When smaller is better: leaf hydraulic conductance and drought vulnerability correlate to leaf size and venation density across four *Coffea arabica* genotypes

Andrea Nardini\textsuperscript{A,C}, Eele Ōunapuu-Pikas\textsuperscript{A,B} and Tadeja Savi\textsuperscript{A}

\textsuperscript{A}Department of Life Sciences, University of Trieste, Via L. Giorgieri 10, 34127 Trieste, Italy.
\textsuperscript{B}Institute of Ecology and Earth Sciences, University of Tartu, Vanemuise 21, 51014 Tartu, Estonia.
\textsuperscript{C}Corresponding author. Email: nardini@units.it

**Abstract.** Leaf hydraulic conductance ($K_{\text{leaf}}$) and drought vulnerability in terms of leaf water potential inducing 50\% loss of $K_{\text{leaf}}$ ($P_{50}$), were assessed in four genotypes of *Coffea arabica* L. We tested three hypotheses: (1) leaf $P_{50}$ is lower in small leaves with higher vein densities; (2) lower $P_{50}$ translates into lower $K_{\text{leaf}}$, limiting gas exchange rates and higher leaf mass per unit area (LMA); (3) $P_{50}$ values are coordinated with symplastic drought tolerance. We found partial support for Hypotheses 1 and 3, but not for Hypothesis 2. Significant correlations existed among leaf size, vein network and drought resistance. Smaller leaves displayed higher major vein density, higher $K_{\text{leaf}}$ and more negative $P_{50}$. $K_{\text{leaf}}$ was correlated with leaf gas exchange rates. A negative relationship was observed between $K_{\text{leaf}}$ and LMA, whereas $P_{50}$ was found to be positively correlated with LMA. Across coffee genotypes, reduced leaf surface area and increased vein density shifts $P_{50}$ towards more negative values while not translating into higher LMA or lower $K_{\text{leaf}}$. Breeding crop varieties for both increased safety of the leaf hydraulic system towards drought-induced dysfunction and high gas exchange rates per unit of leaf area is probably a feasible target for future adaptation of crops to climate change scenarios.

**Additional keywords:** coffee, gas exchange, leaf area, Leaf mass per unit area.

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**Introduction**

Ongoing modifications of global and regional temperatures and precipitation patterns are challenging the productivity and even persistence of several crops in different areas of the globe (Bindi and Olesen 2011; Müller et al. 2011). In this regard, coffee (*Coffea arabica* L.) is one of the most threatened crops and a recent study has shown that even under the most conservative climate change scenarios, the extension of bioclimatically favourable localities for the persistence of coffee populations might decrease by 65\% within 2080 (Davis et al. 2012). This finding is particularly alarming, taking into account that coffee is one of the most traded commodities and thus represents an important basis for the economy of several countries.

On the basis of the above, it is not surprising that several recent studies have investigated the morphoanatomical (Pinheiro et al. 2005; Dias et al. 2007; Cavatte et al. 2012), physiological (DaMattia 2004; Kufa and Burkhardt 2011) and biochemical (Marraccini et al. 2012) characteristics of different *C. arabica* and *Coffea canephora* Pierre genotypes, with the aim of identifying key traits underlying differential drought resistance and thus being potentially useful for plant breeding and cultivar selection in the face of progressive temperature increase and precipitation decrease in coffee cultivation areas (DaMattia et al. 2003; Silva et al. 2013). However, only very limited information is available on the hydraulic traits of different coffee species or genotypes, despite the fact that hydraulic capacity and drought vulnerability are now recognised to play fundamental roles in plant adaptation, maintenance of productivity and survival under drought (Sinclair et al. 2008; Brodribb 2009; Gleason et al. 2012; Nardini et al. 2012a). Tausend et al. (2000) reported that stems of *C. arabica* cv. Typica are more resistant to xylem cavitation than those of the cultivars San Ramon and Yellow Caturra. However, with the exception of the study cited above, we are unaware of similar investigations comparing the plant hydraulics of different coffee genotypes. In particular, information is still missing about the leaf hydraulics of drought-resistant versus drought-vulnerable coffee cultivars and their eventual relationship with morphoanatomical leaf traits.

Leaf hydraulic architecture is a key determinant of the productivity of plants as well as of their responses to environmental stress factors (Brodribb et al. 2010). Leaves represent a very large share of plant hydraulic resistance (Sack and Holbrook 2006) and thus limit gas exchange rates and photosynthetic productivity (Brodribb et al. 2005, 2007; Nardini et al. 2005). Moreover, leaves are very vulnerable to drought-induced xylem cavitation and embolism (Salleo et al. 2001; Johnson et al. 2012) thus representing the Achilles’ heel of plant hydraulics and acting, at the same time, as ‘hydraulic fuses’.
triggering stomatal closure upon the establishment of water stress (Johnson et al. 2011), so that transpiration can be promptly reduced by the plant in order to preserve the hydraulic integrity of stems and roots even under intense or prolonged drought (Bucci et al. 2012).

Leaf water transport capacity is generally expressed in terms of leaf hydraulic conductance scaled on a leaf area \((K_{leaf,area} \text{ mmol} s^{-1} \text{ MPA}^{-1} \text{ m}^{-2})\) or dry mass \((K_{leaf, mass} \text{ mmol} s^{-1} \text{ MPA}^{-1} \text{ kg}^{-1})\) basis (Nardini et al. 2012b; Simonin et al. 2012). On the other hand, leaf hydraulic vulnerability is quantified in terms of \(P_{50}\), which is the leaf water potential inducing 50% loss of \(K_{leaf}\) compared with conductance values measured in well hydrated, near-full turgor leaves. At an interspecific level, high gas exchange and photosynthetic rates are generally coupled to high \(K_{leaf}\) (Brodrribb et al. 2005). Similar relationships between gas exchange rates and \(K_{leaf}\) have been also reported at an intraspecific scale (Gortan et al. 2009). On the other hand, drought tolerance is generally associated with very negative \(P_{50}\) values (Nardini et al. 2012a), although different patterns have been sometimes reported (Bucci et al. 2012). Blackman et al. (2012) reported that the climatic limits for the distribution of different species in Tasmanian forests, with special reference to total precipitation, were closely correlated with species-specific \(P_{50}\) values. Even at a local scale, the pattern of distribution of different maple (Acer) and oak (Quercus) species, as correlated with soil water availability, was shown to be in general agreement with species-specific leaf hydraulic vulnerabilities (Nardini et al. 2012a).

Several studies have reported the existence of a trade-off between hydraulic efficiency and vulnerability at the stem level (e.g. Bucci et al. 2006; Barnard et al. 2011), in that species characterised by higher water transport capacity are also generally more vulnerable to xylem cavitation, and vice versa, although this relationship has not always been confirmed across different species’ assemblages (Froux et al. 2002). Highly cavitation-resistant wood has also been shown to display very high mass density values (Jacobsen et al. 2005; Nardini et al. 2013), implying that increased resistance to drought-induced hydraulic dysfunction may come at the cost of reduced water transport rates to the foliage and an increased cost for stem construction, with both factors possibly contributing to reduced productivity of drought-resistant species or cultivars (Tyree et al. 1998). At the leaf level, \(P_{50}\) has been reported to be independent on \(K_{leaf, area}\), but recent reports have highlighted the trade-off between leaf hydraulic vulnerability and \(K_{leaf, mass}\) (Nardini et al. 2012a), indicating that leaves that are very resistant to drought-induced dysfunction are characterised by reduced hydraulic efficiency per unit of carbon invested, thus possibly translating into higher costs for leaf construction and a reduced growth rate. The mechanistic link between leaf hydraulic vulnerability and hydraulic efficiency on a mass basis is still unclear, although it has been suggested that small leaf size, increased vein density, reduced xylem conduit diameter coupled to thicker conduits wall or higher fractions of thick-walled fibres might all contribute to decrease both \(P_{50}\) and \(K_{leaf, mass}\) (Blackman et al. 2010; Scoffoni et al. 2011; Nardini et al. 2012a; Simonin et al. 2012; Sack and Scoffoni 2013).

All the correlations and trade-offs reported above have been demonstrated at an interspecific level. Although some studies have investigated intra-individual patterns of leaf hydraulic capacity according to crown position (Zwieniecki et al. 2004; Sellin et al. 2011; Öunapuu and Sellin 2013), no information is available about the covariation of \(P_{50}\) and \(K_{leaf}\) at an intraspecific level; for example, within an individual (sun v. shade leaves) or across cultivars of economically important crops. Commercial coffee genotypes display considerable variation in leaf size (Tausend et al. 2000), thus suggesting that significant differences might exist among cultivars in terms of leaf hydraulic capacity and vulnerability.

In the present study, wild-type coffee plants are compared with three cultivated genotypes in order to test the following hypotheses: (1) leaf \(P_{50}\) is more negative in small sized leaves with higher vein densities, implying higher leaf mass per unit of area (LMA); (2) increased resistance to hydraulic dysfunction translates into lower leaf hydraulic efficiency, thus limiting gas exchange rates; (3) leaf hydraulic vulnerability is coordinated to symplastic drought tolerance, as previously reported in interspecific comparisons (Blackman et al. 2010; Vilagrosa et al. 2010).

Materials and methods

Plant material

Experimental plants were part of a large collection of Coffea arabica genotypes and cultivars maintained in a dedicated greenhouse at the Department of Life Sciences, University of Trieste, Italy. Measurements were performed between May and July 2012. During measurements, day–night temperatures oscillated between 27°C and 18°C, and the corresponding relative humidity ranged between 45% and 65%. Midday PPFD values averaged 450 μmol m⁻² s⁻¹.

All plants were >7 years old and were growing in 15-L pots filled with a mixture of soil, sand and compost. Plants were irrigated daily to field capacity, with the exception of the time interval necessary to complete the drought experiment described below. Measurements were performed on four genotypes (five plants per genotype): wild-type (WT, from the Yirgacheffe region, Ethiopia), Bourbon (BO, from Patrocino, Brazil), Maragogype (MA, a somaclone of Catuai yellow from Patrocino, Brazil) and the F2 hybrid Sarchymor × Ethiopica (F2, from Costa Rica).

All measurements were performed on 1-year-old leaves from the outer canopy that had reached full expansion and were fully mature when sampled.

Gas exchange measurements

Stomatal conductance to water vapour (mmol m⁻² s⁻¹) was measured between 1200 and 1300 hours of two selected sunny days. Two leaves per individual for a total of 10 leaves per genotype were measured. Measurements were performed using an SC1 porometer (Decagon Devices, Pullman, WA, USA) with the sensor head clamped in the middle portion of each leaf. The porometer was calibrated daily according to manual specifications. In particular, all calibrations were performed within the greenhouse before measurements started. During gas exchange measurements, PPFD ranged between
420 μmol m⁻² s⁻¹ and 530 μmol m⁻² s⁻¹, air temperature averaged 23.3 ± 0.9°C and the relative humidity was 55.2 ± 2.3%.

Leaf water potential isotherms

Leaf water potential isotherms or pressure–volume (PV) curves (Tyree and Hammel 1972) were measured of one leaf per individual, giving a total of five leaves per genotype. The aim of the experiment was to calculate values of leaf water potential at the turgor loss point ($\Psi_{tg}$) as an indicator of the symplastic drought tolerance of different genotypes. Leaves were sampled early in the morning and their petioles were immersed in distilled water. Leaves were allowed to rehydrate for at least 1 h. Leaves were then wrapped in clingfilm and their initial water potential ($\Psi$) was measured using a pressure chamber (mod. 1505D, PMS Instruments, Albany, OR, USA). Measurements continued only if the initial $\Psi$ was $>-$0.1 MPa, indicating full hydration. Leaves were then immediately weighed on a digital balance and left to dehydrate on the bench before measuring their $\Psi$ and weight again. Measurements were repeated until the relationship between $\Psi$−1 and cumulative water loss became strictly linear, indicating that turgor had been lost and $\Psi$ variations were governed by changes in leaf osmotic potential only.

PV curves were modelled according to Salleo (1983) and $\Psi_{tp}$ was calculated. At the end of the experiments, leaf surface area ($A_{leaf}$) was measured using a leaf area meter (model 3000A, Li-Cor Inc., Lincoln, NE, USA). Finally, the leaves were oven-dried at 70°C for 24 h in order to get their dry mass (DM). PV curves were also used to calculate leaf capacitance ($C_{leaf}$) values as the ratio between leaf water content changes ($\Delta W$) over the corresponding leaf water potential variation ($\Delta \Psi$) i.e. $C_{leaf} = \Delta W / \Delta \Psi$. $C_{leaf}$ was normalised either by $A_{leaf}$ or DM thus obtaining $C_{leaf}$ on an area basis (mmol m⁻² MPa⁻¹) and $C_{leaf}$ on a mass basis (mmol g⁻¹ MPa⁻¹), respectively.

Measurements of $K_{leaf}$ and vulnerability

Leaf hydraulic conductance and vulnerability to drought stress were assessed using the hydraulic rehydron kinetic technique (Nardini et al. 2012b). In particular, $K_{leaf}$ was estimated as based on rehydration time ($t$), initial and final leaf water potential ($\Psi_0$ and $\Psi_f$, respectively), and $C_{leaf}$ (Blackman and Brodribb 2011) as shown in Eqn 1:

$$K_{leaf} = C_{leaf} \times \ln (\Psi_0 / \Psi_f)^{-1} t^{-1}. \quad (1)$$

Terminal twigs bearing one or two leaf couples (coffee has opposite leaves) were sampled from plants between 1200 and 1300 hours and immediately enclosed in a black plastic bag with a piece of wet filter paper inside. This procedure allowed us to stop transpiration and favour the equilibration of $\Psi$ values across all leaves. Preliminary experiments showed that full equilibration was reached within 15 min. After this interval, one leaf was detached and used to measure $\Psi_0$. A second leaf was detached with the petiole immersed in deionised water and left to rehydrate for 30–90 s before measuring $\Psi_f$. $K_{leaf}$ was finally calculated on the basis of Eqn 1, using $C_{leaf}$ either on an area or a mass basis in order to obtain values of $K_{leaf, area}$ (mmol s⁻¹ m⁻² MPa⁻¹) or $K_{leaf, mass}$ (mmol s⁻¹ kg⁻¹ MPa⁻¹), respectively. $K_{leaf}$ was first measured in well irrigated plants and maximum values of leaf hydraulic capacity were calculated as an average of the values obtained in these plants. Next, irrigation was suspended and measurements were repeated at the same time over the next 5–8 days, in order to assess relationships between $K_{leaf}$ and progressively declining $\Psi_0$. All leaves used for hydraulic measurements were measured for their $A_{leaf}$ and DM (see above).

Morphoanatomical measurements

LMA was calculated as DM $A_{leaf}^{-1}$ using all data gathered during the PV and hydraulic experiments (see above). Moreover, leaf vein traits were measured in 10 leaves (two per plant) for each genotype. Images of fresh leaves were first acquired using a scanner. Next, samples of leaf lamina (10 × 10 mm) were cut from each leaf. Samples were maintained in 1 M KOH for 5–7 days, repeatedly washed with deionised water, immersed in 0.5% toluidine blue for 1 min and then washed again in deionised water. Leaf samples were observed under a light microscope (at 4x and 10×; Laborlux D, Leitz GmbH, Stuttgart, Germany) equipped with a digital camera (Leica DC–300F; Leica Camera, AG, Solns, Germany). Scanned or microscope leaf images were acquired and analysed using the software Imagej (http://rsweb.nih.gov/ij/index.html, accessed 14 April 2014) in order to measure both major (first and second order) and minor (third order or higher) vein densities, expressed as vein length per unit of surface area (mm mm⁻¹).

A subset of five leaves per genotype (one leaf per individual) was used to measure leaf thickness ($T_{leaf}$) and mass density ($p_{leaf}$). Leaves were sampled the evening before measurements and fully rehydrated overnight. Leaf fresh volume ($V_{leaf}$) was measured using a water displacement method (Hughes 2005) and $A_{leaf}$ was measured as described above. Samples were oven-dried for 24 h at 70°C and their DM was recorded. Leaf mass density was calculated as $p_{leaf} = DM / V_{leaf} A_{leaf}^{-1}$, whereas the average leaf thickness was calculated as $T_{leaf} = V_{leaf} A_{leaf}^{-1}$.

Anatomical features of the midrib xylem were measured from transverse sections taken from the middle portion of leaves (five leaves per genotype, one section per leaf). Midrib xylem conduit and fibre features were measured as a proxy for the xylem traits of higher-order veins. The density of midrib xylem conduits (De) was calculated by counting all conduits for each midrib and dividing this number by the area of the midrib xylem. Conduit lumen diameter ($D_c$) and wall thickness were measured of at least 10 randomly selected midrib conduits per section. Similar measurements of lumen diameter and wall thickness were also performed for fibres.

Statistics

Statistical analysis was performed using the software packages SigmaStat ver. 2.0 (Systat Software Inc., San Jose, CA, USA) and SPSS ver. 20.0 (IBM Corp., NY, USA). The significance of differences among genotypes was tested using one-way-ANOVA followed by Tukey’s post hoc comparisons. The significance of correlations was tested using the Pearson product–moment coefficient. Regressions or differences were considered to be highly significant if $P \leq 0.05$. Regressions with $P$-values between 0.05 and 0.2 were considered as marginally significant. In order to test for statistically significant differences among genotypes in terms of $K_{leaf}$ response to declining $\Psi_{leaf}$, an ANCOVA with no
interaction was run, followed by comparisons based on Sidak’s correction.

Results

The four coffee genotypes investigated displayed several significant differences in their morphoanatomical and physiological traits, with special reference to leaf hydraulic capacity and vulnerability. $K_{\text{leaf,area}}$ was highest in WT and BO ($4.6 \text{ mmol s}^{-1} \text{ MPa}^{-1} \text{ m}^{-2}$ and $4.9 \text{ mmol s}^{-1} \text{ MPa}^{-1} \text{ m}^{-2}$, respectively) and significantly lower in both MA and F2 ($3.3 \text{ mmol s}^{-1} \text{ MPa}^{-1} \text{ m}^{-2}$ and $3.2 \text{ mmol s}^{-1} \text{ MPa}^{-1} \text{ m}^{-2}$, respectively; Table 1). Similar and even larger differences among the genotypes were recorded in terms of $K_{\text{leaf, mass}}$, with statistically similar values recorded for WT, MA and F2 ($\sim 48$–$60 \text{ mmol s}^{-1} \text{ MPa}^{-1} \text{ kg}^{-1}$) and $\sim 2$-fold higher hydraulic efficiency per unit of dry weight in BO (an average value of $107 \text{ mmol s}^{-1} \text{ MPa}^{-1} \text{ kg}^{-1}$; Table 1).

Table 1. Leaf hydraulic conductance on a leaf surface ($K_{\text{leaf, area}}$) or dry mass ($K_{\text{leaf, mass}}$) basis recorded in irrigated plants, maximum stomatal conductance to water vapour ($\delta_{\text{L}}$), leaf water potential at the turgor loss point ($\Psi_{\text{tlp}}$) and leaf water potential inducing 50% loss of $K_{\text{leaf, area}}$ ($\Psi_{\text{P50}}$) as recorded in four genotypes of Coffea arabica. Means are reported $\pm$ s.d. Different letters indicate statistically significant differences. *, $P < 0.05$; **, $P < 0.01$

<table>
<thead>
<tr>
<th>Genotype</th>
<th>$K_{\text{leaf, area}}$ (mmol s$^{-1}$ MPa$^{-1}$ m$^{-2}$)*</th>
<th>$K_{\text{leaf, mass}}$ (mmol s$^{-1}$ MPa$^{-1}$ kg$^{-1}$)**</th>
<th>$\delta_{\text{L}}$ (mmol m$^{-2}$ s$^{-1}$)*</th>
<th>$\Psi_{\text{tlp}}$ (MPa)*</th>
<th>$\Psi_{\text{P50}}$ (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>$4.6 \pm 1.4$ a</td>
<td>$60.6 \pm 21.3$ a</td>
<td>$228 \pm 49$ a</td>
<td>$-1.49 \pm 0.06$ a</td>
<td>$-1.01$</td>
</tr>
<tr>
<td>Bourbon</td>
<td>$4.9 \pm 1.2$ a</td>
<td>$106.8 \pm 29.3$ b</td>
<td>$298 \pm 74$ b</td>
<td>$-1.71 \pm 0.06$ b</td>
<td>$-1.21$</td>
</tr>
<tr>
<td>Maragogype</td>
<td>$3.3 \pm 0.3$ b</td>
<td>$59.3 \pm 10.2$ a</td>
<td>$145 \pm 41$ c</td>
<td>$-1.39 \pm 0.15$ a</td>
<td>$-0.66$</td>
</tr>
<tr>
<td>F2 hybrid</td>
<td>$3.2 \pm 0.8$ b</td>
<td>$47.8 \pm 8.2$ a</td>
<td>$138 \pm 37$ c</td>
<td>$-0.77 \pm 0.14$ c</td>
<td>$-0.61$</td>
</tr>
</tbody>
</table>

$K_{\text{leaf,area}}$ decreased upon leaf dehydration and was linearly correlated with corresponding $\Psi_{\text{leaf}}$ values in all genotypes (Fig. 1). However, both the maximum $K_{\text{leaf,area}}$ values (see above) and the slopes of the $K_{\text{leaf,area}}$ to $\Psi_{\text{leaf}}$ relationships differed significantly across genotypes, leading to genotype-specific $\Psi_{\text{leaf}}$ values inducing a 50% loss of $K_{\text{leaf,area}}$ ($\Psi_{\text{P50}}$). In fact, the ANCOVA test revealed significant differences ($P < 0.0001$) among genotypes both in terms of the $y$-intercept and in terms of slope, with the exception of the comparison between the slopes of MA and F2, which were not found to be statistically different from each other. On this basis, $\Psi_{\text{P50}}$ was found to be lowest in BO ($-1.2$ MPa) and WT ($-1.0$ MPa) but higher in

![Fig. 1](https://via.placeholder.com/150)

**Fig. 1.** Relationships between leaf hydraulic conductance ($K_{\text{leaf,area}}$) and water potential ($\Psi_{\text{leaf}}$) as measured in four genotypes of Coffea arabica: (a) wild-type; (b) Bourbon; (c) Maragogype; (d) Sarchymor × Ethiopica F2 hybrid. Each point represents a different leaf. The regression line is reported together with $r$ and $P$-values (Pearson’s product–moment correlation).
both MA and F2 (about −0.6 MPa), indicating greater leaf hydraulic vulnerability in these latter cultivars than in the former ones (Table 1).

Stomatal conductance to water vapour, as measured in well watered plants, ranged from 138 mmol s⁻¹ m⁻² to 298 mmol s⁻¹ m⁻² in F2 and BO, respectively. Intermediate values were recorded in the other cultivars. The high stomatal conductance to water vapour values recorded in BO were significantly different from those measured in WT and in both MA and F2, whereas differences between these last two cultivars were not significant (Table 1). PV analysis revealed that BO had the lowest value of $\Psi_{WP}$ (−1.7 MPa), whereas significantly higher (less negative) turgor loss points were measured for WT and MA (−1.5 MPa and −1.4 MPa, respectively) and especially for F2 (−0.8 MPa) (Table 1).

Besides physiological differences, the four genotypes also displayed significant differences with respect to several morphoanatomical traits. $A_{leaf}$ was significantly higher in MA and F2 than in WT and BO, with this latter cultivar displaying the lowest $A_{leaf}$ (Table 2). Similarly, the lowest value of LMA was found in BO and the highest in F2, with intermediate values recorded for the other two genotypes (Table 2). Average $T_{leaf}$ ranged between 0.21 mm in BO and 0.26 mm in F2, this difference being statistically significant, whereas no statistically significant difference was found with respect to $\rho_{leaf}$, which averaged ~0.20 g cm⁻² in all four genotypes.

Minor vein densities ($D_{vein}$) ranged between ~5.6 mm mm⁻² and 7.2 mm mm⁻², as recorded in F2 and BO respectively, with significant differences among genotypes. Similar significant differences were observed for major vein density ($D_{vein}$), which averaged 2 mm mm⁻² in BO but was significantly lower (0.7–1.1 mm mm⁻²) in the other cultivars (Table 2). Midrib $D_{vein}$ were significantly lower in BO (11 μm) than in the other three genotypes, where $D_{vein}$ averaged 14 μm. Similar differences among genotypes were observed in terms of fibre lumen diameter and xylem conduit wall thickness (Table 3). Finally, midrib xylem conduit density ($D_{cell}$) was significantly higher in WT and BO (~2500 conduits mm⁻²) than in MA and F2 (~1900 and 1600 conduits mm⁻², respectively).

Significant correlations were observed among physiological traits as well as between these traits and leaf morphoanatomical features. Stomatal conductance to water vapour was positively correlated with both $A_{leaf}$ and $D_{vein}$ (Fig. 2); these relationships are likely to be driven by the parallel, although marginal, correlation between $K_{leaf}$ and $D_{vein}$ ($r^2 = 0.66$, $P = 0.18$). $K_{leaf}$ was also correlated with both $D_{vein}$ and $D_{vein}$ ($r^2 = 0.92$, $P = 0.04$) and $D_{vein}$ ($r^2 = 0.90$, $P = 0.05$). In turn, $D_{vein}$ was inversely correlated with $A_{leaf}$ (Fig. 3), and $LMA$ ($r^2 = 0.88$, $P = 0.04$), whereas a positive correlation was observed between $A_{leaf}$ and $LMA$ ($r^2 = 0.94$, $P = 0.03$). A negative correlation was also observed between $LMA$ and $D_{vein}$ ($r^2 = 0.88$, $P = 0.06$), but $T_{leaf}$ showed a positive correlation with $LMA$ ($r^2 = 0.86$, $P = 0.06$) and $A_{leaf}$ ($r^2 = 0.96$, $P = 0.02$). As a consequence of these relationships, inverse correlations were observed between $A_{leaf}$ and both $K_{leaf}$ and $D_{vein}$ ($r^2 = 0.83$, $P = 0.08$) and $K_{leaf}$ and $D_{vein}$ ($r^2 = 0.92$, $P = 0.04$). Both $K_{leaf}$ and $D_{vein}$ were inversely correlated with $LMA$ (Fig. 4), although only the latter relationship was statistically significant.

$P_{50}$ was positively correlated with $A_{leaf}$ but inversely correlated with $D_{vein}$ (Fig. 3). Moreover, $P_{50}$ was weakly correlated with another physiological trait related to drought tolerance, namely $\Psi_{WP}$ ($r^2 = 0.66$, $P = 0.19$). Inverse relationships were observed between $P_{50}$ and both $K_{leaf}$ and $K_{leaf}$ (Fig. 4), indicating that drought-resistant genotypes (with a more negative $P_{50}$) also displayed relatively higher leaf hydraulic capacity both on a leaf area and a DM basis. Interestingly, $P_{50}$ showed a positive correlation with $LMA$ ($r^2 = 0.79$, $P = 0.11$), suggesting that lower $P_{50}$ was associated with lower LMA.

### Table 2. Leaf surface area ($A_{leaf}$), leaf mass per unit surface area (LMA), density of major ($D_{major}$) and minor ($D_{minor}$) veins, leaf thickness ($T_{leaf}$) and leaf mass density ($\rho_{leaf}$) as recorded in four genotypes of *Coffee arabica*

<table>
<thead>
<tr>
<th>Genotype</th>
<th>$A_{leaf}$ (cm²)*</th>
<th>LMA (g m⁻²)*</th>
<th>$D_{minor}$ (mm²)*</th>
<th>$D_{major}$ (mm²)*</th>
<th>$T_{leaf}$ (mm)*</th>
<th>$\rho_{leaf}$ (g cm⁻³)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>52 ± 10 a</td>
<td>54.9 ± 7.0 a</td>
<td>5.7 ± 0.4 a</td>
<td>1.06 ± 0.06 a</td>
<td>0.23 ± 0.01 a</td>
<td>0.21 ± 0.02</td>
</tr>
<tr>
<td>Bourbon</td>
<td>17 ± 6 b</td>
<td>44.8 ± 4.6 b</td>
<td>7.2 ± 1.1 b</td>
<td>2.01 ± 0.27 b</td>
<td>0.21 ± 0.01 b</td>
<td>0.23 ± 0.02</td>
</tr>
<tr>
<td>Maragogype</td>
<td>72 ± 24 c</td>
<td>56.0 ± 4.9 a</td>
<td>6.3 ± 1.1 ab</td>
<td>0.74 ± 0.02 a</td>
<td>0.25 ± 0.02 c</td>
<td>0.22 ± 0.03</td>
</tr>
<tr>
<td>F2 hybrid</td>
<td>79 ± 16 c</td>
<td>59.2 ± 6.5 c</td>
<td>5.6 ± 0.5 a</td>
<td>0.87 ± 0.10 a</td>
<td>0.26 ± 0.02 c</td>
<td>0.21 ± 0.04</td>
</tr>
</tbody>
</table>

### Table 3. Midrib xylem conduit diameter ($D_{vein}$), wall thickness ($T_{vein}$) and density ($D_{cell}$), as well as fibre lumen diameter ($D_{fibre}$) and wall thickness ($T_{fibre}$), as recorded in leaves of four genotypes of *Coffee arabica*

<table>
<thead>
<tr>
<th>Genotype</th>
<th>$D_{vein}$ (μm)*</th>
<th>$T_{vein}$ (μm)*</th>
<th>$D_{cell}$ (mm²)*</th>
<th>$D_{fibre}$ (μm)*</th>
<th>$T_{fibre}$ (μm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>13.4 ± 2.4 a</td>
<td>2.0 ± 0.1 a</td>
<td>2547 ± 631 a</td>
<td>5.8 ± 0.3 a</td>
<td>2.8 ± 0.2 a</td>
</tr>
<tr>
<td>Bourbon</td>
<td>10.9 ± 1.1 b</td>
<td>1.7 ± 0.2 b</td>
<td>2664 ± 485 a</td>
<td>4.8 ± 0.3 b</td>
<td>2.9 ± 0.1 a</td>
</tr>
<tr>
<td>Maragogype</td>
<td>14.3 ± 0.6 a</td>
<td>2.2 ± 0.2 a</td>
<td>1973 ± 162 b</td>
<td>6.1 ± 0.4 a</td>
<td>3.1 ± 0.3 a</td>
</tr>
<tr>
<td>F2 hybrid</td>
<td>15.3 ± 0.4 a</td>
<td>2.4 ± 0.2 a</td>
<td>1617 ± 102 b</td>
<td>6.0 ± 0.2 a</td>
<td>3.5 ± 0.3 b</td>
</tr>
</tbody>
</table>
values. Moreover, $P_{50}$ was inversely correlated with $D_{ec}$ ($r^2 = 0.92$, $P = 0.04$) and $D_{ec}$ was positively correlated with $K_{leaf\_area}$ ($r^2 = 0.94$, $P = 0.03$).

**Discussion**

Our data revealed significant variability in both $K_{leaf}$ and drought vulnerability across different coffee genotypes. Although some of the relationships among functional and structural traits unravelled by this study are in agreement with previous interspecific comparisons, at the narrower intraspecific scale of our analysis, some of the previously reported trade-offs between the efficiency and safety of leaf hydraulic systems apparently do not hold or are even reversed.

$K_{leaf\_area}$ basis ranged between $3 \text{ mmol s}^{-1} \text{ MPa}^{-1} \text{ m}^{-2}$ and $\sim 5 \text{ mmol s}^{-1} \text{ MPa}^{-1} \text{ m}^{-2}$, which is in agreement with previous estimates of $K_{leaf\_area}$ in *C. arabica* as based on a high-pressure method (Gascó *et al.* 2004). Interestingly, the values of $K_{leaf\_area}$ recorded for coffee are in the lowest range reported for woody plants (Sack and Holbrook 2006) and are close to those typically recorded in shade-adapted species (Nardini *et al.* 2005; Sack *et al.* 2005), which is in agreement with the native habitat of coffee (Meyer 1965). Nonetheless, significant variation in $K_{leaf\_area}$ and $K_{leaf\_mass}$ was found across genotypes, with BO displaying the highest values for both parameters.

The relatively high hydraulic capacity of BO leaves was consistent with the higher $D_{major\_vein}$ and $D_{minor\_vein}$ (Table 2) recorded in this cultivar with respect to the other genotypes. $D_{major\_vein}$ and $D_{minor\_vein}$ have been suggested to be key traits influencing leaf hydraulics (Nardini and Salleo 2003; Sommerville *et al.* 2012), as reduced vein spacing would significantly shorten the length of the high-resistance pathway from minor vein endings to stomata (Brodribb *et al.* 2007). In turn, increasing $K_{leaf\_area}$ would allow plants to sustain...
higher gas exchange rates, as water vapour lost during transpiration would be more efficiently replaced by liquid water transported through the leaf. Ultimately, high $K_{\text{leaf}}$ and vein densities would contribute to the achievement of high photosynthetic rates, thus favouring plant growth and competitiveness (Brodribb et al. 2005). Our findings confirm these views at an intraspecific scale, as stomatal conductance to water vapour was clearly linked to both $K_{\text{leaf}}$ and $D_{\text{major vein}}$ (Fig. 2), thus suggesting that vein length per unit of leaf area might be a preliminary proxy for estimating $K_{\text{leaf}}$ across coffee genotypes. Moreover, significant correlations emerged between $K_{\text{leaf}}$ and both $D_{\text{major vein}}$ and $D_{\text{minor vein}}$, suggesting that in some coffee genotypes, the development of an extensive vein network was coupled to a decrease of the carbon costs associated with the achievement of sufficient hydraulic efficiency.

In all the genotypes tested, $K_{\text{leaf}}$ sharply decreased upon leaf dehydration, as a likely consequence of embolism development in vein xylem conduits, which has been shown to be among the main mechanisms leading to drought-induced $K_{\text{leaf}}$ decline (Nardini et al. 2003; Johnson et al. 2012). Values of $P_{50}$ ranged between −0.6 MPa and −1.2 MPa, the lowest value being recorded in BO. Previously reported $P_{50}$ values for different woody angiosperms ranged between −0.4 MPa (in Magnolia grandiflora L., Scoffoni et al. 2011) and approximately −5 MPa (in Arbutus menziesii Pursh, Johnson et al. 2009). Hence, C. arabica appears as to be relatively drought-vulnerable species, in accordance with its original habitat and distribution area. Nonetheless, our data demonstrate large and significant intraspecific variability in $P_{50}$ across coffee genotypes. The variation of $\Psi_{\text{lp}}$ values across the cultivars was consistent with the $P_{50}$ trends, confirming the existence of a general coordination of functional traits conferring both apoplastic and symplastic drought resistance (Blackman et al. 2010; Vilagrosa et al. 2010), and also suggesting that turgor loss might also affect $K_{\text{leaf}}$ during dehydration, as suggested recently by Scoffoni et al. (2014).

Taking into account that values of minimum daily water potential for well irrigated field-grown C. arabica plants range between −0.3 MPa and −1.3 MPa (Dias et al. 2007; Kufa and Burkhard 2011; Cavatte et al. 2012), our data would suggest

![Fig. 4. Relationships between leaf water potential inducing 50% loss of leaf hydraulic capacity ($P_{50}$), leaf hydraulic conductance on a surface area ($K_{\text{leaf}}$) or dry mass ($K_{\text{leaf mass}}$) basis, and leaf mass per unit surface area (LMA) as measured in four genotypes of Coffea arabica. WT, wild-type; BO, Bourbon; MA, Maragogype; F2, Sarchymor × Ethiopica hybrid. (a) Relationship between $P_{50}$ and $K_{\text{leaf}}$; (b) relationship between $P_{50}$ and $K_{\text{leaf mass}}$; (c) relationship between $K_{\text{leaf}}$ and LMA; (d) relationship between $K_{\text{leaf mass}}$ and LMA. Each point represents the mean value of parameters for each genotype. The regression lines are reported together with $r$ and $P$-values (Pearson's product–moment correlation).](image-url)
that coffee leaves commonly operate with very narrow or even negative safety margins with respect to $P_{50}$. This is not surprising, as previous studies have shown that daily dynamic water stress can commonly reduce $K_{leaf}$ by $\sim 80\%$ with respect to its maximum values, and nocturnal recovery of $K_{leaf}$ has also been reported (Johnson et al. 2009). Although the $P_{50}$ values recorded in the present study might be different from those of field-grown plants as a consequence of acclimation processes, our data invite one to speculate that a transient $K_{leaf}$ drop in coffee might represent a signal for midday stomatal closure, as suggested by previous studies in different woody plants (Charra-Vaskou et al. 2012).

Variability in $P_{50}$ and $K_{leaf}$ was apparently mainly associated with leaf size and $D_{major, vein}$ (Fig. 3). Significant correlations between vein length per unit of area and leaf drought vulnerability have been already demonstrated at an interspecific level by Scoffoni et al. (2011) and by Nardini et al. (2012a). Such a relationship would be explained by the fact that vascular redundancy based on increased $D_{major, vein}$ confers greater tolerance of leaf hydraulic damage caused by cavitation-induced embolism of vein conduits (Sack et al. 2008). The relationship between leaf size and drought vulnerability observed in this study is in agreement with previous reports, where drought tolerance was found to be higher for $C. canephora$ clones displaying smaller leaves (DaMatta et al. 2003). Interestingly, one of the reported effects of drought stress on drought-sensitive clones was leaf fall preceded by apparent leaf damage (DaMatta et al. 2003), an observation that would be consistent with drought-induced hydraulic impairment of foliage.

In the present as well as in some previous studies, increased $D_{major, vein}$ apparently resulted from leaf size reduction (Edwards 2006; Dunbar-Co et al. 2009), in accordance with a general developmental model of leaf vein establishment that has found support both at inter- and intraspecific scales (Sack et al. 2012), although some studies reported positive correlations among leaf size, vein density and $K_{leaf}$ (Price et al. 2012; Sellin et al. 2012). Other anatomical traits were apparently not or only weakly correlated with both the efficiency and safety of the leaf hydraulic system, with the exception of $D_{ec}$, which partially accounted for the trends in $P_{50}$ and $K_{leaf, area}$. Hence, increased hydraulic capacity and safety towards leaf dehydration were driven by a higher number of veins per unit area as well as by an increased number of xylem conduits per vein, indicating that redundancy of the vascular water transport pathway is a key trait driving variation in leaf hydraulic systems at an intraspecific level.

Previous leaf-level interspecific comparisons reported negative correlations between $K_{leaf, mass}$ and LMA (Simonin et al. 2012) as well as negative correlations between $P_{50}$ and LMA (Nardini et al. 2012a) (i.e. species with higher LMA were characterised by lower $P_{50}$ (higher drought resistance) and $K_{leaf, mass}$ (low hydraulic efficiency per carbon cost)). These findings have suggested that increased hydraulic safety implies an increased carbon cost for construction of a unit of leaf surface area, translating into reduced hydraulic efficiency. It might be hypothesised that these trends may arise as a consequence of the mix of morphoanatomical traits correlated with leaf hydraulic safety, specifically increased vein length per unit of area, a higher number of conduits per vein and increased thickness of xylem conduit and fibre walls (Jordan et al. 2013); all these features imply substantial carbon investment for vein and cell wall construction.

In the present study, however, only the negative relationship between $K_{leaf}$ and LMA was confirmed (Fig. 4), whereas $P_{50}$ was found to be positively correlated with LMA (see Results). Hence, although reduced $A_{leaf}$ and increased $D_{major, vein}$ across coffee genotypes are associated with a shift of $P_{50}$ towards more negative values, this does not translate into higher LMA, leading to functional links among smaller leaf area, lower LMA and lower $P_{50}$. This also suggests that high LMA (and consequently lower $K_{leaf, mass}$) in large-leaved coffee genotypes arises from leaf traits other than leaf venation. A positive correlation was found between LMA and $A_{leaf}$, as well as between $A_{leaf}$ and $T_{leaf}$, whereas $\rho_{leaf}$ was invariant across genotypes. Overall, these data are in agreement with previously reported positive correlations between leaf surface area and LMA (Milla and Reich 2007), which are likely to be associated with the increased costs of improved light interception guaranteed by a larger leaf surface area. Our data further suggest that across closely linked genotypes, the increase of LMA is driven by increased leaf thickness at an invariant leaf mass density.

The relationships discussed above are in overall accordance with the ‘venation theory’ (Blonder et al. 2011; Blonder et al. 2013), recently proposed to explain the physiological trade-offs that are at the basis of the leaf economics spectrum describing the fundamental correlations among traits controlling the light, carbon, nitrogen and water use in vascular plants (Wright et al. 2004). According to this theory, higher vein length per unit of surface area should lead simultaneously to lower $A_{leaf}$, lower LMA (because of decreased leaf thickness), and higher $K_{leaf}$ and gas exchange rates, assuming leaf mass density to be invariant. This latter assumption does not hold true for interspecific comparisons, where leaf density can vary by over threefold (Niinemets 2001; Méndez-Alonzo et al. 2013), leading to correlations just opposite to those predicted by the venation theory. In the case of the four coffee genotypes investigated, however, $\rho_{leaf}$ was constant across genotypes. This basic assumption of the ‘venation theory’ being satisfied, covariation of leaf vein density, size, thickness and LMA were in agreement with the proposed model.

A surprising result of our study is the negative relationship between $P_{50}$ and leaf hydraulic capacity expressed both in terms of $K_{leaf, area}$ and $K_{leaf, mass}$ (Fig. 4). This finding contrasts with one of our starting hypotheses and clearly shows that previously reported trade-offs among hydraulic efficiency, safety and construction costs of plant organs might not hold at an intraspecific level. On the contrary, an unusual pattern towards both higher hydraulic capacity and safety was observed for the cultivar BO. A similar lack of trade-off between hydraulic efficiency and safety at the stem level has been previously reported by Fichot et al. (2010) in a comparison across eight unrelated $Populus deltoides$ Bartr. ex Marsh × $Populus nigra$ L. hybrids.

Overall, our findings are promising in that they suggest that breeding crop varieties for both increased safety of the leaf hydraulic system towards drought-induced hydraulic dysfunction and high gas exchange rates per unit of leaf area...
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