conditions in both lines. The WT showed the same AQP expression profile in dark and light-transpiration conditions, suggesting a light-independent expression of these *PIP* genes (Fig. 6). Furthermore, in the OE line, the expression profile of AQPs and transgenic *VvPIP2;4N* was generally not affected by light; only *VvPIP2;2* was slightly more highly expressed in the dark.

Leaf dehydration dynamics in dark conditions

The dynamics of the dehydration of detached leaves in darkness was observed from the relationship between Ψ_{leaf} and relative water content (RWC). In addition, C was calculated as $\Delta RWC/\Delta\Psi$*dry weight. The linear regression indicated a slightly higher RWC coupled to the decrease in Ψ_{leaf} in OE compared to WT leaves (Fig. 7A). The hyperbole describing Ψ_{leaf} versus C was similar in both lines, showing a reduction of C that was related to the decrease in Ψ_{leaf} (Fig. 7B). Overall, the mean values of C for the two lines confirmed the higher C in OE lines (156 ± 26 for the WT, 261 ± 52 for OE; $P < 0.05$).

Finally, to evaluate whether the observed differences were attributable to anatomical or morphological traits, the pairwise relationships between leaf area, dry weight and fresh weight were

assessed, without identifying any significant differences between WT and OE samples (Fig. S2).

Discussion

In this study, transgenic grapevines that constitutively over-expressed *VvPIP2;4N* under the *Cauliflower mosaic virus* 35S promoter (Perrone et al. 2012) were used to assess the role of this AQP on leaf Rh and C during leaf dehydration and recovery. Many studies in several transgenic plants had previously shown that overexpression of aquaporin genes decreased the Rh (Ding et al. 2004, Lee et al. 2012, Perrone et al. 2012), whereas the silencing of AQPs resulted in an increase in Rh (Siefritz et al. 2004, Sade et al. 2014). However, no information is available concerning the relationship between AQP and C.

The effect of aquaporins on hydraulic resistance (Rh)

As expected, the Rh was lower in OE leaves than WT leaves when a high over-pressure was applied to the leaves (+1) and when recovery was performed in dark conditions. These two results can be ascribed to a direct effect of transgenic *PIP2;4N*, since an increase in PIP2;4N protein in the membranes improves the membrane permeability to water.

Several studies have demonstrated that AQPs expression and activity are regulated in leaves by circadian rhythms (Siefritz et al. 2002, Nardini et al. 2005, Hachez et al. 2008). In this study, the dynamics of the cumulative water outflow from leaves showed an influence of circadian rhythms both in OE and WT leaves (Fig. S1). These differences might have affected the computation of the hydraulic traits (Fig. S3); however, to limit the impact of the biological clock, the experiments were performed at distinct times during the day and averaged together in both genotypes. AQP expression has been assessed just during the morning. However, the impact of circadian rhythms on the extremely high expression of the transgene (meanly 7 times higher than endogenous aquaporins) can be reasonably neglected. In addition, it is known that the 35S gene promoter, controlling expression of our transgene, shows low or no sensitivity to the biological clock (Millar et al. 1992, Xu and Johnson 2001).

The leaf Rh increase with increased dehydration as observed in Fig. 3, where Rh drastically increased from the +0.5 to the +1 treatment, which was reported previously (Sack and Holbrook 2006, Scoffoni et al. 2014). However, this might represent a physical artefact. One hypothesis might be that a pressure of 1 MPa leads to a massive flow of water in the leaf hydraulic system in a short time interval. Probably, the anatomy of the leaf itself (e.g., connectivity between cells, bundle-sheath permeation, petiole conductivity) hinders the runoff of a large amount of water in very short period, leading to an overestimation of the Rh. This phenomenon might explain the different magnitude of the Rh values in the dehydration +1 and recovery treatments.

In the recovery trial (rehydration, Fig. 6), the impact of various stimuli, such as (1) light, (2) ABA and (3) transpiration on the aquaporin activity was studied.

1) The light effect cancelled the differences in Rh observed in the **dark** between WT and OE. Indeed, in WT, the hydraulic resistance decreased from dark to light conditions, whereas this parameter was not affected in OE leaves. This change in the Rh in WT leaves agrees with the increase in leaf conductivity under irradiation previously reported by several authors (Nardini et al. 2010, Sellin et al. 2010, Guyot et al. 2012, Lopez et al. 2013, Prado et al. 2013). Cochard et al. (2007) indicated two potential light-modulated mechanisms of water movement in leaves: activated AQPs in light conditions allows water to move freely in the symplast and apoplast, whereas at low irradiance, deactivated AQPs force the water to move apoplastically, limited by the bundle sheath.

Voicu et al. (2009) highlighted that the light-dependent change in leaf hydraulic conductance in bur oak (*Quercus macro-carpa*) was not linked to any AQP transcriptional changes. Similarly, in this study, we observed only slight differences in the expression profile between WT and OE following changes in the light conditions (Fig. 6). The qRT-PCR data showed clearly that the major difference between WT and OE lines derived exclusively from the high and constitutive expression of transgenic *VvPIP2;4N* in all conditions (Figure 6). Thus, for the OE line, the low levels of Rh in dark conditions were probably linked to the constitutive over-expression of *VvPIP2;4N*. Some type of contrasting regulation can be hypothesized between the light-mediated activation of leaf AQPs (as in WT) and *VvPIP2;4N*. In WT plants, light activates AQPs depressing the Rh recorded upon dark condition, whereas in OE plants the effect of *VvPIP2;4N* (a root-specific AQP isoform, presumably insensitive to light modulation) is masked from the light-activation of the other leaf aquaporins.

2) ABA modulates AQP activity, having opposite effects on root and leaf AQPs: downregulating the bundle-sheath AQPs and thus limiting hydraulic conductivity in leaf (Pantin et al. 2013, Shatil-Cohen et al. 2011), and upregulating the AQP isoforms and increasing the hydraulic conductivity in the root (Jang et al. 2004, Hose et al. 2000, Thompson et al. 2007, Parent et al. 2009). In this study, no significant differences in Rh between OE and WT were observed in the presence of ABA. However, comparing the light transp and light transp ABA treatments, Rh values were twice as high in WT, even if this difference was not statistically significant, whereas no changes were observed in OE leaves. We can speculate that ABA caused a closed conformation of PIP2;4N or inhibited the expression of all AQP isoforms in WT leaves. This downregulation might also occur in OE leaves, but additionally and in contrast, ABA might promote the expression or open conformation of PIP2;4N. The PIP2;4N protein is a root-specific AQP, and therefore, is putatively upregulated by ABA; its presence might have led to a lack of increase in Rh. The first ABA signaling transduction pathway that mediates water transport in roots has been recently demonstrated in maize (Fan et al. 2015). Notably, the post-translational regulation of ZmPIP through ABA signaling appears to be particularly important to regulate root hydraulic conductivity. This might also be the case for transgenic VvPIP2;4N aquaporin in leaf: since it is under the control of a constitutive promoter (35s), ABA might promote its activity in a phosphorylation-dependent manner. Moreover, Chitarra et al. (2014) demonstrated that ABA promoted *VvPIP2;4N* expression in vessel-associated cells (VACs), but not in whole petiole tissue.

Similarly to the VACs, the leaf bundle sheath cells regulate exchange between the xylem and other parenchyma cells. The ABA-induced upregulation of *VvPIP2;4* at this level might easily explain the lack of rise of Rh level in the transgenic leaves.

A secondary effect of ABA was observed in the recovery of the final Ψ_{leaf} after 1 h of rehydration (Table 1). In contrast to the Light trans treatment (final Ψ_{leaf} = –0.23 MPa for WT and –0.35 MPa for OE), ABA promoted a more rapid recovery of Ψ_{leaf} in OE leaves than WT leaves (–0.19 MPa for WT and –0.17 MPa for OE). A positive effect of ABA on Ψ_{leaf} recuperation has been already described by Lovisolo et al. (2008) and Chitarra et al. (2014) in grapevine. In the OE leaves, the better recovery of Ψ_{leaf} appeared to be coupled to a low hydraulic resistance, in agreement with data reported by Martre et al. (2002).

3) In this study, we observed no effect of transpiration on changes in leaf Rh, contrary to that reported for AQPs in root (Sakurai-Ishikawa et al. 2011, Laur and Hacke 2013). This might be due to the low vapor-pressure deficit in the laboratory atmosphere or to the real absence of an effect of this parameter in leaf.

The effect of aquaporins on hydraulic capacitance (C)

The C plays an important role in drought tolerance, as does Rh, by affecting the amount of water destined to buffer the change in the transpiration stream elicited by the atmospheric conditions and especially the ability to extend survival after stomatal closure (Bartlett et al. 2012, Gleason et al. 2014). Since sapwood has been recognized as the major source of stored water, several studies have addressed the importance of C in this tissue in depth. Generally, C varies in sapwood between 40 and 900 kg m^{-3} MPa^{-1} (Scholz et al. 2007, Čermák et al. 2007, McCulloh et al. 2014) and is inversely related to the wood density. The contribution to the total daily transpiration reported in literature varies between 5% and 45% (Goldstein et al. 1997, Phillips et al. 2003, Verbeeck et al. 2007). However, the importance of the leaf C was highlighted by Gleason et al. (2014), who suggested that the majority of water lost during dehydration derives from leaves. By comparing the C in grapevine leaves in this study (up to 250 mg H_2O g^{-1} DW MPa^{-1}), classifies them within the upper half of the ranking proposed by Blackman and Brodribb (2011), among species with a low C, although the measured units are not the same (the conversion was performed by considering that 1 m^2 of leaf area corresponds to 31 g DW, from Fig. S2).

A novel aspect highlighted by this study is the effect of AQP on C, which, as far as we know, has never been reported so far. The ability of a tissue to be a capacitor, and to buffer the xylem tension and prevent embolism was debated as an important trait that might distinguish plants and their susceptibly to water stress (Sperry et al. 2008, McCulloh et al. 2014).

In the pressurization tests, differences in C were observed between WT and OE leaves only when the pressure applied was low (+0.5 MPa, Fig. 3B), in contrast to what was observed for Rh (Fig. 3A). The absence of effect when the applied pressure reached +1 MPa might be due to the high dehydration

imposed on the leaves. Indeed, C is a variable parameter that decreases together with water status (Fig. 7B), potentially becoming comparable in the two lines when the leaves were dehydrated to –1 MPa.

In the recovery trials (Fig. 5B), WT leaves showed the lowest C in dark conditions compared with the other treatments; probably, the high Rh observed in WT hindered the water uptake and thus, the recovery of C. In theory, a longer recovery time might lead to the complete recovery of C in WT leaves, although the leaves were in dark conditions. However, in dark conditions, the final Ψ_{leaf} values fully recovered in both lines; even though this occurred in WT without a complete recovery in C. Thus, in this latter case, the amount of water inside the WT leaves was lower than that in OE leaves, and probably, the amount of water outflow pressurizing once more the leaf could be lower than that observed during the first pressurization.

The aim of the last experiment was to check whether OE leaves dehydrate more rapidly in dark conditions (where major differences between WT and OE leaves were observed). However, the overexpression of *VvPIP2;4N* did not lead to a more rapid leaf dehydration, and the computed C ($\Delta RWC/\Delta\Psi$*dry weight) confirmed higher values in the OE line.

Hypothesis and ecological significance

Using transgenic plants ant the novel method proposed in this study, a positive relationship between C and AQPs in grapevine leaves was demonstrated. The mechanisms underlying this interaction are not yet clear, however, an initial hypothetical mechanism might involve the reflection coefficient (□) of the plasma-membrane. This parameter is considered to be the ability of a channel (AQP in our case) to be permeable to, or reflect a solute. It is determined by the arginine selectivity filter at the end of the pore (Zeuthen et al. 2013) and by the solute size. In the membrane of transgenic plants in this study, the higher concentration of AQPs can lead to a higher permeation of small solutes (Gomes et al. 2009), resulting in a low reflection coefficient, and consequently allowing a higher water flow (coupled to osmolyte flow) through the lipid bilayer. Although this consideration depends on the contribution of *VvPIP2;4N* to the total water transport across the plasma membrane. The theoretical framework for the implication of σ on C is provided as supplemental information.

Recently, Maurel et al. (2015) proposed a pivotal role for AQPs in buffering cell osmoregulation, by reviewing and re-interpreting AQP function as osmo-sensor in guard cells during stomatal movements or in growing pollen tubes. The mechanism was only speculated, but an AQP-mediated increasing cell C, conferring to the cells higher buffer capacitance, could give light to this still un-described phenomenon.

A second hypothesis, which does not exclude the first, is that AQPs might connect several cells, and increase the volume of the reservoir. In grapevine, AQPs can increase the link between the symplast and apoplast in the areoles, the enclosed areas between the interconnected veins, thereby improving the leaf capacitance. In figure 8 a schematic representation of the leaf hydraulic pathway is represented with its simplification (right side), showing the increase of the total capacitor due to the

sum of different capacitors.

Previously, studies have attributed a role to AQPs in iso/anisohydric behavior, due to changes in leaf conductance (Sade et al. 2009, Vandeleur et al. 2009, Chaumont and Tyremann 2014). In other studies (Ogasa et al. 2013, McCulloh et al. 2014), plants are categorized according to their C, for their investment in structural features to maintain the transpiration stream (anisohydry) or their sensitivities to embolisms (Tombesi et al. 2014).

Perrone et al. (2012) considered the 'Brachetto' WT as an anisohydric cultivar, and the transgenic lines could be interpreted as being even more anisohydric. In this study, AQPs conferred a greater C, and hence, a greater degree of anisohydry, highlighting the possible link between C, Rh, aquaporins and iso/anisohydric responses to water stress. Clearly, the implication of Rh and C on whole leaf hydraulics deserves further attention.

Author contributions

H.C., C.L. and M.V. conceived and planned the study. M.V. performed the experiment and analyzed the data. M.V. wrote the first draft of the manuscript. G.G. and I.P. produced plant materials, carried out the molecular analysis and reviewed the manuscript. A.P. helped in the physical dissertations. H.C. theorized the hypothesis presented and reviewed the manuscript. C.L. reviewed the manuscript and obtained funds to support the project.

Acknowledgements – M.V. wishes to thank the staff of INRA of Clermont Ferrand for their great assistance, and Philippe Label for providing ABA; Francesca Cardinale and Silvia Cavalletto for reviewing the manuscript and for 'physics' explanations, respectively, and Sonia Livigni for discussions on post-translational regulation and for her understanding. M.V. was partly granted during his PhD by SIGEVI project (PSR, misura 124, azione 1) founding by Piedmont region and UE.

References

Angeles G, Bond B, Boyer JS, Brodribb T, Brooks JR, Burns MJ, Cavender-Bares J, Clearwater M, Cochard H, Comstock J, Davis SD, Domec J-C, Donovan L, Ewers F, Gartner B, Hacke U, Hinckley T, Holbrook NM, Jones HG, Kavanagh K, Law B, Lopez-Portillo J, Lovisolo C, Martin T, Martinez-Vilalta J, Mayr S, Meinzer FC, Melcher P, Mencuccini M, Mulkey S, Nardini A, Neufeld HS, Passioura J, Pockman WT, Pratt RB, Rambal S, Richter H, Sack L, Salleo S, Schubert A, Schulte P, Sparks JP, Sperry J, Teskey R, Tyree M (2004) The cohesion-tension theory. New Phytol 163: 451–452

Bartlett MK, Scoffoni C, Sack L (2012) The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. Ecol Lett 15: 393–405

Blackman CJ, Brodribb TJ (2011) Two measures of leaf capacitance: insights into the water transport pathway and hydraulic conductance in leaves. Funct Plant Biol 38: 118–126

Čermák J, Kučera J, Bauerle WL, Phillips N, Hinckley TM (2007) Tree water storage and its diurnal

dynamics related to sap flow and changes in stem volume in old-growth Douglas-fir trees. Tree Physiol 27: 181–198

Chaumont F and Tyerman SD (2014) Aquaporins: highly regulated channels controlling plant water relations. Plant Physiol 164: 1600–1618

Chitarra W, Balestrini R, Vitali M, Pagliarani C, Perrone I, Schubert A, Lovisolo C (2014) Gene expression in vessel-associated cells upon xylem embolism repair in *Vitis vinifera* L. petioles. Planta 239: 887–899

Cochard H, Venisse JS, Barigah TS, Brunel N, Herbette S, Guilliot A, Tyree MT, Sakr S (2007) Putative role of aquaporins in variable hydraulic conductance of leaves in response to light. Plant Physiol 143: 122–133

Comstock JP (2002) Hydraulic and chemical signalling in the control of stomatal conductance and transpiration. J Exp Bot 53: 195–200

Ding X, Iwasaki I, Kitagawa Y (2004) Overexpression of a lily PIP1 gene in tobacco increased the osmotic water permeability of leaf cells. Plant Cell Environ 27: 177–186

Dixon HH (1914) Transpiration and the ascent of sap in plants. Macmillan, London

Fan W, Li J, Jia J, Wang F, Cao C, Hu J, Mu Z (2015) Pyrabactin regulates root hydraulic properties in maize seedlings by affecting PIP aquaporins in a phosphorylation-dependent manner. Plant Physiol Bioch 94: 28–34

Flexas J, Scoffoni C, Gago J, Sack L (2013) Leaf mesophyll conductance and leaf hydraulic conductance: an introduction to their measurement and coordination. J Exp Bot 64: 3965–3981

Gleason SM, Blackman CJ, Cook AM, Laws CA, Westoby M (2014) Whole-plant capacitance, embolism resistance and slow transpiration rates all contribute to longer desiccation times in woody angiosperms from arid and wet habitats. Tree Physiol 34: 275–284

Goldstein G, Andrade JL, Meinzer FC, Holbrook NM, Cavelier J, Jackson P, Silvera K (1997) Stem water storage and diurnal patterns of water use in tropical forest canopy trees. Plant Cell Environ 21: 397–406

Gomes D, Agasse A, Thiébaud P, Delrot S, Gerós H, Chaumont F (2009) Aquaporins are multifunctional water and solute transporters highly divergent in living organisms. Biochimica Biophysica Acta 1788: 1213–1228

Guyot G, Scoffoni C, Sack L (2012) Combined impacts of irradiance and dehydration on leaf hydraulic conductance: insights into vulnerability and stomatal control. Plant Cell Environ 35: 857–871

Hachez C, Heinen RB, Draye X, Chaumont F (2008) The expression pattern of plasma membrane aquaporins in maize leaf highlights their role in hydraulic regulation. Plant Mol Biol 68: 337–353

Honert TH van den (1948) Water transport in plants as a catenary process. Discuss Faraday Soc 3: 146–153

Hose E, Steudle E, Hartung W (2000) Abscisic acid and hydraulic conductivity of maize roots: a study

using cell- and root-pressure probes. Planta 211: 874–882

Jang JY, Kim DG, Kim YO, Kim JS, Kang H (2004) An expression analysis of a gene family encoding plasma membrane aquaporins in response to abiotic stresses in *Arabidopsis thaliana*. Plant Mol Biol 54: 713–725

Kaldenhoff R, Ribas-Carbo M, Sans JF, Lovisolo C, Heckwolf M, Uehlein N (2008) Aquaporins and plant water balance. Plant Cell Environ 31: 658–666

Koide RT, Robichaux RH, Morse SR, Smith CM (2000) Plant water status, hydraulic resistance and capacitance. In: Pearcy RW, Ehleringer JR, Mooney HA, Rundel PW (eds) Plant Physiological Ecology: Field Methods and Instrumentation. Kluwer, Dordrecht, The Netherlands, pp. 161–183

Lange OL, Nobel PS, Osmond CB, Ziegler H (1982) Physiological plant ecology II; water relations and carbon assimilation. Encyclopedia of Plant Physiology, vol. 12B. Springer, Berlin Heidelberg New York

Lee SH, Chung GC, Jang JY, Ahn SJ, Zwiazek JJ (2012) Overexpression of PIP2;5 aquaporin alleviates effects of low root temperature on cell hydraulic conductivity and growth in Arabidopsis. Plant Physiol 159: 477–488

Lopez D, Venisse JS, Fumanal B, Chaumont F, Guillot E, Daniels MJ, Cochard H, Julien JL, Gousset-Dupont A (2013) Aquaporins and leaf hydraulics: Poplar sheds new light. Plant Cell Physiol 54: 1963–1975

Laur J, Hacke UG (2013) Transpirational demand affects aquaporin expression in poplar roots. J Exp Bot 64: 2283–2293

Lovisolo C, Perrone I, Hartung W, Schubert A (2008) An abscisic acid-related reduced transpiration promotes gradual embolism repair when grapevines are rehydrated after drought. New Phytol 180: 642–651

Martinez-Ballesta MdC, Carvajal M (2014) New challenges in plant aquaporin biotechnology. Plant Sci 217–218: 71–77

Martre P, Morillon R, Barrieu F, North GB, Nobel PS, Chrispeels MJ (2002) Plasma membrane aquaporins play a significant role during recovery from water deficit. Plant Physiol 130: 2101–2110

Maurel C, Boursiac Y, Luu D-T, Santoni V, Shahzad Z, Verdoucq L (2015) Aquaporins in Plants. Physiol Rev 95: 1321–1358

McCulloh AK, Daniel MJ, Frederick CM, David RW (2014) The dynamic pipeline: hydraulic capacitance and xylem hydraulic safety in four tall conifer species. Plant Cell Environ 37: 1171–1183

Millar AJ, Short SR, Chua NH, Kay SA (1992) A novel circadian phenotype based on firefly luciferase expression in transgenic plants. Plant Cell 4: 1075–1087

Nardini A, Salleo S, Andri S. (2005) Circadian regulation of leaf hydraulic conductance in sunflower (*Helianthus annuus* cv. Margot). Plant Cell Environ 28: 750–759

Nardini A, Raimondo F, Lo Gullo MA, Salleo S (2010) Leaf miners help us understand leaf hydraulic design. Plant Cell Environ 33: 1091–1100

Ogasa M, Miki N, Murakami Y, Yoshikawa K (2013) Recovery performance in xylem hydraulic conductivity is correlated with cavitation resistance for temperate deciduous tree species. Tree Physiol 33: 335–344

Pantin F, Monnet F, Jannaud D, Costa JM, Renaud J, Muller B, Simonneau T, Genty B (2013) The dual effect of abscisic acid on stomata. New Phytol 197: 65–72

Parent B, Hachez C, Redondo E, Simonneau T, Chaumont F, Tardieu F (2009) Drought and abscisic acid effects on aquaporin content translate into changes in hydraulic conductivity and leaf growth rate: a trans-scale approach. Plant Physiol 149: 2000–2012

Péret B, Li G, Zhao J, Band LR, Voß U, Postaire O, Luu DT, Da Ines O, Casimiro I, Lucas M, Darren MW, Lazzerini L, Nacry P, King JR, Jensen OE, Schaffer AR, Maurel C, Bennet MJ (2012) Auxin regulates aquaporin function to facilitate lateral root emergence. Nature Cell Biol 14: 991–998

Perrone I, Gambino G, Chitarra W, Vitali M, Pagliarani C, Riccomagno N, Balestrini R, Kaldenhoff R, Uehlein N, Gribaudo I, Schubert A, Lovisolo L (2012) The grapevine root-specific aquaporin VvPIP2;4N controls root hydraulic conductance and leaf gas exchange upon irrigation but not under water stress. Plant Physiol 160: 965–977

Phillips NG, Ryan MG, Bond BJ, McDowell NG, Hinckley TM, Čermák J (2003) Reliance of stored water increases with tree size in three species in the Pacific Northwest. Tree Physiol 23: 237–245

Postaire O, Tournaire-Roux C, Grondin A, Boursiac Y, Morillon R, Schäffner AR, Maurel C (2010) A PIP1 aquaporin contributes to hydrostatic pressure-induced water transport in both the root and rosette of Arabidopsis. Plant Physiology 152: 1418–1430

Prado K, Maurel C (2013) Regulation of leaf hydraulics: from molecular to whole plant levels. Front Plant Sci 4: 255

Prado K, Boursiac Y, Tournaire-Roux C, Monneuse JM, Postaire O, Da Ines O, Schäffner AR, Hem S, Santoni V, Maurel C (2013) Regulation of Arabidopsis leaf hydraulics involves light-dependent phosphorylation of aquaporins in veins. Plant Cell 25: 1029–1039

Robichaux HR (1984) Variation in the tissue water relation of two sympatric Hawaiian Dubautia species and their natural hybrid. Oecologia 65: 75–81

Sack L, Cowan PD, Jaikumar N, Holbrook NM (2003) The "hydrology" of leaves: co-ordination of structure and function in temperate woody species. Plant Cell Environ 26: 1343–1356

Sack L, Holbrook NM (2006) Leaf hydraulics. Annu Rev Plant Biol 57: 361–381

Sade N, Vinocur BJ, Diber A, Shatil A, Ronen G, Nissan H, Wallach R, Karchi H, Moshelion M (2009) Improving plant stress tolerance and yield production: is the tonoplast aquaporin SlTIP2;2 a key to isohydric to anisohydric conversion? New Phytol 181: 651–661

Sade N, Shatil A, Attia Z, Maurel C, Boursiac Y, Kelly G, Granot D, Yaaran A, Lerner S, Moshelion

M (2014) The role of plasma membrane aquaporins in regulating the bundle sheath-mesophyll continuum and leaf hydraulics. Plant Physiol 166: 1609–1620

Sakurai-Ishikawa J, Murai-Hatano M, Hayashi H, Ahamed A, Fukushi K, Matsumoto T, Kitagawa Y (2011) Transpiration from shoots triggers diurnal changes in root aquaporin expression. Plant Cell Environ 34: 1150–1163

Scoffoni C, Vuong C, Diep S, Cochard H, Sack L (2014) Leaf shrinkage with dehydration: coordination with hydraulic vulnerability and drought tolerance. Plant Physiol 164: 1772–1788

Siefritz F, Otto B, Bienert GP, van der Krol A, Kaldenhoff R. (2004) The plasma membrane aquaporin NtAQP1 is a key component of the leaf unfolding mechanism in tobacco. Plant J 37: 147–155

Siefritz F, Tyree MT, Lovisolo C, Schubert A, Kaldenhoff R (2002) PIP1 Plasma Membrane Aquaporins in Tobacco: From Cellular Effects to Function in Plants. Plant Cell 14: 869–876

Shatil-Cohen A, Attia Z, Moshelion M (2011) Bundle-sheath cell regulation of xylem-mesophyll water transport via aquaporins under drought stress: a target of xylem-borne ABA? Plant J 67: 72–80

Sellin A, Ounapuu E, Karusion A (2010) Experimental evidence supporting the concept of light-mediated modulation of stem hydraulic conductance. Tree Physiol 30: 1528–1535

Sperry JS, Adler FR, Campbell GS, Comstock JP (1998) Limitation of plant water use by rhizosphere and xylem conductance: results from a model. Plant Cell Environ 21: 347–359

Sperry JS, Meinzer FC, McCulloh AK (2008) Safety and efficiency conflicts in hydraulic architecture: scaling from tissues to trees. Plant Cell Environ 31: 632–645

Thompson AJ, Andrews J, Mulholland BJ, McKee JM, Hilton HW, Horridge JS, Farquhar GD, Smeeton RC, Smillie IR, Black CR, Taylor IB (2007) Overproduction of abscisic acid in tomato increases transpiration efficiency and root hydraulic conductivity and influences leaf expansion. Plant Physiol 143: 1905–1917

Tombesi S, Nardini A, Farinelli D, Palliotti A (2014) Relationships between stomatal behavior, xylem vulnerability to cavitation and leaf water relations in two cultivars of *Vitis vinifera*. Physiol Plant 152: 453–464

Tyree MT, Hammel HT (1972) The measurement of the turgor pressure and the water relations of plants by the pressure bomb technique. J Exp Bot 23: 267–282

Tyree MT, Ewers WF (1991) The hydraulic architecture of tree and other woody plants. New Phytol 119: 345–360

Vandeleur RK, Mayo G, Shelden MC, Gilliham M, Kaiser BN, Tyerman SD (2009) The role of plasma membrane intrinsic protein aquaporins in water transport through roots: diurnal and drought stress responses reveal different strategies between isohydric and anisohydric cultivars of grapevine. Plant Physiol 149: 445–460

Vandeleur RK, Sullivan W, Athman A, Jordans C, Gilliham M, Kaiser BN, Tyerman SD (2014) Rapid shoot-to-root signalling regulates root hydraulic conductance via aquaporins. Plant Cell Environ

37: 520–538

Verbeeck H, Steppe K, Nadezhdina N, Op de Beeck M, Deckmyn G, Meiresonne L, Lemeur R, Cermák J, Ceulemans R, Janssens IA (2007) Stored water use and transpiration in Scots pine: a modeling analysis with ANAFORE. Tree Physiol 27: 1671–1685

Voicu MC, Cooke JE, Zwiazek JJ (2009) Aquaporin gene expression and apoplastic water flow in bur oak (*Quercus macro-carpa*) leaves in relation to the light response of leaf hydraulic conductance. J Exp Bot 60: 4063–4075

Xu Y, Johnson CH (2001) A clock-and light-regulated gene that links the circadian oscillator to LHCB gene expression. Plant Cell 13: 1411–1426

Zeuthen T, Alsterfjord M, Beitz E, MacAulay N (2013) Osmotic water transport in aquaporins: evidence for a stochastic mechanism. J Physiol 591: 5017–5029

Supporting information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Time-course of the cumulative water flow out of leaves in WT leaves in morning, noon, afternoon.

Fig. S2. Relationship between dry weight and leaf area and fresh weight.

Fig. S3. Hydraulic resistance and capacitance values, averaging data obtained during morning, noon and afternoon period.

Fig. S4. Höfler diagram showing the changes in whole leaf water potential (Ψ), pressure potential (P) and osmotic potential (Π) as a function of relative symplasmic water content.

Fig. S5. Effect of the reflection coefficient σ on the relative change in leaf capacitance.

Appendix S1. We provide here the theoretical framework demonstrating the relation between the bulk leaf reflection coefficient σ and the bulk leaf capacitance C (contains Fig. S4 and S5).

Edited by J. Flexas

Figure legends

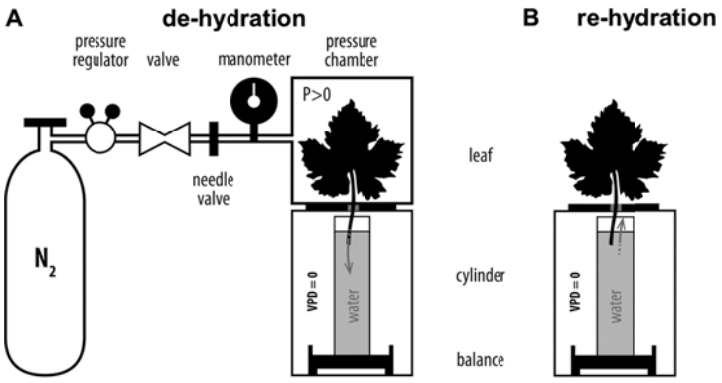


Fig. 1. The experimental setup used in this study. Nitrogen gas, regulated through valves and monitored by a manometer, was used to compress the leaf in the pressure chamber. Water flowing out from the petiole increased the weight of the water-filled cylinder (left). Following rehydration (right), the chamber was removed, allowing the rehydration of the previously pressurized leaf. The rehydration was conducted under different stimuli (light, transpiration, ABA).

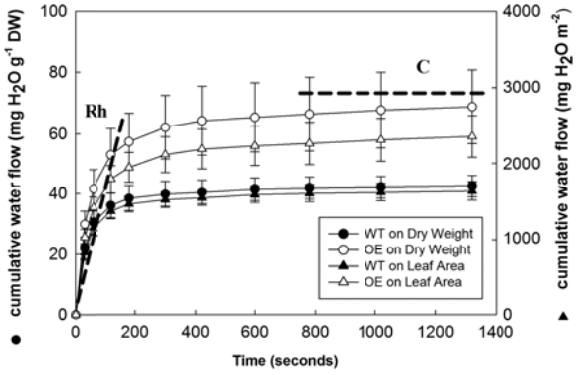


Fig. 2. Time-course of the cumulative water flow out of leaves in WT leaves (filled symbols) and OE leaves (empty symbols) in the +0.5 experiment. Data were normalized according to the dry weight (circles, left y-axis) or leaf area (triangles, right y-axis). Symbols represent the means ± SE. (n = 6).

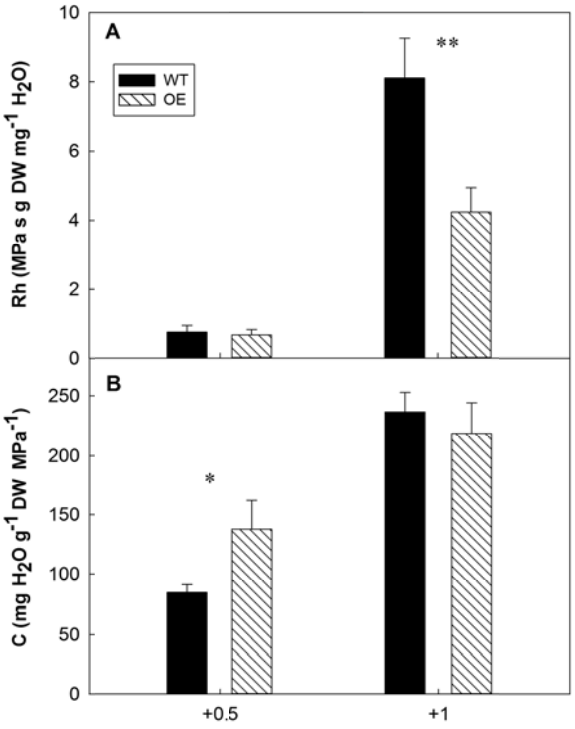


Fig. 3. (A) Hydraulic resistance (Rh), and (B) capacitance in WT leaves (black columns) and OE leaves (grey columns) obtained by pressurizing the leaves as described in dehydration phase for the experimental design: rehydrated leaves pressurized to +0.5 MPa (+0.5) or +1.0 MPa (+1). Columns represent the means (n = 6 for +0.5 and n = 16 for +1.0) ± SE Means were obtained by averaging the measurements performed at different times of day (8:00–10.00; 11:00–13.00; 14:00–16.00). Asterisks mark significant differences between means (* = $P < 0.05$ ** = $P < 0.01$).

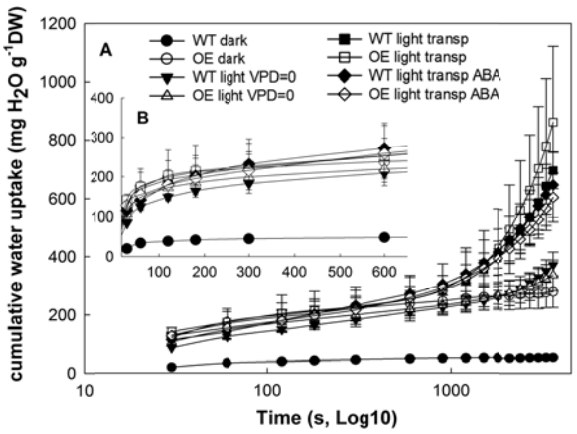


Fig. 4. (A) Time-course of the water flow into the petioles of WT leaves (filled symbols) and OE leaves (empty symbols) in the four recovery treatments: dark (circles), light low transpirative

conditions (VPD≈0; triangles), light transpirative conditions (square) and light transpirative conditions in the presence of ABA (rhombus) (n=4). (B) highlights the first 600 s of the time-course (the mean values of of Rh and C are displayed in Fig. 5).

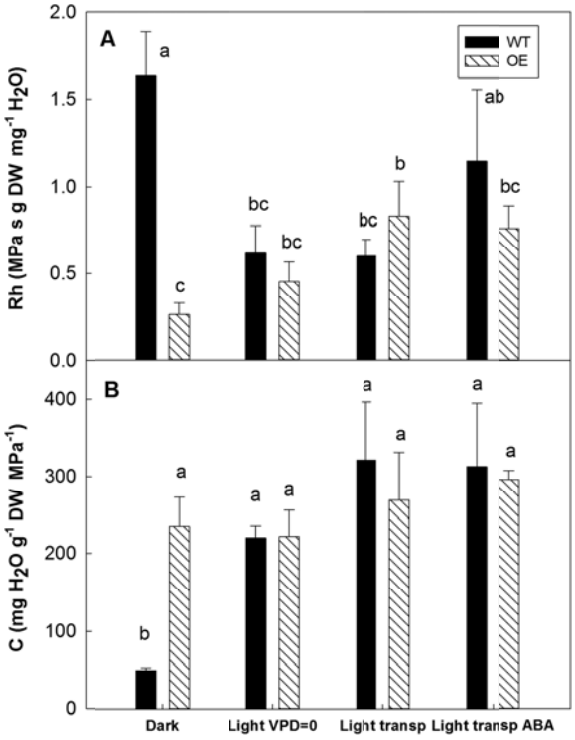


Fig. 5. (A) Hydraulic resistance (Rh), and (B) capacitance in WT (black columns) and OE (grey columns) obtained from the rehydration of leaves after dehydration to Ψ_{leaf} = –1 MPa (+1), as described in the rehydration phase of the experimental design. Recovery treatments were performed in dark, in light low transpirative, light transpirative or light transpirative conditions after adding ABA (final concentration 100 µmol) to the cylinder where the cut petioles were submerged. Columns represent the means (n = 4) ± SE. Different letters mark significant differences ($P < 0.05$) between means according to ANOVA after data normalization. In frame b, $P < 0.001$.

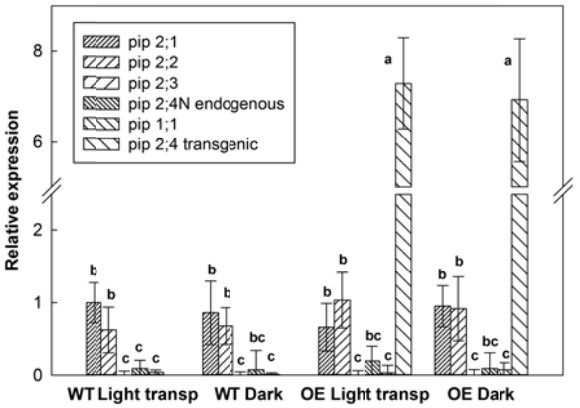


Fig. 6. Expression of endogenous and transgenic *PIP*-type AQP genes in WT and OE lines under dark and light transpirative conditions in rehydrated leaves. Relative expression levels of *VvPIP1;1*, *VvPIP2;1*, *VvPIP2;2*, *VvPIP2;3*, endogenous *VvPIP2;4N*, and transgenic *VvPIP2;4N* were determined by qRT-PCR in leaves. The PCR data were normalized with those for UBI transcripts. Data are expressed as the mean ± SE; different letters denote significant differences at $P \leq 0.05$.

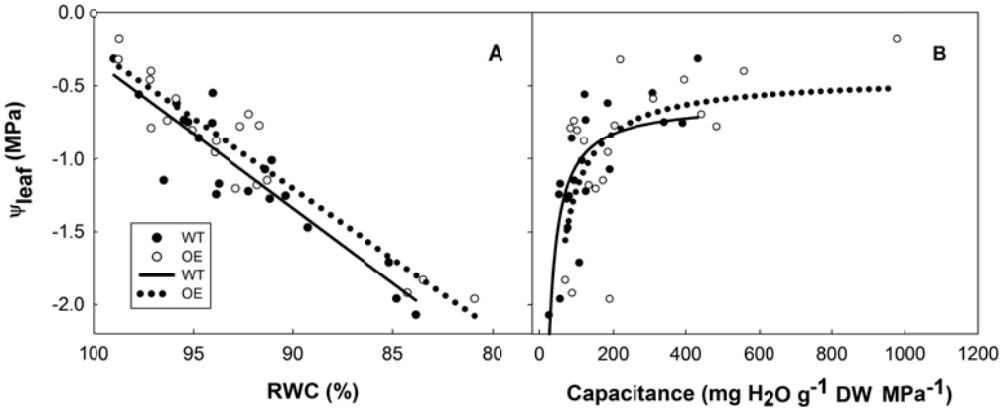


Fig. 7. Relationship between Ψ_{leaf} and RWC (A) and Ψ_{leaf} and C (B). Data were obtained from leaves allowed to dehydrate in darkness in the laboratory atmosphere. Filled circles represent the WT leaves; open circles represent OE leaves. Solid and dotted lines correspond to the regression of WT and OE, respectively.

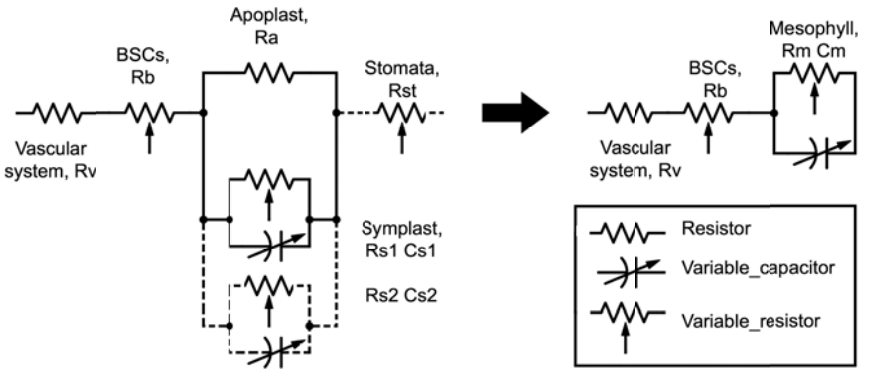


Fig. 8. Schematic representation of the leaf hydraulic pathway. The leaf is divided in several compartments represented by resistors (vascular system, Rv; Bundle sheath cells, BSCs Rb; apoplast, Ra; symplast Rs1 Rs2; and stomata Rst) and capacitors (symplast, Cs1 Cs2). Dashed lines indicate additional parts that can be added to the system. When transpiration is stopped, the system could be simplified by summing the capacitors and the reciprocal resistors (right side).

Supporting information

Figure S1: time-course of the cumulative water flow out of leaves in WT leaves (filled symbols) and OE leaves (empty symbols) in the +0.5 experiment. Data (corresponding to the ones in Fig. 2) were plotted according to the time of the day when the experiment was performed: morning (8:00–10:00, circles), noon (11:00–13:00, triangles) and afternoon (14:00–16:00, squares). Symbols represent the means ± SE (n = 2).

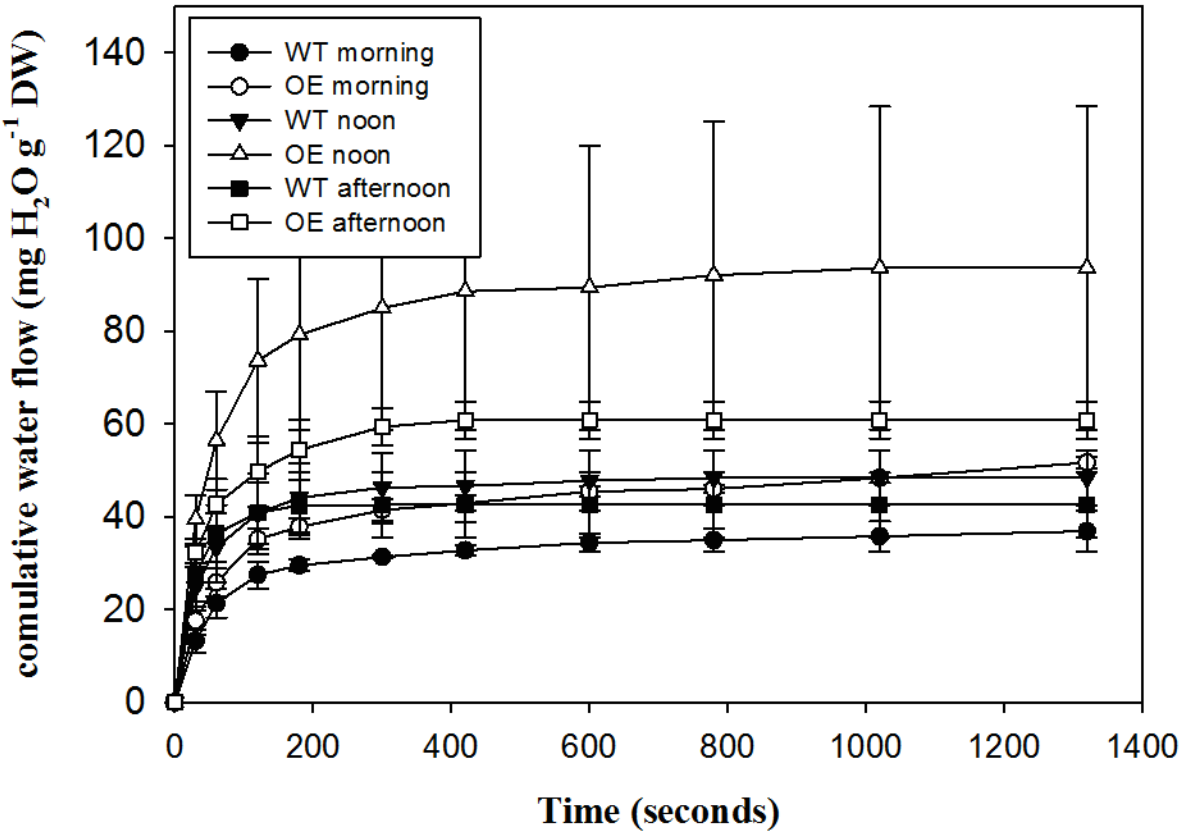


Figure S2: relationship between dry weight and leaf area (circles) and fresh weight and leaf area (triangles) in WT (filled symbols) and OE leaves (empty symbols). Regression lines are shown for WT (solid trend line) and OE (dotted trend line).

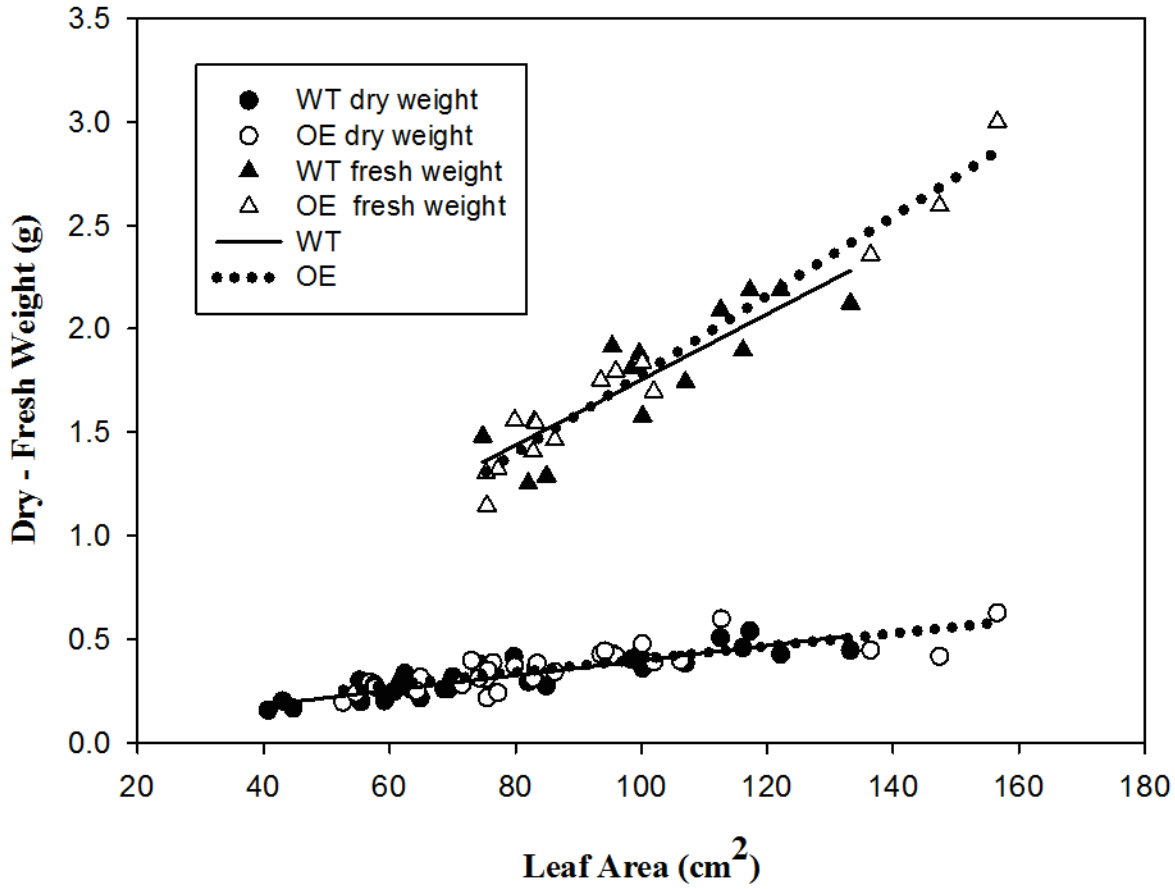
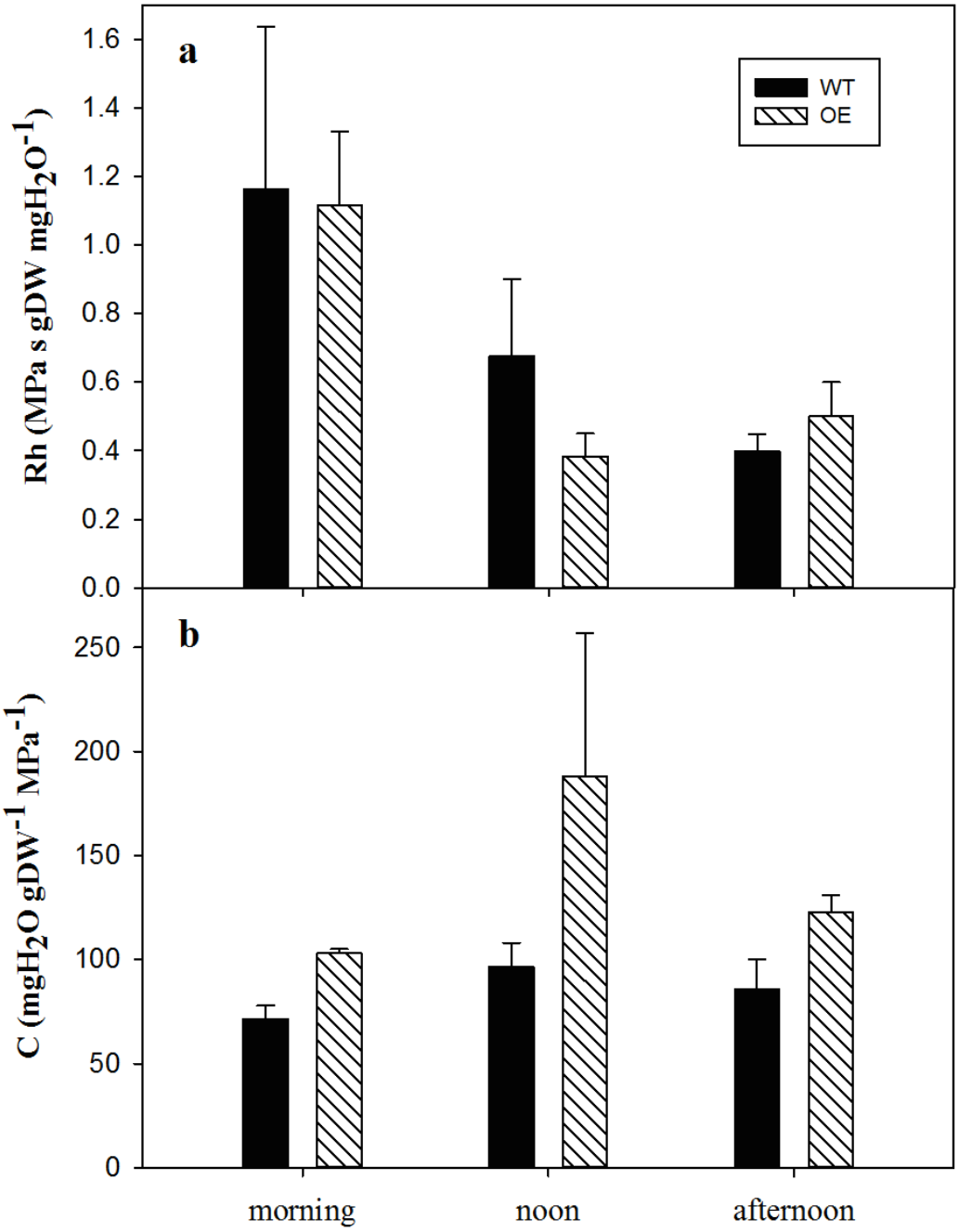


Figure S3: a) hydraulic resistance (Rh), and b) capacitance (C) in WT leaves (black columns) and OE leaves (grey columns) obtained by pressurising the leaves to +0.5 MPa (+0.5). Columns represent the means (n = 2) ± SE. Means were obtained by averaging the measurements performed at different times of day (morning 8:00–10.00; noon 11:00–13.00; afternoon 14:00–16.00 hours). Averages were obtained from the same datasets of Fig. 2 and 3 (only +0.5 treatment) and S1.



Appendix S1

We provide here the theoretical framework demonstrating the relation between the bulk leaf reflection coefficient σ and the bulk leaf capacitance C. The demonstration is based on a reanalyze of the Pressure-Volume curve theory (Tyree and Hamel 1972).

Whole leaf water potential Ψ is usually considered as the algebraic sum of the pressure turgor potential P and the osmotic potential Π:

$$\Psi=P+\Pi \qquad (s1)$$

However, this equation is correct only when the reflection coefficient is equal to one. If σ less than unity then:

$$\Psi=P+\sigma\Pi \qquad (s2)$$

When a leaf dehydrates, its relative water content (RWC) decreases and the total relative loss of water R is equal to:

$$R=1-RWC \qquad (s3)$$

Assuming a constant apoplasmic water content fraction (af), we can compute the relative water content loss R_s of the symplasmic compartment as:

$$R_s=R/(1-af) \qquad (s4)$$

Rs is equal to 0 when the leaf is fully turgid and equal to 1 when the symplasmic compartment is empty.

Leaf capacitance C is defined as:

$$C=dR_s/d\Psi \qquad (s5)$$

or, as a proxy, as:

$$C=(R_s(\Psi_1) - R_s(\Psi_2)) / (\Psi_2- \Psi_1) \qquad (s6)$$

We will focus here on our +0.5 experiment, where $\Psi_1 = 0$ and $\Psi_1 = -0.5$MPa

Defining Π_0 as the osmotic potential at full leaf turgor and ε as the bulk leaf modulus of elasticity, we can express P and Π as a function of R_s as:

$P = -\sigma(\Pi_0 + \varepsilon R_s);\ P>0$ (s7)

$\Pi = \sigma\Pi_0 / (1-R_s)$ (s8)

Equations s1, s7 and s8 are used to construct an Höfler diagram (figure s1):

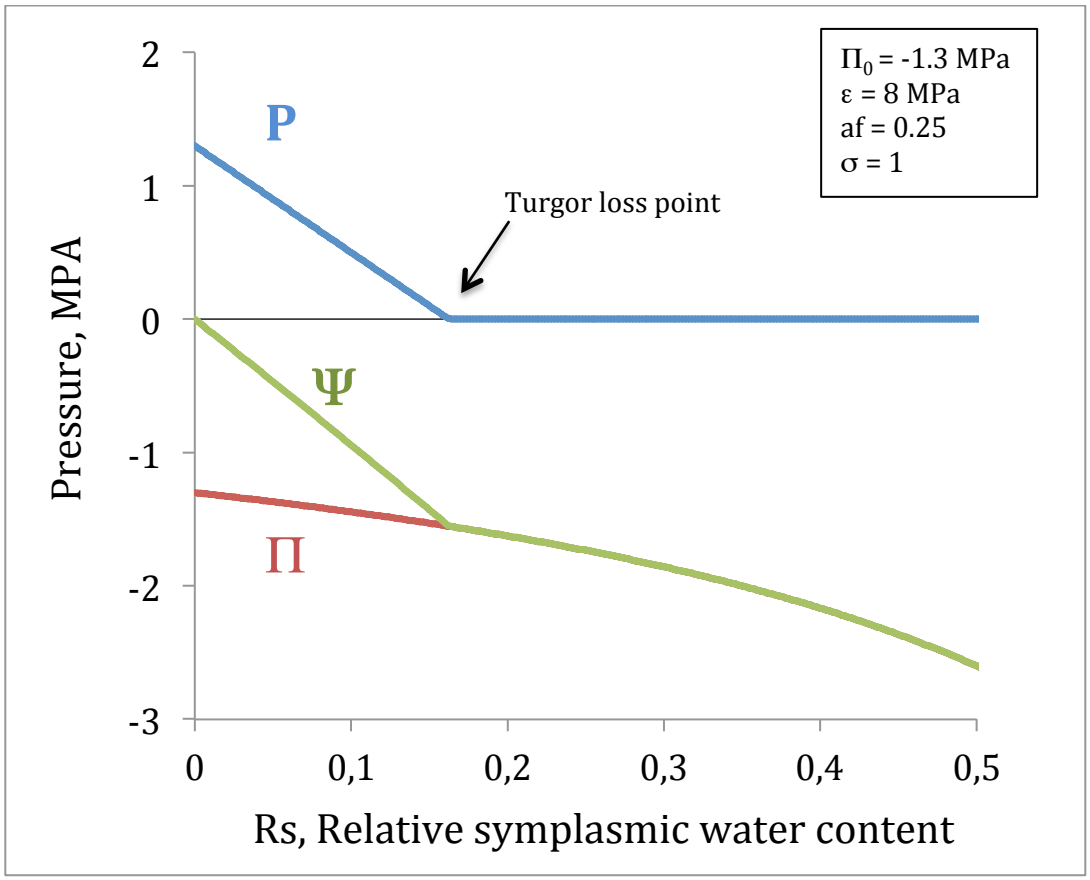


Figure S4: Höfler diagram showing the changes in whole leaf water potential (Ψ), pressure potential (P) and osmotic potential (Π) as a function of relative symplasmic water content. The parameters used to construct the diagram are shown in the insert. These parameters were obtained on *Vitis* leaves similar to those used in this study.

Combining s1, s7 and s8 we have:

$\Psi = -\sigma(\Pi_0 + \varepsilon R_s) + \sigma\Pi_0 / (1-R_s)$ for P>0 (s9)

$\Psi = \sigma\Pi_0 / (1-R_s)$ for $P=0$ (s10)

By solving equations s9 and s10 we can express R_s as a function of Ψ as:

$$R_s = \frac{\sigma(\varepsilon - \Pi_0) - \Psi - \sqrt{(\Psi + \sigma(\Pi_0 - \varepsilon))^2 + 4\sigma\varepsilon\Psi}}{2\sigma\varepsilon} \quad \text{for } P>0 \text{ (s11)}$$

$R_s = 1 - \sigma\Pi_0/\Psi$ for $P=0$ (s12)

Exact solutions of C can then be derived from s11 and s12 using s5 or s6 (not shown).
A proxy of C can also be obtained if we assume that for low R_s values equation s8 can be approximated by:

$\Pi \approx \sigma\Pi_0$ (s13)

then, by combining s2, s7 and s13 we have:

$\Psi \approx -\sigma\varepsilon R_s$ (s14)

then it comes:

$C \approx 1/\sigma\varepsilon$ (s15)

The relative change of whole leaf capacitance C_{rel} when the reflection coefficient decreases from 1 to σ is:

$C_{rel} \approx 1/\sigma$ (s16)

The relations between C_{rel} derived from s11 and s16 and σ are shown in figure s2. The approximation is robust but valid only when R_s is low and σ is high (>0.5).
Therefore, dividing σ by two will double approximately the whole leaf capacitance and this effect is largely independent of ε, Π_0 and Ψ.

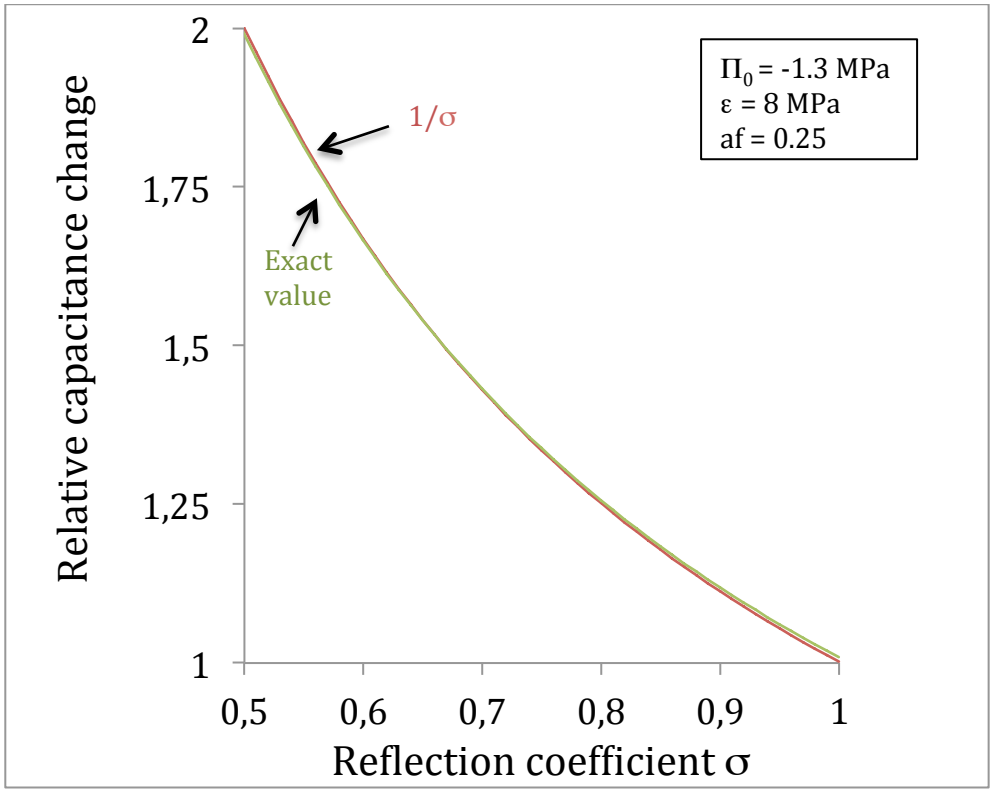


Figure S5. Effect of the reflection coefficient σ on the relative change in leaf capacitance. The exact relation is shown in green and the approximation given in equation s16 is shown in red.