RESEARCH PAPER

Leaf hydraulic conductance is coordinated with leaf morpho-anatomical traits and nitrogen status in the genus Oryza

Dongliang Xiong, Tingting Yu, Tong Zhang, Yong Li, Shaobing Peng and Jianliang Huang*

National Key Laboratory of Crop Genetic Improvement, MOA Key Laboratory of Crop Ecophysiology and Farming System in the Middle Reaches of the Yangtze River, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, Hubei 430070, China

* To whom correspondence should be addressed. E-mail: jhuang@mail.hzau.edu.cn

Received 10 March 2014; Revised 3 September 2014; Accepted 17 September 2014

Abstract

Leaf hydraulic conductance ($K_{\text{leaf}}$) is a major determinant of photosynthetic rate in plants. Previous work has assessed the relationships between leaf morpho-anatomical traits and $K_{\text{leaf}}$ with woody species, but there has been very little focus on cereal crops. The genus Oryza, which includes rice (Oryza sativa) and wild species (such as O. rufipogon cv. Griff), is ideal material for identifying leaf features associated with $K_{\text{leaf}}$ and gas exchange. Leaf morpho-anatomical traits, $K_{\text{leaf}}$, leaf N content per leaf area, and CO$_2$ diffusion efficiency were investigated in 11 Oryza cultivars. $K_{\text{leaf}}$ was positively correlated with leaf thickness and related traits, and therefore positively correlated with leaf mass per area and leaf N content per leaf area, and negatively with inter-veinal distance. $K_{\text{leaf}}$ was also positively correlated with leaf area and its related traits, and therefore negatively correlated with the proportion of minor vein length per area. In addition, coordination between $K_{\text{leaf}}$ and CO$_2$ diffusion conductance in leaves was observed. We conclude that leaf morpho-anatomical traits and N content per leaf area strongly influence $K_{\text{leaf}}$. Our results suggest that more detailed anatomical and structural studies are needed to elucidate the impacts of leaf feature traits on $K_{\text{leaf}}$ and gas exchange in grasses.

Key words: CO$_2$ diffusion conductance, leaf anatomy, leaf hydraulic conductance, leaf N content per leaf area, photosynthesis, rice.

Introduction

Leaf hydraulics is the major bottleneck of the overall plant hydraulic system, and therefore the fundamental factor restricting gas exchange and biomass production (Sack et al., 2003; Sack and Holbrook, 2006). The efficiency of water transport through the leaf to the evaporating surface of the mesophyll is quantified by leaf hydraulic conductance ($K_{\text{leaf}}$), which is generally expressed on a leaf area base (Sack and Holbrook, 2006). Decreases in $K_{\text{leaf}}$ usually cause leaves to become less hydrated (corresponding to a low leaf water potential), a response often associated with stomatal closure and, consequently, reduced CO$_2$ assimilation (Sperry, 2000; Johnson et al., 2009). This reduction occurs partly because CO$_2$ and water exchange between leaves and air share a common pathway through stomatal pores. The coupling of stomatal conductance ($g_s$) to CO$_2$ and water vapour leads to strong coordination between $g_s$ and $K_{\text{leaf}}$ (Sack et al., 2003; Brodribb et al., 2005; Sack and Holbrook, 2006). Furthermore, owing to the tight coupling between $g_s$ and photosynthetic rate ($A$) in C$_3$ species (Wong et al., 1979), a positive relationship between $K_{\text{leaf}}$ and $A$ is reported (Brodribb et al., 2005; Franks, 2006; Brodribb et al., 2007; Flexas et al., 2013b).

Previous studies have found that $K_{\text{leaf}}$ varies greatly between species, ranging 65-fold from the lowest to highest value (Sack and Holbrook, 2006). Interspecific variation in $K_{\text{leaf}}$ reflects differences in the morpho-anatomy of leaves, as well as pathways through the outside xylem to evaporation sites. In plants, leaf vein systems, as distinct water transport systems, vary greatly in arrangement, density, vascular bundle features, and xylem conduits within the bundles (Sack and Scoffoni, 2013). In the past two decades, increasing numbers of studies have focused on the relationship between $K_{\text{leaf}}$ and venation architecture, expressed as vein length per
area (VLA). Positive and negative relationships between 
\( K_{\text{leaf}} \) and VLA have been reported (Nardini 
\textit{et al.}, 2012; Sack and Scoffoni, 2012), although no relationship was found in another study (Flexas 
\textit{et al.}, 2013b). Most of these studies, however, were conducted with woody species, and very few focused on cereal crops such as rice.

Outside the xylem, there are three main pathways for water flow: apoplastic, symplastic, and transcellular (Sack and Holbrook, 2006). Several early studies suggested that water exits the xylem mainly through the apoplastic pathway, because of high resistance in the symplastic and transcellular pathways. However, many recent studies have shown that aquaporins have a positive effect on water transport across the membranes of bundle sheath and mesophyll cells (Martre 
\textit{et al.}, 2002; Sack 
\textit{et al.}, 2004). Dye and cell pressure probe experiments also suggest that the symplastic and transcellular pathways play a vital role in water transport in plants (Murphy and Smith, 1998; North 
\textit{et al.}, 2013). Furthermore, the distance that water travels from veins to stomata (\( D_v \)), which is usually expressed as the distance between veins and stomata (\( D_s \)) in the leaf cross section (Brodribb 
\textit{et al.}, 2007; North 
\textit{et al.}, 2013), has been suggested to be an important trait affecting \( K_{\text{leaf}} \). Although water movement in mesophyll tissues is now widely recognized, how leaf mesophyll architecture contributes to water flux in the mesophyll and water evaporation at the cell wall surface remains unclear (Sack and Holbrook, 2006; Flexas 
\textit{et al.}, 2013b; North 
\textit{et al.}, 2013).

N is a vitally important element for plants, and it profoundly influences leaf anatomical and functional traits (Rademacher and Nelson, 2001; Lee 
\textit{et al.}, 2011). Previous studies have shown that leaf N promotes \( A \) by increasing Rubisco content and \( CO_2 \) diffusion conductance (Imai 
\textit{et al.}, 2008; Franks 
\textit{et al.}, 2009). However, the correlation of leaf N content per leaf area with \( K_{\text{leaf}} \) remains to be investigated. Studying the interactions between leaf N status and \( K_{\text{leaf}} \) may help determine the effects of N on rice leaf morpho-anatomical traits associated with \( K_{\text{leaf}} \) and \( CO_2 \) movement in leaves.

\textit{Oryza} spp. are distributed worldwide, and they exhibit a wide range of phenotypes. This diversity is an important resource (Giuliani 
\textit{et al.}, 2013) that is being utilized to improve rice yield and other agronomic traits, particularly in unfavourable environments. In the present study, four cultivated and seven wild cultivars in the genus \textit{Oryza} were investigated with the aims of: (i) identifying the variation in leaf morpho-anatomical traits and \( K_{\text{leaf}} \), (ii) investigating whether leaf morpho-anatomical traits and leaf N status influence \( K_{\text{leaf}} \), and (iii) determining the relationship between \( K_{\text{leaf}} \) and gas exchange.

**Materials and methods**

\textit{Plant materials}

Four rice (\textit{O. sativa} L.) cultivars and seven wild cultivars in the genus \textit{Oryza} (Table 1; these were provided by the National Key Laboratory of Crop Genetic Improvement) were investigated in a pot experiment at Huazhong Agricultural University, Wuhan, China. Three hills of seedlings were grown in 15.0 l pots filled with 13.0 kg soil. N, P, and K were applied as basal fertilizers at a rate of 3.0 g, 1.95 g and 1.95 g per pot, respectively. There were three pots per cultivar.

| Cultivar | Species | Area (cm²) | Length (cm) | Width (cm) | VLA (mm mm⁻¹) | VLA \text{water} (mm mm⁻¹) | LMA (g m⁻²) | \( K_{\text{leaf}} \) (mmol m⁻² s⁻¹ MPa⁻¹) |
|---------|---------|------------|-------------|------------|--------------|----------------|-------------|---------------------------------------|
| Shanyou 63 | \textit{O. sativa} | 54.2 ± 3.2 | 46.2 ± 3.0 | 1.2 | 3.91 ± 0.2 | 3.02 ± 0.2 | 2.3 | 0.40 ± 0.08 |
| Huanghai26 | \textit{O. sativa} | 31.4 ± 2.1 | 28.0 ± 2.1 | 1.3 | 3.96 ± 0.1 | 3.06 ± 0.1 | 2.3 | 0.40 ± 0.08 |
| Nipponbare | \textit{O. sativa} | 42.7 ± 4.7 | 44.5 ± 4.2 | 1.4 | 5.18 ± 0.3 | 4.13 ± 0.3 | 2.3 | 0.40 ± 0.08 |
| Aus | \textit{O. rufipogon} | 12.3 ± 2.2 | 25.1 ± 2.2 | 1.0 | 4.13 ± 0.3 | 3.00 ± 0.3 | 2.3 | 0.40 ± 0.08 |
| Koshihikari | \textit{O. rufipogon} | 31.4 ± 0.7 | 41.0 ± 0.7 | 1.5 | 3.96 ± 0.1 | 3.06 ± 0.1 | 2.3 | 0.40 ± 0.08 |
| I08 | \textit{O. rufipogon} | 12.3 ± 2.2 | 25.1 ± 2.2 | 1.0 | 4.13 ± 0.3 | 3.00 ± 0.3 | 2.3 | 0.40 ± 0.08 |
| I90 | \textit{O. rufipogon} | 31.4 ± 0.7 | 41.0 ± 0.7 | 1.5 | 3.96 ± 0.1 | 3.06 ± 0.1 | 2.3 | 0.40 ± 0.08 |
| Koshihikari | \textit{O. rufipogon} | 31.4 ± 0.7 | 41.0 ± 0.7 | 1.5 | 3.96 ± 0.1 | 3.06 ± 0.1 | 2.3 | 0.40 ± 0.08 |

\( * \) Values are mean ± SD; **, \( P < 0.001 \).
Throughout their growth, plants were well watered and a water depth of at least 2 cm was maintained. Pests were controlled using chemical pesticides.

Gas exchange measurements
To avoid the effect of fluctuation in outdoor air temperature, light intensity, and humidity on gas exchange measurement, measurement was done between 9.30 and 15.30 in an environmentally controlled room with an air temperature of 27.8 ± 2.1°C, a photosynthetic photon flux density (PPFD) at the leaf surface of 1200 ± 47 μmol m−2 s−1 (artificial light source), and relative humidity of 77.4 ± 5.3%. Measurements were taken on newly and fully expanded leaves of three plants for each cultivar after they were acclimated for ~1.5 h. Gas exchange and chlorophyll fluorescence were simultaneously measured using an LI-6400XT portable photosynthesis system equipped with a leaf chamber (LI-COR, NE, USA). Leaf temperature during measurements was maintained at 28°C. In the leaf chamber, PPFD was maintained at 1500 μmol m−2 s−1, and leaf-to-air vapour pressure deficit at 1.1–1.4 kPa; CO₂ concentration was adjusted to 400 μmol m−2 s−1 with a CO₂ mixture. After equilibration to a steady state, A, gₛ, steady-state fluorescence (Fₛ), and maximum fluorescence (Fₚₛ) were recorded. The actual photochemical efficiency of photosystem II (Φₚₛ) was calculated as follows:

$$\Phi_{\text{PSII}} = \frac{(F_{\text{m}} - F_{\text{s}})}{F_{\text{m}}}$$

Electron transport rate (J) was calculated as follows:

$$J = \Phi_{\text{PSII}} \cdot \text{PPFD} \cdot \alpha \beta$$

where α is the leaf absorptance and β represents the distribution of electrons between PSI and PSII.

Light response curves were determined under low O₂ concentration (<2%) for estimating α and β. The gas exchange system was immediately switched to low O₂ concentration (<2%) without removing the leaves from the chamber. Simultaneous measurements of light response curves and chlorophyll fluorescence were then performed. During the measurements, chamber conditions were the same as those described above, except that PPFD was controlled across a series: 2000, 1200, 800, 400, 250, 200, 150, 100, 50, 20, and 0 μmol m−2 s−1. After reaching a steady state, parameters of gas exchange and chlorophyll fluorescence were simultaneously recorded. The values of αβ and daytime respiration rate (Rₛ) were calculated as the slope and intercept, respectively, of the linear regression of A on PPFD-Φₚₛ/4 (Yim et al., 2009). Our αβ values are consistent with the values estimated from the slope between Φₚₛ and Φₚ₃, with varying light intensity under non-photorespiratory conditions (O₂ < 1%) (Supplementary Figure S1).

The variable J method described in Harley et al. (1992) was used to calculate mesophyll conductance of CO₂ (gₘ) and CO₂ concentration in the chloroplast (Cₛ). Cₛ was calculated as follows:

$$C_S = \frac{\Gamma^* (J + 8(A + R_S))}{J - 4(A + R_S)}$$

where Cₚ represents the intercellular CO₂ concentration.

Leaf hydraulic conductance
Kₜₕₙ was measured using the evaporative flux method (Sack et al., 2002; Brodribb et al., 2007; Guoyt et al., 2012; Sack and Scoffoni, 2012). Three to nine leaves of each cultivar were excised in water and placed under conditions favourable to transpiration (i.e. PPFD of 1200 μmol m−2 s−1 and air temperature of 28°C) with the petiole attached to a potometer. When leaves reached a transpirational steady state, the transpirational flux rate (E) was recorded. The leaf area was then measured using a leaf area meter (LI-Cor 3000C, LI-COR, NE, USA) and leaf length and width were measured quickly using a plastic ruler. The leaves were detached and cut into small sections, immediately followed by leaf water potential (Ψₛ) measurement using a WP4C Dewpoint Potentiometer (Decagon, Pullman, WA, USA). Kₜₙ was calculated as follows:

$$K_{\text{leaf}} = \frac{E}{0 - \Psi_{\text{leaf}}}$$

VLA and leaf thickness
Three leaves per cultivar were cleared in 20% aqueous NaOH after their widths were recorded. Three sections of leaf lamina of ~5.0 mm length were excised from the middle portion of each leaf, stained, and mounted in glycerol for the determination of vein density. According to Scarpella et al. (2003) and Smillie et al. (2012), rice vascular bundles can be categorized into three types based on their size: midrib, large veins, and minor veins. In the present study, the numbers of major veins (sum of midrib and large veins) and minor veins, and the inter-vein distance (IVD, distance between two minor veins), were recorded using a microscope at 40× magnification. The proportion of minor vein length was calculated as the percentage of minor vein length per area (VLAₘᵢₗᵢᵣₒ) over VLA. The leaf thickness (Tᵥₑᵃₙ) was measured using a DTG03 digital thickness gauge (Digital Micrometers Ltd, Sheffield, UK).

Leaf N content per leaf area
After Ψₛ measurement, leaves were oven-dried at 80°C to constant weight, and ground using a mixer mill homogenizer (MM400, Retsch, Germany). Approximately 5.0 mg was used to measure N content per leaf area using an NC analyzer (IsopPrime100 IRMS, Isoprinte Ltd, UK).

Statistical analyses
One-way analysis of variance (ANOVA) and multiple regression analysis were applied to assess the significance of cultivar effect with SAS 9.2 (SAS Institute Inc., USA). Regression analyses between parameters were performed using SigmaPlot 12 (SPSS Inc., Chicago, IL, USA). All regressions were fitted by both linear and power models, and the model with higher regression coefficient was selected.

Results

 Differences in leaf morpho-anatomical traits and Kₜₙ across cultivars
There were very large variations in leaf morpho-anatomical traits in the genus Oryza (Table 1). The differences were 6.9-fold in leaf area (ranging from a minimum of 18.4 cm² in Rhi to a maximum of 127.3 cm² in Lat), 4.6-fold in leaf length, and 5.8-fold in leaf width. With respect to leaf veins, VLA, VLA_major, and VLA_minor were significantly different across
cultivars. There was a 2.3-fold difference in VLA (minimum in Rhi and maximum in Wcr). The difference in leaf mass per area (LMA) was 1.8-fold (minimum in Rhi and maximum in Lat), and the difference in \(K_{\text{leaf}}\) was 3.7-fold (minimum in Rhi and maximum in Lat).

**Relationships among leaf morpho-anatomic traits, leaf N, and \(K_{\text{leaf}}\)**

Across all cultivars, \(K_{\text{leaf}}\) was positively correlated with leaf area (\(r = 0.80, P < 0.01\)), leaf length (\(r = 0.62, P < 0.05\)), and leaf width (\(r = 0.66, P < 0.05\)) (Fig. 1). No significant correlation was observed between \(K_{\text{leaf}}\) and VLA, VLA\(_{\text{major}}\), or VLA\(_{\text{minor}}\). However, a positive correlation (\(r = 0.86, P < 0.01\)) between the proportion of minor vein length and \(K_{\text{leaf}}\) was observed (Fig. 2). In addition, \(K_{\text{leaf}}\) was positively correlated with LMA (\(r = 0.83, P < 0.01\)), IVD (\(r = 0.92, P < 0.01\)), \(T_{\text{leaf}}\) (\(r = 0.67, P < 0.05\)) (Fig. 3), and leaf N content per leaf area (\(r = 0.86, P < 0.01\)) (Fig. 4). IVD and \(T_{\text{leaf}}\) were positively correlated with leaf N content per leaf area, while VLA was independent of leaf N content per leaf area (Fig. 5). In order to identify the direct effects of leaf N content per leaf area on \(K_{\text{leaf}}\), a multiple regression analysis was performed between \(K_{\text{leaf}}\) and leaf N content per leaf area, \(T_{\text{leaf}}\), and IVD. Our results show that \(K_{\text{leaf}}\) tightly correlated with N content per leaf area (\(P = 0.015\)) compared with \(T_{\text{leaf}}\) (\(P = 0.673\)) and IVD (\(P = 0.052\)).

**Relationship between \(K_{\text{leaf}}\) and gas exchange**

There were very large variations in \(A, g_s, \) and \(g_m\) in the genus *Oryza* (Supplementary Figure S2). The \(g_m\) estimated by a combination of gas-exchange and chlorophyll fluorescence methods showed a linear relationship with the value estimated from the \(A-C\) curve-fitting method (Supplementary Figure S1). Across all cultivars, a positive correlation (\(r = 0.63, P < 0.05\)) was found between \(A\) and \(K_{\text{leaf}}\) (Table 2; Supplementary Figure S2). \(A\) was closely related to total CO\(_2\) diffusion conductance (\(g_t\)) (\(r = 0.85, P < 0.01\)), \(g_s\) (\(r = 0.86, P < 0.01\)) and \(g_m\) (\(r = 0.73, P < 0.01\)). The \(g_t\) was positively correlated with both \(g_s\) (\(r = 0.92, P < 0.001\)) and \(g_m\) (\(r = 0.92, P < 0.001\)). There was a strong relationship between \(g_s\) and \(g_m\). \(K_{\text{leaf}}\) was positively correlated with \(g_t\) (\(r = 0.88, P < 0.01\)), \(g_s\) (\(r = 0.75, P < 0.01\)), and \(g_m\) (\(r = 0.77, P < 0.01\)).

**Discussion**

**Relationship between \(A\) and \(K_{\text{leaf}}\)**

Improving photosynthesis is central to improving crop yield. In C\(_3\) plants, an important determinant of photosynthesis is the CO\(_2\) concentration in the chloroplast. (Evans and Von Caemmerer, 1996; Flexas et al., 2008; Franks et al., 2009; Flexas et al., 2013a). Previous studies have shown correlations between \(A\) and \(K_{\text{leaf}}\) across a wide range of species (Brodribb et al., 2007; Flexas et al., 2013b). In the present study, \(A\) was correlated with \(K_{\text{leaf}}\) in the genus *Oryza* (Table 2). During photosynthesis, CO\(_2\) must move from outside the leaf through the stomata to the sub-stomatal internal cavities, and from there to the site of carboxylation inside the chloroplast through leaf mesophyll (Evans et al., 2009; von Caemmerer and Evans, 2010; Flexas et al., 2012). Opening the stomata would benefit photosynthesis in the presence of sufficiently high intercellular CO\(_2\) concentration. However, maintaining open stomata depends on leaf water supply capacity, which is determined by \(K_{\text{leaf}}\). Under normal conditions, \(K_{\text{leaf}}\) is limited by leaf anatomy (Sack et al., 2003; Sack and Holbrook, 2006).

**Relationship between \(K_{\text{leaf}}\) and leaf morpho-anatomical traits**

Across a large variation in leaf area, we observed a positive correlation between \(K_{\text{leaf}}\) and leaf area (Fig. 1), as was also observed in *Acer* and *Quercus* spp. (Nardini et al., 2012). However, our results were contrary to those of Simonin et al. (2012), who showed, by summarizing published data, that \(K_{\text{leaf}}\) was independent of variations in leaf area. There are two reasons for the discrepancy between our results and those of Simonin et al. (2012). Firstly, our results were derived from the genus *Oryza*, which has a homologous hydraulic architecture, and the relatively expanded (leaf area and leaf thickness increasing) leaf needs to evolve stronger water transportation ability, because vein xylem conductivity tends to increase with leaf size. However, the result reported by Simonin et al. (2012) was derived from a wide range of plant species with a multiplicity of leaf hydraulic architectures, masking the effects of leaf area and leaf thickness on \(K_{\text{leaf}}\). Secondly, the large variation in leaf area in the present study was contributed by
Leaf hydraulic conductance related to leaf morpho-anatomical traits and leaf N status

Lat (Table 1; Fig. 1), which caused a significant correlation between $K_{leaf}$ and leaf area.

In the present study, a strong positive correlation was observed between $K_{leaf}$ and LMA (Fig. 3). If LMA is considered as the sum of the mass of different leaf tissues per unit of leaf area, variation in LMA occurs via changes in leaf tissue composition. Blonder et al. (2011), on the basis of a mathematic model, hypothesized that high VLA results in high LMA. However, Sack et al. (2013) contested this by compiling a large database, reporting that, in fact, vein xylem and sclerenchyma accounted for <10% of leaf volume per area and thus did not contribute strongly and directly to either leaf thickness or leaf density (Sack et al., 2013). In the present study with the genus Oryza, no relationship between VLA and LMA was observed (Supplementary Figure S3). Additionally, especially within species, LMA correlates with $T_{leaf}$, which is derived from layers of mesophyll cells. Our result indicates that the variation in LMA resulted from changing proportions of mesophyll tissue rather than from changes in VLA in monocots.

There are conflicting reports on the relationship between $K_{leaf}$ and VLA (Scoffoni et al., 2011; Carins Murphy et al., 2012; Flexas et al., 2013b). In the present study, we found that $K_{leaf}$ was not correlated with VLA, VLA$_{major}$, or VLA$_{minor}$. However, $K_{leaf}$ significantly increased with an increasing proportion of minor vein length in the genus Oryza (Fig. 2). In monocots, the water in major veins, as in minor veins, exits into the surrounding tissue, instead of into minor veins. Minor veins have a large surface area for exchange of xylem water with the surrounding mesophyll, and a short distance through which water travels outside the xylem (Sack and Holbrook, 2006). These results suggest that $K_{leaf}$ in the genus

Fig. 2. Relationships between leaf hydraulic conductance ($K_{leaf}$) and (A) VLA$_{major}$, (B) VLA$_{minor}$, (C) VLA, and (D) proportion of minor vein length. Values shown are mean ± SD, and data in (D) were fitted by power regression. Regression coefficients and significance are shown when $P < 0.05$ (**, $P < 0.01$; ns, not significant).

Fig. 3. Relationship between $K_{leaf}$ and (A) LMA, (B) IVD, and (C) $T_{leaf}$. Values shown are mean ± SD, and data were fitted by power adjustment. Regression coefficients and significance are shown when $P < 0.05$ (*, $P < 0.05$; **, $P < 0.01$).
The water must first move through the bundle sheath, which is made up of parenchymatous cells wrapped around the veins, to mesophyll cells, and then diffuse into the intercellular airspace; or directly diffuse to intercellular airspace. Finally, the water escapes into the atmosphere via stomatal pores. The distance travelled by the water within leaves has been quantified in several ways (Brodribb et al., 2007; Noblin et al., 2008; North et al., 2013), such as by measuring $D_m$ and IVD. Brodribb et al. (2007) reported that $K_{leaf}$ had a strongly negative relationship with $D_m$ (in monocots IVD = 0.5 $D_m$) across species with a wide range of habitats and leaf structures. Furthermore, the relationships between $K_{leaf}$ and IVD depend on water travel pathways and the water vapour concentration gradient between the intercellular airspace and atmosphere. In fact, the water in leaves turns into water vapour at mesophyll cell walls exposed to intercellular air space (Sack and Holbrook, 2006). Thus, if the liquid water supplement in leaves is not a limiting factor, an increase in $K_{leaf}$ may occur via an increase in the mesophyll cell wall area exposed to the intercellular airspace. Indeed, Nardini et al. (2012) reported that $K_{leaf}$ was enhanced by an increase in mesophyll porosity (the fraction of leaf mesophyll volume occupied by intercellular air space) under high irradiance.

The value of mesophyll porosity is relatively stable within the genus Oryza (Giuliani et al., 2013). In other words, the volume of intercellular air space per leaf area depends on the proportion of mesophyll tissue in leaves. In rice, it has been shown that the proportion of mesophyll tissue in leaves is related to IVD (Smillie et al., 2012) and $T_{leaf}$ (Sack et al., 2003). Early studies hypothesized that $K_{leaf}$ in thick leaves should decline with increasing pathway length outside the xylem. However, experimental results show that $K_{leaf}$ correlates with $T_{leaf}$ across species, and across sun and shade leaves within a given species (Sack et al., 2003; Zhang and Cao, 2009). This is because thicker leaves have more parallel flow pathways outside the xylem. Here we demonstrated that increases in IVD and $T_{leaf}$ benefit $K_{leaf}$ in the genus Oryza (Fig. 5).

**Effects of leaf N status on $K_{leaf}$**

N significantly influences rice leaf anatomy, structure, and function (Lee et al., 2011). In the present study, leaf N content per leaf area had a significant positive effect on $K_{leaf}$ (Fig. 4). Increased IVD and $T_{leaf}$ under high N supplementation (data not shown) facilitates water evaporation at the cell wall surface, and this response could be one of the reasons

---

**Table 2. Coefficients of correlations**

|      | $K_{leaf}$ | $A$ | $g_t$ | $g_s$ | $g_m$ |
|------|------------|-----|-------|-------|-------|
| $K_{leaf}$ | 1.00*** | | 0.63* | 0.88** | 0.75** |
| $A$ | | 1.00*** | 0.85** | 0.86** | 0.73** |
| $g_t$ | | | 1.00*** | 0.92*** | 0.94*** |
| $g_s$ | | | | 1.00*** | 0.73** |
| $g_m$ | | | | | 1.00*** |

*a*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. 

---

**Fig. 4.** Correlation of leaf N content per leaf area and $K_{leaf}$. Values shown are mean ± SD, and data were fitted by power adjustment. Regression coefficients and significance are shown when $P$ was <0.05 (**, $P < 0.01$).

**Fig. 5.** Effect of leaf N content per leaf area on (A) VLA, (B) IVD, and (C) $T_{leaf}$. Values shown are mean ± SD, and data in (B) and (C) were fitted by power adjustment. Regression coefficients and significance are shown when $P < 0.05$ (ns, not significant; *, $P < 0.05$; **, $P < 0.01$).

**Oryza** may be driven by the cross-sectional conductivity of veins and outside xylem conductance (McKown et al., 2010; Sommerville et al., 2012).

After leaving the xylem, water must pass through liquid and gas phases before it reaches the sub-stomatal cavities.
why $K_{\text{leaf}}$ increased with increasing N content per leaf area in leaves. Moreover, water flux across bundle sheath and mesophyll cells travels through either apoplastic, or cell-to-cell pathways, or both (Sack and Holbrook, 2006). In the cell-to-cell pathway, water molecules diffuse either across the plasma membrane or through plasmodesmata. Water channels, plasma membrane-intrinsic aquaporins (PIPs), play an important role in this process (Maggio and Joly, 1995; Pou et al., 2013). Several studies have shown that PIP expression varies with N supply (Clarkson et al., 2000; Guo et al., 2007). It is reasonable to speculate that in rice PIPs are regulated by leaf N content per leaf area.

**Relationship between $K_{\text{leaf}}$ and $g_m$**

Inside leaves, $K_{\text{leaf}}$ and $g_m$ are two traits which play central roles in determining gas exchange and plant performance (Sack and Holbrook, 2006; Flexas et al., 2013b). However, very few studies have focused on their coordination; rather, the two traits have been studied independently in the past two decades. Recently, by summarizing the published data, Flexas et al. (2013b) reported that $K_{\text{leaf}}$ was correlated with $g_m$. In the present study, we found coordination of $K_{\text{leaf}}$ and $g_m$ in the genus *Oryza*, which provides further evidence that water and CO$_2$ diffusion in the leaf share common pathways (Table 6). Many studies have found that $g_m$ correlates with certain leaf structural traits in some species, particularly with the mesophyll cell surface area exposed to intercellular airspace per leaf area ($S_m$) (Flexas et al., 2008; Evans et al., 2009; Flexas et al., 2012). This correlation occurs because increasing $S_m$ provides more pathways in parallel for CO$_2$ diffusion. In fact, the mesophyll surface exposed to the intercellular airspace is the site at which water changes from liquid to vapour via evaporation. Cell wall thickness has been recognized as another important limiting factor for CO$_2$ diffusion in the leaf. Interestingly, thick mesophyll cell walls may increase the extra-xylem apoplast path length, thereby increase $K_{\text{leaf}}$. Further, membrane PIPs are known to facilitate transmembrane water transport as well as CO$_2$ transport. For instance, Otto et al. (2010) reported a trade-off between water and CO$_2$ permeability through membranes, depending on the proportion of PIP1 and PIP2 present.

Similar responses of $g_m$ and $K_{\text{leaf}}$ to various environmental factors, including temperature, light, leaf N status (Fig. 4; Supplementary Figure S4), and leaf water status, provide another line of evidence for their relationships (Flexas et al., 2013b). However, the relative effects of leaf structural traits on $g_m$ and $K_{\text{leaf}}$ and the coordinated dynamics of $g_m$ and $K_{\text{leaf}}$ under various environmental conditions, needs to be clarified in the future.

In conclusion, there were significantly positive relationships between $K_{\text{leaf}}$ and LMA, leaf area, proportion of minor vein length, IVD, $T_{\text{leaf}}$ and leaf N content per leaf area in the genus *Oryza*, but $K_{\text{leaf}}$ was independent of VLA. High $K_{\text{leaf}}$ was associated with high $A_{\frac{g}{S}}$, $g_s$, and $g_m$. Our results indicate that leaf morpho-anatomical traits and leaf N content per leaf area had significant effects on $K_{\text{leaf}}$ and suggest that more detailed anatomical and structural studies are needed to elucidate the impacts of leaf feature traits on $K_{\text{leaf}}$ and gas exchange in grasses.

**Supplementary material**

Supplementary data can be found at *JXB* online.

**Supplementary Figure S1.** Relationship between $\alpha \beta$ values obtained using Yin’s method (Yin et al., 2009) and the $\Phi_{\text{PSII}}$ and $\Phi_{\text{CO}_2}$ slope method; and between $g_m$ values estimated from a combination method with gas-exchange and Chl fluorescence, and the $A-\bar{C}_i$ curve-fitting method (b).

**Supplementary Figure S2.** Relationship between $K_{\text{leaf}}$ and $A_{\frac{g}{S}}$, $g_s$, and $g_m$.

**Supplementary Figure S3.** Relationship between VLA and LMA in the genus *Oryza*.

**Supplementary Figure S4.** Relationship between leaf N concentration and both $g_s$ and $g_m$.

**Funding**

This work was supported by the Programme for Changjiang Scholars and Innovative Research Team in the University of China (IRT1247), Special Fund for Agro-scientific Research in the Public Interest of China from the Ministry of Agriculture (No. 201203096), and Fundamental Research Funds for the Central Universities (2012SC13).

**References**

Bernacchi CJ, Portis AR, Nakano H, von Caemmerer S, Long SP. 2002. Temperature response of mesophyll conductance. Implications for the determination of Rubisco enzyme kinetics and for limitations to photosynthesis in vivo. *Plant Physiology* 130, 1992–1998.

Blonder B, Violle C, Bentley LP, Enquist BJ. 2011. Venation networks and the origin of the leaf economics spectrum. *Ecology Letters* 14, 91–100.

Brodribb TJ, Feild TS, Jordan GJ. 2007. Leaf maximum photosynthetic rate and venation are linked by hydraulics. *Plant Physiology* 144, 1890–1898.

Brodribb TJ, Holbrook NM, Zwieniecki MA, Palma B. 2005. Leaf hydraulic capacity in ferns, conifers and angiosperms: impacts on photosynthetic maxima. *New Phytologist* 165, 839–846.

Carins Murphy MR, Jordan GJ, Brodribb TJ. 2012. Differential leaf expansion can enable hydraulic acclimation to sun and shade. *Plant, Cell and Environment* 35, 1407–1418.

Clarkson DT, Carvajal M, Henzler T, Waterhouse RN, Smyth AJ, Cooke DT, Steudle E. 2000. Root hydraulic conductance: diurnal aquaporin expression and the effects of nutrient stress. *Journal of Experimental Botany* 51, 61–70.

Evans JR, Kaldenhoff R, Genty B, Terashima I. 2009. Resistances along the CO$_2$ diffusion pathway inside leaves. *Journal Of Experimental Botany* 60, 2235–2248.

Evans JR, Von Caemmerer S. 1992–1998. Temperature response of mesophyll conductance. *Plant Physiology* 130, 1992–1998.

Flexas J, Barbour MM, Brendel O et al. 2012. Mesophyll diffusion conductance to CO$_2$: An unappreciated central player in photosynthesis. *Plant Science* 193–194, 70–84.

Flexas J, Niinemets U, Galle A et al. 2013a. Diffusional conductances to CO$_2$ as a target for increasing photosynthesis and photosynthetic water-use efficiency. *Photosynthesis Research* 117, 45–59.

Flexas J, Ribas-Carbó M, Díaz-Aspejo A, Galmés J, Medrano H. 2008. Mesophyll conductance to CO$_2$: current knowledge and future prospects. *Plant, Cell and Environment* 31, 602–621.
Flexas J, Scoffoni C, Gago J, Sack L. 2013b. Leaf mesophyll conductance and leaf hydraulic conductance: an introduction to their measurement and coordination. Journal of Experimental Botany 64, 3965–3981.

Franks PJ. 2006. Higher rates of leaf gas exchange are associated with higher leaf hydraulic pressure gradients. Plant, Cell and Environment 29, 584–592.

Franks PJ, Drake PL, Beerling DJ. 2009. Plasticity in maximum stomatal conductance constrained by negative correlation between stomatal size and density: an analysis using Eucalyptus globulus. Plant, Cell and Environment 32, 1737–1748.

Giuliani R, Koteyeva N, Voznesenskaya E, Evans MA, Cousins AB, Edwards GE. 2013. Coordination of leaf photosynthesis, transpiration, and structural traits in rice and wild relatives (genus Oryza). Plant Physiology 162, 1632–1651.

Guo S, Kaldenhoff R, Uehlein N, Sattelmacher B, Brueck H. 2007. Relationship between water and nitrogen uptake in nitrate-and ammonium-supplied Phaseolus vulgaris L. plants. Journal of Plant Nutrition and Soil Science 170, 73–80.

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 and Environment, 35, 857–871.

Harley PC, Loreto F, Di Marco G, Sharkey TD. 1998. Theoretical considerations when estimating the mesophyll conductance to CO2 flux by analysis of the response of photosynthesis to CO2. Plant Physiology 98, 1429–1436.

Imai K, Suzuki Y, Mae T, Makino A. 2008. Changes in the synthesis of rubisco in rice leaves in relation to senescence and N influx. Annals of Botany 101, 135–144.

Johnson DM, Woodruff DR, McCulloh KA, Meinzer FC. 2009. Leaf hydraulic conductance, measured in situ, declines and recovers daily: leaf hydraulics, water potential and stomatal conductance in four temperate and three tropical tree species. Tree Physiology 29, 879–887.

Lee Y-J, Yang C-M, Chang K-W, Shen Y. 2011. Effects of nitrogen status on leaf anatomy, chlorophyll content and canopy reflectance of paddy rice. Botanical Studies 52, 295–303.

Maggio A, Joly RJ. 1995. Effects of mercuric chloride on the hydraulic conductivity of tomato root systems (evidence for a channel-mediated water pathway). Plant Physiology 109, 331–335.

Martré 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 Physiology 130, 2101–2110.

McKown AD, Cochard H, Sack L. 2010. Decoding leaf hydraulics with a spatially explicit model: principles of venation architecture and implications for its evolution. The American Society of Naturalists 175, 447–460.

Murphy R, Smith J. 1998. Determination of cell water-relation parameters using the pressure probe: extended theory and practice of the pressure-clamp technique. Plant, Cell and Environment 21, 637–657.

Nardini A, Pedà G, Roca NL. 2012. Trade-offs between leaf hydraulic capacity and drought vulnerability: morpho-anatomical bases, carbon costs and ecological consequences. New Phytologist 196, 788–798.

Noblin X, Mahadevan L, Coomaraswamy I, Weitz D, Holbrook N, Zwieniecki M. 2008. Optimal vein density for a tank brinelliat: axial and radial pathways for moving and conserving water. Frontiers in Plant Science 4, 78.

Otto B, Uehlein N, Sdorra S, Fischer M, Ayaz M, Belasteguig-Macadam X, Heckwolf M, Lachnit M, Pede N, Priem N. 2010. Aquaporin tetramer composition modifies the function of tobacco aquaporins. The Journal of Biological Chemistry 285, 31253–31260.

Pou A, Medrano H, Flexas J, Tyerman SD. 2013. A putative role for TIP and PIP aquaporins in dynamics of leaf hydraulic and stomatal conductances in grapevine under water stress and re-wetting. Plant, Cell and Environment 36, 828–843.

Rademacher IF, Nelson CJ. 2001. Nitrogen Effects on Leaf Anatomy within the Intercalary Meristems of Tall Fescue Leaf Blades. Annals of Botany 88, 983–993.

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 and Environment 26, 1343–1356.

Sack L, Holbrook NM. 2006. Leaf hydraulics. Annual Review Of Plant Biology 57, 361–381.

Sack L, Melcher PJ, Zwieniecki MA, Holbrook NM. 2002. The hydraulic conductance of the angiosperm leaf lamina: a comparison of three measurement methods. Journal of Experimental Botany 53, 2177–2184.

Sack L, Scoffoni C. 2012. Measurement of leaf hydraulic conductance and stomatal conductance and their responses to irradiance and dehydration using the Evaporative Flux Method (EFM). Journal of Visualized Experiments 70, 4179.

Sack L, Scoffoni C. 2013. Leaf venation: structure, function, development, evolution, ecology and applications in the past, present and future. New Phytologist 198, 983–1000.

Sack L, Scoffoni C, John GP, Poorter H, Mason CM, Mendez-Alonzo R, Donovan LA. 2013. How do leaf veins influence the worldwide leaf economic spectrum? Review and synthesis. Journal of Experimental Botany 64, 4053–4080.

Sack L, Streeter CM, Holbrook NM. 2004. Hydraulic analysis of water flow through leaves of sugar maple and red oak. Plant Physiology 134, 1824–1833.

Scarpella E, Rueb S, Meijer AH. 2003. The RADICLELESS1 gene is required for vascular pattern formation in rice. Development 130, 645–658.

Scoffoni C, Rawls M, McKown A, Cochard H, Sack L. 2011. Decline of leaf hydraulic conductance with dehydration: relationship to leaf size and venation architecture. Plant Physiology 156, 882–893.

Simonin KA, Limm EB, Dawson TE. 2012. Hydraulic conductance of leaves correlates with leaf lifespan: implications for lifetime carbon gain. New Phytologist 193, 939–947.

Smillie IRA, Pyke KA, Murchie EH. 2012. Variation in vein density and mesophyll cell architecture in a rice deletion mutant population. Journal of Experimental Botany 63, 4563–4570.

Sommerville KE, Sack L, Ball MC. 2012. Hydraulic conductance of Acacia phylloides (foliage) is driven by primary nerve (vein) conductance and density. Plant, Cell and Environment 35, 158–168.

Sperry JS. 2000. Hydraulic constraints on plant gas exchange. Agricultural and Forest Meteorology 104, 13–23.

von Caemmerer S, Evans JR. 2010. Enhancing C3 photosynthesis. Plant Physiology 154, 589–592.

Warren CR, Dreyer E. 2006. Temperature response of photosynthesis and internal conductance to CO2: results from two independent approaches. Journal of Experimental Botany 57, 3057–3067.

Wong SC, Cowan IR, Farquhar GD. 1979. Stomatal conductance correlates with photosynthetic capacity. Nature 282, 424–426.

Yin X, Struik PC, Romero P, Harbinson J, Evers JB, PE VDP, Vos J. 2009. Using combined measurements of gas exchange and chlorophyll fluorescence to estimate parameters of a biochemical C3 photosynthesis model: a critical appraisal and a new integrated approach applied to leaves in a wheat (Triticum aestivum) canopy. Plant, Cell and Environment 32, 448–464.

Zhang JL, Cao KF. 2009. Stem hydraulics mediates leaf water status, carbon gain, nutrient use efficiencies and plant growth rates across dipterocarp species. Functional Ecology 23, 658–667.