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*Functional Plant Biology*

### Supplementary Material

#### **Linking structure to function: the connection between mesophyll structure and intrinsic water use efficiency**

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# Supplement

## S1 References used in Figure2

### Panel A

Trueba S, Théroux-Rancourt G, Earles JM, Buckley TN, Love D, Johnson DM, Brodersen CR. 2022. The 3D construction of leaves is coordinated with water use efficiency in conifers. *New Phytologist* 233: 851–861

### Panel B

Ouyang W, Struik PC, Yin X, Yang J. 2017. Stomatal conductance, mesophyll conductance, and transpiration efficiency in relation to leaf anatomy in rice and wheat genotypes under drought. *Journal of Experimental Botany* 68: 5191-5205

Pandey S, Kumar N, Kushwaha R. 2006. Morpho-anatomical and physiological leaf traits of two alpine herbs, *Podophyllum hexandrum* and *Rheum emodi* in the Western Himalaya under different irradiances. *Photosynthetica* 44: 11–16

Trueba S, Théroux-Rancourt G, Earles JM, Buckley TN, Love D, Johnson DM, Brodersen CR. 2022. The 3D construction of leaves is coordinated with water use efficiency in conifers. *New Phytologist* 233: 851–861

Yang Z-H, Huang W, Yang Q-Y, Chang W, Zhang S-B. 2017. Anatomical and diffusional determinants inside leaves explain the difference in photosynthetic capacity between *Cypripedium* and *Paphiopedilum*, Orchidaceae. *Photosynthesis research* 136: 315-328

### Panel C

Trueba S, Théroux-Rancourt G, Earles JM, Buckley TN, Love D, Johnson DM, Brodersen CR. 2022. The 3D construction of leaves is coordinated with water use efficiency in conifers. *New Phytologist* 233: 851–861

### Panel D

Fini A, Loreto F, Tattini M, Giordano C, Ferrini F, Brunetti C, Centritto M (2016) Mesophyll conductance plays a central role in leaf functioning of Oleaceae species exposed to contrasting sunlight irradiance. *Physiologia Plantarum* 157: 54–68

Kawase M, Hanba YT, Katsuhara M. 2013. The photosynthetic response of tobacco plants overexpressing ice plant aquaporin McMIPB to a soil water deficit and high vapor pressure deficit. *Journal of Plant Research* 126: 517–527

Kolbe AR, Cousins AB. 2017. Mesophyll conductance in *Zea mays* responds transiently to CO<sub>2</sub> availability: implications for transpiration efficiency in C4 crops. *New Phytologist* 217: 1463-1474

Ouyang W, Struik PC, Yin X, Yang J. 2017. Stomatal conductance, mesophyll conductance, and transpiration efficiency in relation to leaf anatomy in rice and wheat genotypes under drought. *Journal of Experimental Botany* 68: 5191-5205

Pandey S, Kumar N, Kushwaha R. 2006. Morpho-anatomical and physiological leaf traits of two alpine herbs, *Podophyllum hexandrum* and *Rheum emodi* in the Western Himalaya under different irradiances. *Photosynthetica* 44: 11–16

Trueba S, Théroux-Rancourt G, Earles JM, Buckley TN, Love D, Johnson DM, Brodersen CR. 2022. The 3D construction of leaves is coordinated with water use efficiency in conifers. *New Phytologist* 233: 851–861

Watanabe M., Kamimaki Y., Mori M., Okabe S., Arakawa I., Kinose Y., Nabaka S., Izuta T. 2018. Mesophyll conductance to CO<sub>2</sub> in leaves of Siebold's beech (*Fagus crenata*) seedlings under elevated ozone. *Journal of Plant Research* 131: 907-914

Xiong D, Huang J, Peng S, Li Y. 2017. A few enlarged chloroplasts are less efficient in photosynthesis than a large population of small chloroplasts in *Arabidopsis thaliana*. *Scientific reports* 7: Article 5782.

Yang Z-H, Huang W, Yang Q-Y, Chang W, Zhang S-B. 2017. Anatomical and diffusional determinants inside leaves explain the difference in photosynthetic capacity between *Cypripedium* and *Paphiopedilum*, Orchidaceae. *Photosynthesis research* 136: 315-328

## S2 Relationship between $S_c$ and $S_m$

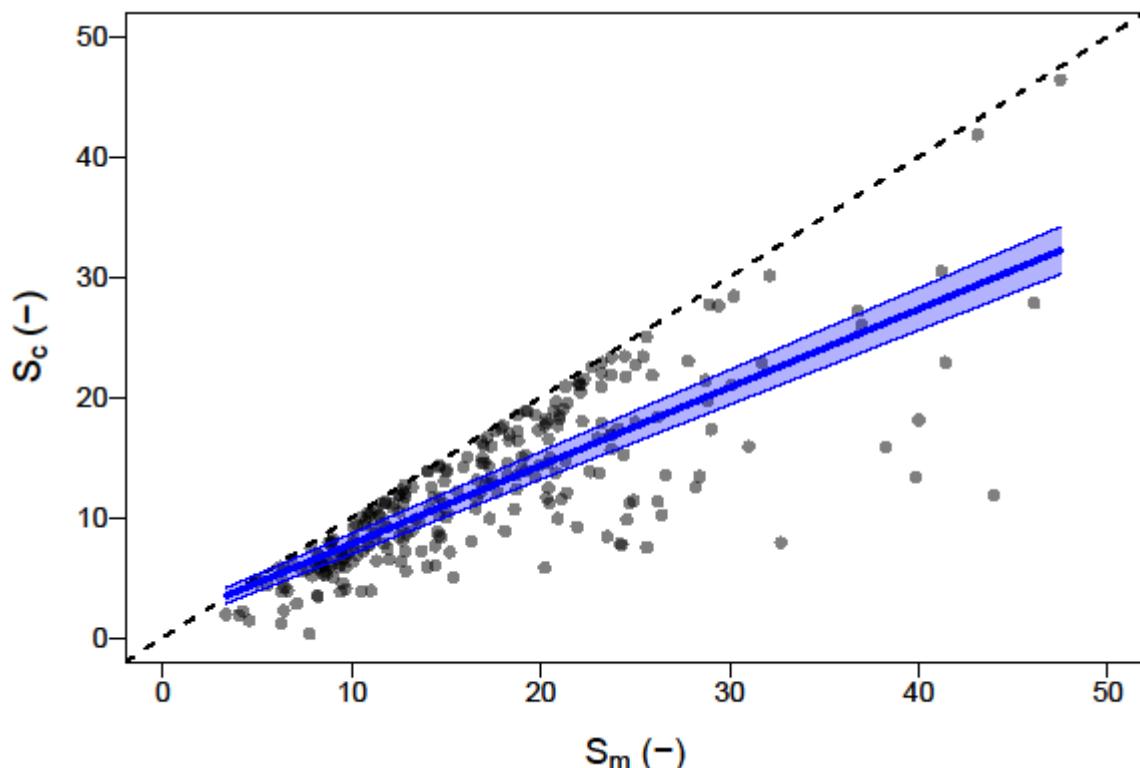


Fig. S1 Surface area of chloroplast exposed to IAS ( $S_c$ ) as a function of mesophyll surface area per unit of total leaf area ( $S_m$ ).

### References used in Figure S1

Adachi S., Nakae T., Uchida M., Soda K., Takai T., Oi T., Yamamoto T., Ookawa T., Miyake H., Yano M., Hirasawa T. 2013. The mesophyll anatomy enhancing CO<sub>2</sub> diffusion is a key trait for improving rice photosynthesis. Journal of Experimental Botany 64: 1061–1072

Barbour M.M., Evans J.R., Simonin K.A., von Caemmerer S. 2016. Online CO<sub>2</sub> and H<sub>2</sub>O oxygen isotope fractionation allows estimation of mesophyll conductance in C4 plants, and reveals that mesophyll conductance decreases as leaves age in both C4 and C3 plants. New Phytologist 210: 875–889

Ellsworth PV, Ellsworth PZ, Koteyeva NK, Cousins A.B. 2018 Cell wall properties in *Oryza sativa* influence mesophyll CO<sub>2</sub> conductance. New Phytologist 219: 66-76

Evans J.R., Caemmerer von S., Setchell B.A. & Hudson G.S. 1994. The relationship between CO<sub>2</sub> transfer conductance and leaf anatomy in transgenic tobacco with a reduced content of rubisco. Australian Journal of Plant Physiology 21: 475–495

Fini A, Loreto F, Tattini M, Giordano C, Ferrini F, Brunetti C, Centritto M. 2016 Mesophyll conductance plays a central role in leaf functioning of Oleaceae species exposed to contrasting sunlight irradiance. Physiologia Plantarum 157: 54–68

Galmés J., Ochogavía J.M., Gago J., Roldán E.J., Cifre J. & Conesa M.À. 2013 Leaf responses to drought stress in Mediterranean accessions of *Solanum lycopersicum*: anatomical adaptations in relation to gas exchange parameters. *Plant, Cell & Environment* 36: 920–935

Giuliani, R., Koteyeva, N., Voznesenskaya, E., Evans, M.A., Cousins, A.B., Edwards, G.E. 2013 Coordination of leaf photosynthesis, transpiration, and structural traits in rice and wild relatives (Genus *Oryza*). *Plant Physiology* 162: 1632–1651

Han, J. , Lei, Z. , Zhang, Y. , Yi, X. , Zhang, W., Zhang, Y. 2019. Drought introduced variability of mesophyll conductance in *Gossypium* and its relationship with leaf anatomy. *Physiologia Plantarum* 166: 873-887

Hanba Y.T., Kogami H., Terashima I. 2002. The effect of growth irradiance on leaf anatomy and photosynthesis in *Acer* species differing in light demand. *Plant, Cell & Environment* 25: 1021–1030

Hanba YT, Shibasaki M, Hayashi Y, Hayakawa T, Kasamo K, Terashima I, Katsuhara M. 2004. Overexpression of the barley aquaporin HvPIP2;1 increases internal CO<sub>2</sub> conductance and CO<sub>2</sub> assimilation in the leaves of transgenic rice plants. *Plant and Cell Physiology* 45: 521–529

Javier Peguero-Pina J., Sisó S., Sancho-Knapik D., Diaz-Espejo A., Flexas J., Galmés J., Gil-Pelegrín E. 2016. Leaf morphological and physiological adaptations of a deciduous oak (*Quercus faginea* Lam.) to the Mediterranean climate: a comparison with a closely related temperate species (*Quercus robur* L.). *Tree Physiology* 36, 287–299

Kawase M, Hanba YT, Katsuhara M. 2013. The photosynthetic response of tobacco plants overexpressing ice plant aquaporin McMIPB to a soil water deficit and high vapor pressure deficit. *Journal of Plant Research* 126: 517–527

Kogami H, Hanba YT, Kibe T, Terashima I, Masuzawa T 2001. CO<sub>2</sub> transfer conductance, leaf structure and carbon isotope composition of *Polygonum cuspidatum* leaves from low and high altitudes. *Plant, Cell & Environment* 24: 529–538

Lu Z., Ren T., Pan Y., Li X., Cong R., Lu J. 2016. Differences on photosynthetic limitations between leaf margins and leaf centers under potassium deficiency for *Brassica napus* L. *Scientific Reports* 6: Article 21725

Maxwell K, Caemmerer von S, Evans JR 1997. Is a low internal conductance to CO<sub>2</sub> diffusion a consequence of succulence in plants with Crassulacean Acid Metabolism? *Australian Journal of Plant Physiology* 24: 777–786

Milla-Moreno E.A., McKown A.D., Guy R.D., Soolanayakanahally R.Y. 2016. Leaf mass per area predicts palisade structural properties linked to mesophyll conductance in balsam poplar (*Populus balsamifera* L.). *Botany* 94: 225–239

Miyazawa S.-I., Terashima I. 2001. Slow development of leaf photosynthesis in an evergreen broad-leaved tree, *Castanopsis sieboldii*: relationships between leaf anatomical characteristics and photosynthetic rate. *Plant, Cell & Environment* 24, 279–291

Miyazawa S.-I., Yoshimura S., Shinzaki Y., Maeshima M., Miyake C. 2008. Deactivation of aquaporins decreases internal conductance to CO<sub>2</sub> diffusion in tobacco leaves grown under long-term drought. *Functional Plant Biology*: 35: 553-564

Muller, O., Oguchi, R., Hirose, T., Werger, M.J.A., Hikosaka, K. 2009. The leaf anatomy of a broad-leaved evergreen allows an increase in leaf nitrogen content in winter. *Physiologia Plantarum* 136: 299–309

Nishida K., Kodama N., Yonemura S., Hanba Y.T. 2015. Rapid response of leaf photosynthesis in two fern species *Pteridium aquilinum* and *Thelypteris dentata* to changes in CO<sub>2</sub> measured by tunable diode laser absorption spectroscopy. *Journal of Plant Research* 128: 777–789

Nomura N., Setoguchi H., Takaso T. 2006. Functional consequences of stenophyllly for leaf productivity: comparison of the anatomy and physiology of a rheophyte, *Farfugium japonicum* var. luchuence, and a related non-rheophyte, *F. japonicum* (Asteraceae). *Journal of Plant Research*: 119, 645–656

Oguchi R., Hikosaka K., Hirose T. 2005. Leaf anatomy as a constraint for photosynthetic acclimation: differential responses in leaf anatomy to increasing growth irradiance among three deciduous trees. *Plant, Cell & Environment* 28: 916–927

Oguchi R, Hikosaka K, Hirose T 2003. Does the photosynthetic light-acclimation need change in leaf anatomy? *Plant, Cell & Environment* 26: 505–512

Oguchi R., Hikosaka K., Hiura T., Hirose T. 2006. Leaf anatomy and light acclimation in woody seedlings after gap formation in a cool-temperate deciduous forest. *Oecologia* 149: 571–582

Ouyang W, Struik PC, Yin X, Yang J. 2017. Stomatal conductance, mesophyll conductance, and transpiration efficiency in relation to leaf anatomy in rice and wheat genotypes under drought. *Journal of Experimental Botany* 68: 5191-5205

Pandey, S. & Kushwaha, R. 2005. Leaf anatomy and photosynthetic acclimation in *Valeriana jatamansi* L. grown under high and low irradiance. *Photosynthetica* 43: 85–90

Pandey S, Kumar N, Kushwaha R. 2006. Morpho-anatomical and physiological leaf traits of two alpine herbs, *Podophyllum hexandrum* and *Rheum emodi* in the Western Himalaya under different irradiances. *Photosynthetica* 44: 11–16

Peguero-Pina J.J., Sisó S., Flexas J., Galmés J., García-Nogales A., Niinemets Ü., Sancho-Knapik D., Saz M.Á., Gil-Pelegín E. 2017. Cell-level anatomical characteristics explain high mesophyll conductance and photosynthetic capacity in sclerophyllous Mediterranean oaks. *New Phytologist* 214: 585–596

Sáez PL, Bravo LA, Cavieres LA, Vallejos V, Sanhueza C, Font-Carrascosa M, Gil-Pelegín E, Peguero-Pina JJ, Galmés J. 2017. Photosynthetic limitations in two Antarctic vascular plants: importance of leaf anatomical traits and Rubisco kinetic parameters. *Journal of Experimental Botany* 68: 2871–2883

Scafaro A.P., von Caemmerer S., Evans J.R., Atwell B.J. 2011. Temperature response of mesophyll conductance in cultivated and wild *Oryza* species with contrasting mesophyll cell wall thickness. *Plant, Cell & Environment* 34, 1999–2008

Syvertsen J.P., Lloyd J., McConchie C., Kriedemann P.E., Farquhar G.D. 1995. On the relationship between leaf anatomy and CO<sub>2</sub> diffusion through the mesophyll of hypostomatous leaves. *Plant, Cell & Environment* 18: 149–157

Thérioux-Rancourt G., Gilbert M.E. 2016. The light response of mesophyll conductance is controlled by structure across leaf profiles. *Plant, Cell & Environment* 40: 726–740

Tholen D., Boom C., Noguchi K.O., Ueda S., Katase T., Terashima I. 2008. The chloroplast avoidance response decreases internal conductance to CO<sub>2</sub> diffusion in *Arabidopsis thaliana* leaves. *Plant, Cell & Environment* 31, 1688–1700

Tomás M., Flexas J., Copolovici L., Galmés J., Hallik L., Medrano H., Ribas-Carbó M., Tosens T., Vislap V., Niinemets Ü. 2013. Importance of leaf anatomy in determining mesophyll diffusion conductance to CO<sub>2</sub> across species: quantitative limitations and scaling up by models. *Journal of Experimental Botany* 64: 2269–2281

Vyas, P., Bisht, M.S., Miyazawa, S.-I., Yano, S., Noguchi, K., Terashima, I., Funayama-Noguchi, S. 2007. Effects of polyploidy on photosynthetic properties and anatomy in leaves of *Phlox drummondii*. *Functional Plant Biology* 34: 673–682

Xiong D., Liu X., Liu L., Douthe C., Li Y., Peng S., Huang J. 2015. Rapid responses of mesophyll conductance to changes of CO<sub>2</sub> concentration, temperature and irradiance are affected by N supplements in rice. *Plant, Cell & Environment* 38: 2541–2550

Xiong, D., Flexas, J., Yu, T., Peng, S., Huang, J. 2017. Leaf anatomy mediates coordination of leaf hydraulic conductance and mesophyll conductance to CO<sub>2</sub> in *Oryza*. *New Phytologist* 213: 572–583

Xiong D, Huang J, Peng S, Li Y. 2017. A few enlarged chloroplasts are less efficient in photosynthesis than a large population of small chloroplasts in *Arabidopsis thaliana*. *Scientific reports* 7: Article 5782

Yang Z-H, Huang W, Yang Q-Y, Chang W, Zhang S-B. 2017. Anatomical and diffusional determinants inside leaves explain the difference in photosynthetic capacity between *Cypripedium* and *Paphiopedilum*, Orchidaceae. *Photosynthesis research* 136: 315–328