SUPPLEMENTAL FIGURE LEGENDS

Figure S1. SEM images illustrating leaf surfaces of two *Oryza* species which were used to calculate stomatal density and size. Analyses showed *O. alta* (A,B) has low density and size of stomates on each side of the lamina; *O. barthii* (C,D) had among the highest stomatal density and size in both lamina surfaces. Scale bars = 200 μm from A to D. See Supplemental Table S3 for data on stomata across *Oryza* accessions.

Figure S2. A, example of light microscopy image of leaf cross-section (*O. australiensis*, 21) used to estimate the leaf mesophyll volume occupied by Intercellular Air Space (IAS, in red). B, Schematic drawing showing the method of estimating the extent of cell surface lobing as cell perimeter tortuosity. This was calculated as arc-chord ratio: black line is the perimeter of mesophyll cell section (arc) and red line is the perimeter of the cell section circumscribed area (chord). ch, chloroplast; m, mitochondria.

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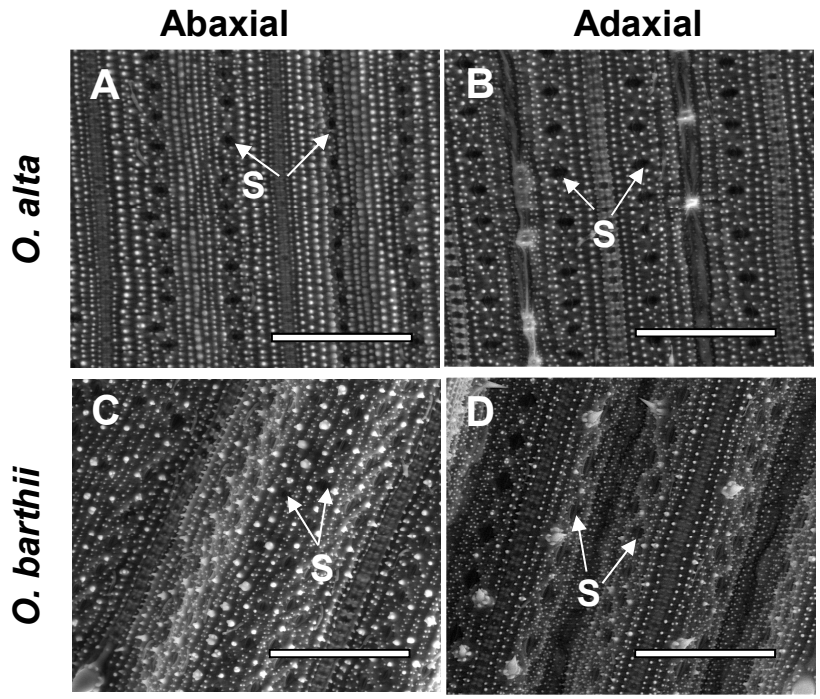

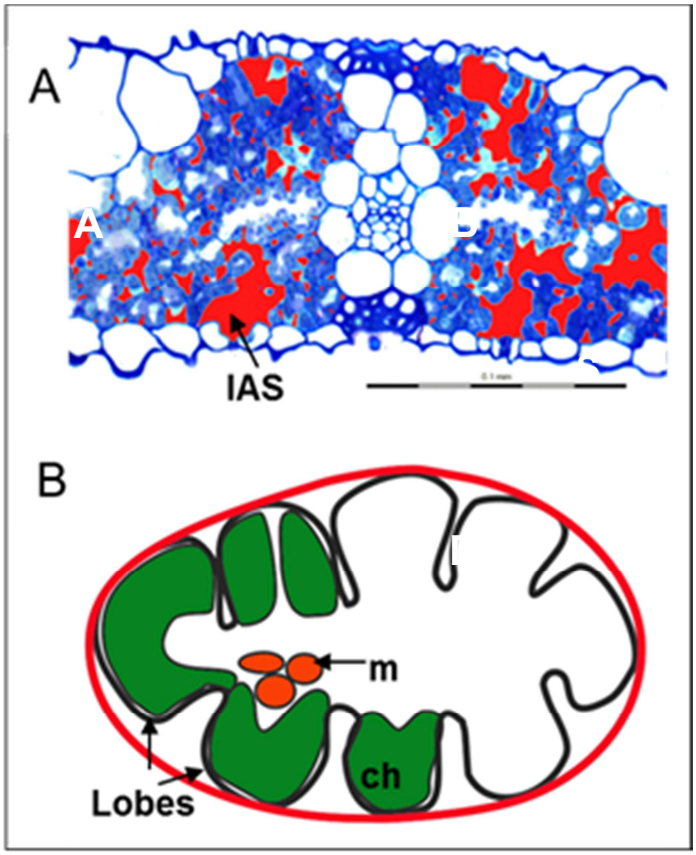

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Supplemental Materials and Methods S1. Estimate of cell volume from values of a_{cell}.

The mean (luminal) M cell section area in leaf cross-sections (a_{cell}, μm^2) was determined for each accession (n= 3). In addition, in each leaf cross section, the three highest a_{cell} were taken as M cell median sections (a_{cell_med}, μm^2). Across *Oryza* accessions, mean a_{cell_med} (n= 3) and a_{cell} had a close positive correlation (r= 0.83).

Leaf M cells in *Oryza* were assumed to be prolate spheroids; in the leaf cross section, they have the major axis (2a) twice the length of the other two minor axes ($2b_1=2b_2=a$), as in Sage and Sage (2009) and Scafaro *et al*. (2011). In each leaf cross section, representative major and minor M cell section semiaxes (a and b, respectively) were calculated based on

$$a_{cell\_med} = \pi * a * b \qquad \text{(Eq. S1)}$$

and they were used to compute cell volume (Vol_{cell}, μm^3) as

$$Vol_{cell} = \frac{4}{3}\pi * a * b^2 \qquad \text{(Eq. S2)}$$

Cell surface lobing was not taken into account. Mean Vol_{cell} and a_{cell} across *Oryza* accessions had a close positive correlation (r = 0.83; P< 0.05). In addition, in each leaf cross section, the M cell surface area (a_{cell_surf}) corresponding to Vol_{cell} was calculated as

$$a_{cell\_surf} = 2\pi * b^2 + 2\pi \frac{ab}{\varepsilon} * \sin^{-1}\varepsilon \qquad \text{(Eq. S3)}$$

where a and b are the major and minor M cell section semiaxes; and $\varepsilon = (\sqrt{a^2 - b^2})/a =$ 0.8660 is the eccentricity parameter. Equations S2 and S3 were taken from Beyer, 1978. a_{cell_surf} was edited to take into account for Lob_{cell}. The mean P/a_{cell} ratio (see list of leaf traits in Table 2) and the mean a_{cell_surf} /Vol_{cell} ratio across *Oryza* accessions showed a positive correlation (r= 0.60).

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Supplemental Materials and Methods S2. Magnitude of the CO_2 diffusion leaks into and from the Fluorometer Leaf Chamber and its effect on A and C_i calculation

A quantitative analysis of CO_2 leakage into and from the LI-COR 6400XT Fluorometer Leaf Chamber was performed, as suggested by LI 6400XT manual (LI-COR Biosciences, 2008) and following Flexas *et al*. (2007) and Rodegheiro *et al*. (2007) suggestions. A portion of a dead and dry leaf of *Oryza sativa* IR64 was enclosed in the cuvette having one neoprene (below) and one polyethylene (above) foam gasket; synthetic rubber (*Terostat* IX, HenkelTechnologies, Düsseldorf, Germany) was used to improve the sealing. Air surrounding the cuvette was ventilated: the ambient CO_2 molar fraction nearby the chamber was constant during the test (400 μmol CO_2 mol^{-1} air). The same molar bulk flow rate through the chamber which was adopted for leaf measurements was employed (300 μmol air s^{-1}); chamber block temperature was set at 30ºC. Drierite knob was turned to bypass, so that there would be corresponding H_2O molar fraction (mmol H_2O mol^{-1} air) inside the cuvette and in the surrounding air.

Several C_a values over the range from 40 to 1500 μmol CO_2 mol^{-1} air were imposed to the LI-COR 6400XT equipment; at each C_a, the CO_2 molar concentrations measured by chamber's inlet and outlet gas analyzers (C_r and C_s, μmol CO_2 mol^{-1} air, respectively) were recorded. Three replicates were taken. The gradients between C_r and C_s [$(C_r\text{-}C_s)_{leak}$, μmol CO_2 mol^{-1} air] were calculated and plotted versus C_s: a linear model was adopted to fit the data

$$(C_r\text{-}C_s)_{leak} = -0.0008C_s + 0.3630 \quad (R^2 = 0.96;\ P < 0.001) \quad \text{(Eq. S4)}$$

From the leaf gas exchange measurements taken on the *Oryza* accessions the value of A was

then calculated (μmol CO_2 m^{-2} s^{-1}) at each imposed C_a, *i.e.* at each C_s, as

$$A = \frac{F(C_r - C_s)}{100S} - C_s E + \frac{F(C_r - C_s)_{leak}}{100S} \quad \text{(Eq. S5)}$$

according to the LI-COR 6400XT manual (LI-COR Biosciences, 2008); F is the molar flow rate (μmol air s^{-1}); (C_r -C_s) is the gradient of CO_2 molar concentration (μmol CO_2 mol^{-1} air) measured by chamber's inlet and outlet gas analyzers; S is the surface area of the chamber lumen (m^2); E is the transpiration rate per unit leaf surface area (mol H_2O m^{-2} s^{-1}); and $(C_r\text{-}C_s)_{leak}$ is the differential of CO_2 molar concentration (μmol CO_2 mol^{-1} air) due to CO_2 diffusion leaks which was provided by Eq. S4.

As reported by Flexas *et al*. (1997), CO_2 leaks into and from the leaf chamber affect A and C_i in opposite direction; see the LI-COR 6400XT manual (LI-COR Biosciences, 2008) for the equation 1-18 (which includes A) adopted to calculate C_i.

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Supplemental Materials and Methods S3

Fick's laws applied to estimate g_m

Based on (First and Second) Fick's Laws (Nobel, 2009), under the assumptions of isothermal and isobaric conditions and of absence of sources and sinks along the one-dimensional diffusion

path, the flow for species *j* in a solution through a barrier can be calculated as:

$$J_j = \frac{D_j * K_j * A_b * (C_{j1} - C_{j2})}{d} \qquad \text{(Eq. S6)}$$

where J_j is the flux for species *j* (mol s^{-1}), D is the diffusion coefficient (m^2 s^{-1}), K_J is the partition coefficient, A_b is the barrier cross-sectional area (m^2), C_j is the concentration of j before and after the barrier (mol j m^{-3} air), d is the path length (m).

When the species is CO_2 that diffuses in the leaf mesophyll from Intercellular Air Space (IAS) to chloroplast stroma in a liquid solution through cell wall and membranes, the barrier cross-section area A_b could be represented by the mesophyll cell surface area exposed to IAS expressed per unit (one side) leaf surface area (S_{mes}, μm^2 μm^{-2}) and the CO_2 flux density, which corresponds to net photosynthetic rate per unit leaf surface area (*A*, mol CO_2 m^{-2} s^{-1}), could be calculated as:

$$A = \frac{D_{CO2} * K_{CO2} * S_{mes} * (C_i - C_c)}{d} \qquad \text{(Eq. S7)}$$

where C_i and C_c are intercellular and chloroplastic CO_2 concentration (mol CO_2 m^{-3} air), respectively. Given that the equation based on Fick's Laws can usually be applied to calculate mesophyll conductance to CO_2 diffusion (g_m, m s^{-1})

$$g_m = \frac{A}{(C_i - C_c)} \qquad \text{(Eq. S8)}$$

and replacing *A* with Eq. S7, g_m corresponds to

$$g_m = \frac{D_{CO2} * K_{CO2} * S_{mes}}{d} \qquad \text{(Eq. S9)}$$

and

$$\frac{g_m}{S_{mes}} = \frac{D_{CO2} * K_{CO2}}{d} \qquad \text{(Eq. S10)}$$

where the second term corresponds to permeability to CO_2 (m s^{-1}) from Intercellular Air Space to

chloroplasts in a liquid solution through cell wall and membranes (Nobel, 2009). In this term, d is the path length for CO_2 to move from IAS to chloroplasts. In the present study $Thick_{cw}$ is considered a significant component of d, based on the evidence that the chloroplasts during the light time are anchored against the cell membrane/walls that directly face the IAS (Takagi *et al.*, 2009).

Vice-versa, S_{mes}/g_m can be taken as a measure of the reciprocal of permeability to CO_2, *i.e.* of diffusive resistance in the liquid phase, from the IAS to chloroplasts. Note in this study, S_{mes}/g_m was used, which has a very high correlation with S_{chl}/g_m (estimated by Evans *et al.*, 2009) due to the high percentage of chloroplast covering the cell walls exposed to IAS.

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