Anatomical features, efficient photosystem, and Rubisco activity contribute to high leaf photosynthesis in wild rice

Jyotirmaya Mathan[1#], Anuradha Singh[1#], Vikram Jathar[1], Aashish Ranjan[1*]

[1]National Institute of Plant Genome Research, Aruna Asaf Ali Marg, New Delhi-110067, India.

# These authors contributed equally to the work

***Address for correspondence**

Aashish Ranjan

Tel: 91-11-26735117

Email: aranjan@nipgr.ac.in

Total word count for the main body of the text: 6,407

Summary: 198

Introduction: 1,142

Materials and Methods: 1,304

Results: 2,167

Discussion: 1,794

Acknowledgements: 71

6 figures (6 color figures)

Supplemental information: 6 figures, 6 Table

Summary

- The importance of increasing photosynthetic efficiency for sustainable crop yield increases to feed the growing world population is well recognized. The genus *Oryza*, including cultivated rice and wild relatives, offers tremendous genetic variability to explore photosynthetic differences and underlying developmental, photochemical, and biochemical basis.
- We quantified leaf photosynthesis and related physiological parameters for ten rice genotypes, six cultivated rice and four wild relatives. Further, we identified differences in anatomical traits, photochemical features and Rubisco activity across the selected rice accessions.
- Wild rice species with high leaf photosynthesis per unit area had striking anatomical features, such as larger mesophyll cells with more chloroplasts, larger and closer veins, and a smaller number of mesophyll cells between two consecutive veins. In addition, photosynthetically efficient wild rice species showed an efficient Photosystem II as well as higher carboxylation activity of Rubisco compared to less efficient cultivated varieties, such as IR 64.
- Our results show the existence of desirable variations in mesophyll and vein features, Photosystem II efficiency, and Rubisco activity in the rice system itself that could possibly be targeted for higher leaf photosynthesis. Detailed genetic and molecular understanding of these traits shall be instrumental in increasing photosynthetic efficiency of cultivated rice.

Keywords: Chlorophyll fluorescence, Leaf anatomy, Photosynthesis, Photosystem II (PSII), Rubisco, Wild rice.

Introduction

A remarkable increase in food grain production is needed to meet the demand of the growing world population that is expected to reach 10 billion by 2050 (Alexandratos & Bruinsma, 2012). Crop yield increases due to extensive breeding and agronomic practices is approaching a plateau over the last decade (Peng *et al*., 2008; Lawson *et al*., 2012). The yield increases due to the breeding for semi-dwarf varieties, resulting in optimization of photosynthates' allocation, during the green revolution have also largely been saturated (Jansson *et al.,* 2018). The further increases in crop yield and productivity, therefore, requires improving photosynthetic efficiency of crop plants (Zhu *et al.*, 2010; Lawson *et al*., 2012; Ort *et al.*, 2015; Simkin *et al.*, 2019).

Photosynthesis, the key biochemical process fixing atmospheric CO_2 in the form of carbohydrates, is a multifaceted process that has contributions from leaf physiological, developmental, and biochemical features along with ambient environmental conditions (Long *et al.,* 2006; Mathan *et al.,* 2016). Stomatal conductance (g_s), carboxylation efficiency (CE), transpiration (E), and water-use efficiency (WUE) are critical physiological parameters determining leaf gaseous exchange and water relationship that eventually affect the leaf photosynthesis (Farquhar & Sharkey, 1982). Extrinsic factors, such as light and CO_2 concentration, influence photosynthetic performance by regulating the activity of light-harvesting complexes (LHCs) and Rubisco as well as stomatal opening (Leong & Anderson, 1984; Eichelmann & Laisk, 2002; Hiyama *et al.*, 2017). In addition, the diurnal timing of the day influences the photosynthesis by mid-day stomatal closure and photorespiration (Zhao *et al.*, 2010).

Developmental characteristics of leaf are strong determinants of photosynthesis (Mathan *et al.,* 2016). Flag leaf width, flag leaf thickness, and specific leaf area showed a positive correlation with intrinsic photosynthetic capacity, thus effecting overall crop growth and yield (Liu *et al.*, 2015). Anatomical features such as size, lobbing, and surface area of mesophyll cells, are critical for proper light distribution and CO_2 diffusion within a leaf (Flexas *et al.*, 2008; Sage & Sage, 2009; Tholen *et al.,* 2012). Similarly, leaf vasculature serves as a channel for distribution of water and minerals for photosynthesis and mobilizes photoassimilated CO_2 from leaves to various sink organs (Sack & Scoffoni, 2013). Vein density and interveinal distance have been shown to influence CO_2 fixation efficiency (Ogle, 2003; Amiard *et al.,* 2005; Ueno *et al.,* 2006; Brodribb *et al.,* 2007).Thus, investigating relationships between leaf anatomy and photosynthetic features, and identification of genetic

79 regulators of desirable anatomical features could help in optimizing crop photosynthetic
80 efficiency (Evans, 2013).

81 Photochemical features, such as organization of light-harvesting complexes, quantum
82 efficiency of photosystem, electron transport rate, and photochemical and non-photochemical
83 quenching are key factors for photosynthetic efficiency (Murchie and Lawson, 2013; Wu e*t*
84 *al.,* 2019). Chlorophyll molecules absorb light energy to drive photosynthesis or fluorescence.
85 Maintaining a higher level of chlorophyll content for longer duration in a leaf is one of the
86 suggested strategies for increasing net photosynthesis and crop production (Huang *et al.*,
87 2013). Quantification of chlorophyll fluorescence is used to differentiate photochemical
88 quenching and non-photochemical quenching, and to evaluate the efficiency of Photosystem
89 II (PSII) reaction centres (Kalaji *et al.*, 2016). Approaches to increase light harvesting by
90 PSII reaction centres and photochemical quenching shall be effective in increasing crop
91 photosynthetic efficiency (Horton & Ruban, 2005; Cardona *et al.,* 2018).

92 Different steps of CO_2 photoassimilation are under complex genetic and
93 environmental regulation. Transcriptional control of photosynthesis and associated leaf
94 features is suggested to have potential for improving photosynthetic assimilation (Wang *et al.*,
95 2017). Transcription factors, such as GLK1, HY5, ATAF1, ORE1, and PIF, have been shown
96 to activate the photosynthetic gene expression in crop plants (Kobayashi *et al.,* 2012; Toledo-
97 Ortiz *et al.,* 2014; Wang *et al.*, 2017). Golden2-like (GLK) transcription factor positively
98 regulates the chloroplast development and also binds directly to the promoter of LHC genes
99 (Fitter *et al.*, 2002; Waters *et al.*, 2009). Similarly, HY5 and PIF4/5 are important regulators
100 controlling the regulatory network governing photosynthetic gene expression and senescence,
101 respectively (Kindgren *et al.*, 2012). However, genetic rewiring of transcription factors
102 towards improving crop photosynthetic performance remains elusive.

103 Over the past years, several studies have extensively elaborated the biochemical
104 understanding of photosynthesis (Tamoi *et al.*, 2006; Fermani *et al.*, 2007; Buchanan *et al.*,
105 2012). There have been examples for increasing photosynthetic efficiency, resulting in
106 increased yield and/or biomass. One such example is the evolution of C_4 plants, where
107 specific developmental and biochemical features promote efficient photoassimilation of CO_2
108 (Kajala *et al.*, 2011; Koteyeva *et al.*, 2011; Wang *et al.*, 2011; Kapralov *et al.*, 2012). Free air
109 CO_2 enrichment increases leaf photosynthesis in C_3 plants (Rogers *et al.*, 2004; Sasaki *et al.*,
110 2007; Högy *et al.*, 2013). Optimization of additional parameters, such as decreasing Rubisco
111 oxygenase activity, increasing RuBP regeneration, suppressing photorespiration, and
112 increasing photoprotection of the photosynthesis have also been suggested to improve

113 photosynthetic efficiency (Long *et al.*, 2006; Murchie & Niyogi, 2011; Rosenthal *et al.*,
114 2011; Furbank *et al.*, 2015).

115 Engineering C_4 photosynthesis in C_3 plants has been envisioned as a potential strategy
116 towards enhancing crop photosynthetic performance (Häusler *et al.*, 2002). However,
117 bringing in C_4 photosynthesis in C_3 plants is challenging due to evolutionary, developmental,
118 and biochemical constraints (von Caemmerer *et al.,* 2012; Schuler *et al.,* 2016; Reeves *et al.,*
119 2017). The natural genetic variation in leaf photosynthesis, associated developmental,
120 photochemical, and biochemical features, and underlying genetic regulation in crop plants is
121 an overlooked and untapped resource that could potentially be rewarding for increasing leaf
122 photosynthesis (Flood *et al.,* 2011; Gu *et al.,* 2014; van Bezouw *et al.,* 2019). Rice, with two
123 cultivated species: *Oryza sativa* (Asian rice with two subspecies: indica and japonica) and
124 *Oryza glaberrima* (African rice), and many related wild rice species, provides an excellent
125 system to investigate photosynthetic differences, and underlying genetic basis. Many of the
126 wild rice species have been used in crop breeding programs towards improving biotic and
127 abiotic stress tolerance of the cultivated rice (Sanchez *et al.,* 2013; Sang & Ge, 2013; Atwell
128 *et al.,* 2014; Palmgren *et al.,* 2015; Wing *et al.,* 2018). Interestingly, some of the wild rice
129 species (*O. rufipogon*, *O. latifolia*, and *O. australiensis*) are reported to accumulate higher
130 biomass than cultivated varieties, and introgression from a wild *Oryza* species to cultivated
131 rice has also been shown to increase biomass (Yeo *et al.*, 1994; Zhao *et al.*, 2008). However,
132 a systematic effort to identify the photosynthetic differences, linking the differences to
133 developmental, photochemical, and biochemical features, and investigating the underlying
134 genetic and molecular basis is warranted.

135 We examined a set of cultivated (including both subspecies of *Oryza sativa* and *Oryza*
136 *glaberrima*) and wild rice species, differing in biomass accumulation, to find out strong
137 differences in leaf photosynthesis. We, further, identified the developmental, photochemical,
138 and biochemical differences among the selected photosynthetically different accessions.
139 Expression pattern of genes encoding photosynthetic components, chlorophyll-biosynthetic
140 enzymes and transcription factors, and Rubisco enzyme activity corroborated the leaf
141 photosynthesis differences across cultivated and wild rice.
142
143
144
145
146

147 **Material and Methods**

148 *Plant material*

149 Ten rice genotypes, consisting of four varieties of *Oryza sativa* ssp. *indica* (IR 64, Swarna,
150 Nagina 22, and Vandana), a variety from *Oryza sativa* ssp. *japonica* (Nipponbare), African
151 cultivated rice *Oryza glaberrima*, and four wild rice species, *Oryza rufipogon, Oryza*
152 *punctata, Oryza latifolia,* and *Oryza australiensis* were grown in Kharif 2016 and 2017 at
153 NIPGR research farm (Table S1). These genotypes were also grown in pots under controlled
154 growth chambers with the temperature (28 ºC), humidity (70-90 %), and day length (14h: 10h,
155 light: dark). Physiological, developmental, photochemical, and biochemical traits were
156 evaluated at heading stage in the widest part of the flag leaves.

157

158 *Photosynthesis rate and related physiological trait measurements*

159 Flag leaf photosynthesis was measured at the full heading stage with a portable
160 photosynthesis system (LI-6400XT equipped with a 6400-40 Leaf Chamber Fluorometer (LI-
161 COR Biosciences). Measurement was conducted on a clear day between 9.00 h to 11.00 h
162 under ambient CO_2 level (400 µmol mol^{-1}), air temperature (30 ºC), leaf temperature (27 ºC),
163 and PAR (1,000 µmol m^{-2} s^{-1}) conditions. Net photosynthesis rate (P_N), and related
164 physiological parameters such as stomatal conductance (g_s), intercellular CO_2 concentration
165 (Ci), transpiration rate (E), water use efficiency (WUE; ratios P_N : E), and carboxylation
166 efficiency (CE; ratios P_N : Ci) were recorded once the expanded leaf was enclosed in the LI-
167 COR leaf chamber. In order to examine the effect of light intensity and CO_2 concentration,
168 leaf photosynthesis was measured at different photosynthetically active radiations (PAR; 0,
169 300, 500, 700, 1000, 1200, and 1500 µmol m^{-2} s^{-1}), and at intercellular CO_2 concentration (Ci;
170 0, 400, 600, 800, 1000, 1200, and 1400 µmol mol^{-1}). Further, leaf photosynthesis was
171 measured at every hour from 9.00 h to 15.00 h to examine the diurnal effect on P_N for the
172 selected cultivated and wild rice as described in Feldman *et al.* (2017).

173

174 *Visualization and quantification of leaf developmental traits*

175 Morphological trait measurements such as flag leaf width (FLW), leaf thickness (FLT) was
176 carried out manually from the widest part of the leaves from ten individual rice plants for
177 each accession. For anatomical traits visualization, thin sections were cut manually and
178 stained with toluidine blue with some modification of the method as described in Lux *et al.*,
179 2005. Briefly, thin sections were mounted with 50 % glycerol on a tissue culture plate,
180 chlorophyll pigments were cleared using 85 % (w/v) lactic acid saturated with chloral hydrate,

181 heated on a water bath at 70 ºC for 1 hour and washed several times with distilled water. To
182 visualize mesophyll cells, sections were incubated in absolute alcohol for overnight, stained
183 with 1% toluidine blue in 1% (w/v) disodium tetraborate for 15 sec. Finally, thin sections
184 were visualized using a bright field microscope (LMI, UK). The quantification of leaf
185 anatomical features was performed using Fiji-Image J software (Wayne Rasband, National
186 Institute of Health, USA) as described in Chatterjee *et al.* (2016).
187
188 *Chloroplast ultrastructure*
189 Freshly harvested flag leaf segments (1 × 1 mm) were fixed under fixative buffer (3%
190 glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2) for 2 hours and then treated with 1%
191 osmium tetroxide overnight at 4 ºC. The fixed segments were dehydrated in a graded acetone
192 series and embedded in epoxy resin (Spurr, 1969). Ultrathin sections were cut with diamond
193 knives, stained with uranyl acetate and lead citrate double staining procedure as described in
194 Reynolds, 1963. Chloroplasts of the uppermost part of the leaf sections were viewed using a
195 digital camera (BH-2, Olympus) equipped in transmission electron microscopy at 1000X
196 magnification (JEM 1230; JEOL).
197
198 *Leaf chlorophyll measurement*
199 Total chlorophyll content (TCC) were measured in 80 % acetone following the procedure of
200 Kiran *et al.* (2013). Briefly, flag leaf (50 mg) was incubated in 5 ml ice-cold 80 % acetone for
201 48 h, centrifuged at 5,000 × g for 5 min and absorbance was recorded at 663 nm and 645 nm.
202 The pigment concentration was calculated according to (Lichtenthaler & Wellburn, 1983)
203 and expressed in mg g^{-1} FW.
204
205 *Leaf Chlorophyll Fluorescence Measurements*
206 Leaf chlorophyll (chl) fluorescence was measured using the LI-6400XT fluorometer (LI-
207 COR Biosciences) according to the method described by Toth *et al.* (2011). Plants were
208 grown under controlled growth conditions, dark-adapted for at least 45 min before the
209 measurement of F_v/F_m (ratio of the variable to maximum fluorescence - the quantum
210 efficiency of open PSII centers). After the initial or minimum fluorescence (F_o) was measure,
211 plants were illuminated with a 0.8 sec saturated pulse of light provided by an array of light-
212 emitting diodes (LEDs) [mixture of red (90 %) and blue (10 %) lights at 650 nm peak
213 wavelength, 1000 µmol m^{-2} s^{-1}] focused on a circular 2.0 cm^2 leaf surface area. Maximum
214 fluorescence (F_m) was measured, and F_v/F_m calculated as (F_m-F_o/F_m). Plants were then placed

215 under natural irradiance, and the steady-state of fluorescence (F_s) was recorded. A second
216 saturating pulse at 1000 µmol m^{-2} s^{-1} was imposed to determine the maximal fluorescence
217 level in light-adapted leaves (F_m'). Then, the actinic light was removed, and the minimal
218 fluorescence level in the light-adapted state (F_o') was determined through of illuminating the
219 leaf with 1 sec of far-red light. Using light fluorescence parameters, we calculated: (a) ratio
220 of variable to maximum fluorescence - the quantum efficiency of open PSII centers (F_v/F_m) in
221 dark-adapted state, (b) the actual PSII efficiency (φPSII), (c) photochemical quenching
222 coefficient (qP), and (d) the photosynthetic electron transport rate (ETR) in light-adapted
223 state as described in Maxwell & Johnson (2000).
224
225 *RNA isolation and expression analysis*
226 Total RNA was isolated from flag leaf and leaf 4 at primordial 4 stages (P4) (van Campen *et*
227 *al.,* 2016). Tissues were harvested, immediately frozen in liquid nitrogen. Total RNA
228 extraction (TRIzol reagent), DNase treatment (DNase enzyme), and cDNA synthesis
229 (RevertAid First-strand cDNA synthesis kit) were performed according to the manufacturer
230 protocol (Thermo Fisher Scientific, USA). The PowerUp™ SYBR® Green fluorescence dye
231 and specific primer sets (Table S6) were used for quantitative gene expression analysis on
232 CFX connect Real-time PCR detection system (Bio-Rad, CA, USA). The expression analysis
233 was confirmed by analyzing two biological replicates, with three technical repeats each. Rice
234 actin was used as an internal control gene for normalization of gene expression. The relative
235 change or fold change was calculated following the method of (Livak & Schmittgen, 2001).
236
237 *Rubisco activation assay*
238 Rubisco extraction and activity (Vcmax) was performed following the procedure described in
239 Kubien *et al.* (2011). Briefly, leaf tissue (3.6 × 1.0 cm^2) from illuminated leaves were excised
240 from five plants of each species and homogenized in extraction buffer [EB, 5 mM DTT, 10
241 mg ml^{-1} PVPP, 2 mg ml^{-1} BSA, 2 mg ml^{-1} PEG, 2% (v/v) Tween-80, 12 mM 6-aminocaproic
242 acid, 2.4 mM benzamidine in HEPES stock) at 4 ºC. The homogenate centrifuged for 1 min
243 at ~16,000 × g and pellet was discarded. To assess the activity of Rubisco from leaf extract,
244 50 µl of leaf extract was incubated with 1,550 ml of the EME buffer, 40 µl 10 mM of NADH
245 (final concentration 200 µM), 80 µl of 250 mM $NaH^{12}CO_3$ (10 mM), 200 µl of
246 ATP/phosphocreatine (1 and 5 mM, respectively), 40 µl of coupling enzymes (Carbonic
247 anhydrase 500U, Glyceraldehyde-3-P dehydrogenase 50U, 3-Phosphoglycerate kinase 50U,
248 Triose-P Isomerase/Glycerol-3-P dehydrogenase 400/40U), 40 µl of 25 mM RuBP (500 µM),

249 mixed gently, and monitored to A_{340} nm continuously for 0 to 180 sec using a UV–Vis
250 spectrophotometer.
251
252 *Statistical analysis*
253 One-way analysis of variance (ANOVA) followed by statistical significance were grouped
254 according to post-hoc Tukey HSD calculation (*P < 0.05, **P < 0.01). Least significant
255 difference was calculated by Fisher's test (α = 0.05). Pearson correlation matrices were
256 calculated based on mean values of each leaf developmental and physiological trait for each
257 accession to evaluate the trait-to-trait associations. The level of significance (α) of correlation
258 coefficients was determined at *P < 0.05 (± 0.666; degree of freedom (df; n-2; 9-2 = 7).
259
260 **Results**
261 **Quantification of leaf photosynthesis and physiological parameters**
262 Cultivated and wild rice species showing remarkable differences for biomass
263 accumulation for the same crop growth period under field conditions as well as controlled
264 growth chambers were examined for leaf photosynthesis (P_N) and related physiological
265 features (Fig. S1). The mean P_N value for the wild rice species, *O. latifolia*, *O. australiensis*,
266 and *O. rufipogon*, and African cultivated rice *Oryza glaberrima* were found to be
267 significantly higher than the cultivated rice varieties, such as IR64, Nagina 22 and Vandana
268 (Fig. 1a). Among the selected accessions, *O. australiensis* and *O. latifolia* showed highest P_N,
269 23.50 and 24.18 μmol CO_2 m^{-2} s^{-1}, respectively. Also, we observed remarkable variations for
270 P_N among the cultivated varieties. For instance, one of the high yielding *indica* variety
271 Swarna and *japonica* variety Nipponbare displayed higher P_N compared to other cultivated
272 varieties.
273 We further examined other important physiological traits of the selected accessions.
274 As expected, wild rice species, *O. latifolia* and *O. australiensis* with very high P_N, showed
275 higher stomatal conductance (g_s), and intercellular CO_2 concentration (Ci) (Fig. 1b,c). In
276 addition, these species along with *O. rufipogon* displayed higher carboxylation efficiency
277 (CE) than those of cultivated rice (Fig. 1f). Interestingly, *O. australiensis*, one of the two wild
278 species with highest P_N, had relatively lower transpiration rate (E), resulting in highest water
279 use efficiency (WUE) for the species (Fig. 1d,e). Wild species *O. punctata* and *O. sativa* ssp
280 *indica* cv. Vandana also exhibited lower E compared to other genotypes (Fig. 1d), and
281 corresponding higher WUE of Vandana (Fig. 1e). WUE of *O. punctata* was still relatively
282 lower due to lower P_N of the species (Fig. 1a,e).

283 Pearson correlation analysis showed a strong positive correlation between P_N with g_s
284 (*r = 0.665*), Ci (*r = 0.632*), and CE (*r = 0.656*) (Fig. S2). Higher WUE results from higher
285 P_N coupled with lower E, which is evident from the strong positive correlation between WUE
286 and P_N (*r = 0.618*) and a negative correlation between WUE and E (r = *-0.589*). A poor
287 correlation was observed between P_N and E (*r = 0.248*) (Fig. S2). We, further, quantified leaf
288 photosynthesis and related physiological traits at early vegetative stage under controlled
289 conditions in two independent experiments. Similar to our observations at full heading stage
290 under field conditions, wild rice species (excluding *O. punctata*) and *O. glaberrima* showed
291 higher P_N and g_s compared to cultivated rice since the beginning of vegetative stage (Table
292 S2). Taken together, these results showed significant differences in photosynthetic traits
293 across cultivated and wild rice, with *O. australiensis* showing higher WUE along with
294 remarkably higher leaf photosynthesis than cultivated rice varieties. We then selected
295 representative cultivated rice accessions (IR 64, Nipponbare, and *O. glaberrima*) and wild
296 rice species (*O. rufipogon, O.latifolia,* and *O. australiensis)* for detailed analyses.
297
298 **Effects of light intensity, CO_2 concentration, and diurnal time on photosynthesis and**
299 **related physiological traits**
300 Light intensity and CO_2 concentration are the two most important factors determining
301 leaf photosynthesis. Therefore, we examined the effect of different light intensity and CO_2
302 concentration on P_N, g_s, E, WUE, P_N (max), Ci and CE of selected wild and cultivated rice
303 accessions (Fig. 2; Fig. S3). All the species showed a steeper initial slope, instantaneous
304 enhancement followed by a plateau for leaf photosynthesis per unit area with an increasing
305 photosynthetically active radiation (PAR) and CO_2 concentration (Fig. 2a,b-I). Wild rice
306 species, *O. latifolia* and *O. australiensis*, together with *O. glaberrima* and *O. sativa* cv.
307 Nipponbare exhibited higher P_N at the beginning, with clearly higher P_N at PAR > 1000 µmol
308 m^{-2} s^{-1} (Fig. 2a-I). Similarly, P_N in response to increasing CO_2 concentration segregated the
309 selected genotypes under two distinct groups: *O. australiensis, O. latifolia* and *O. glaberrima*
310 with higher P_N, and cultivated varieties with lower P_N. The genotypes showed the pattern of
311 g_s similar to that of P_N in response to changing light intensity (Fig. 2a-II). The g_s, however,
312 showed a decreasing trend over increasing CO_2 concentration, likely due to the closure of
313 stomata (Fig. 2b-II). Nonetheless, P_N showed an increasing trend at higher concentration of
314 CO_2. Interestingly, wild rice species were able to maintain higher g_s at different light intensity
315 and CO_2 concentration. Cultivated varieties showed increased E, thus reduced WUE, in
316 response to increasing light intensity (Fig. 2a, III –IV). In contrast, the wild species, *O.*

317 *australiensis* and *O. latifolia*, showed lower E, and hence higher WUE, compared to
318 cultivated varieties at different light intensities. Reducing E, and hence increased WUE, was
319 detected for all the genotypes beyond 400 µmol mol^{-1} CO_2 concentration (Fig. 2b, III –IV).
320 The leaf photosynthesis has been shown to peak up before noon, and subsequently
321 decreases (Hirasawa & Hsiao, 1999; Murchie *et al.,* 1999; Gu *et al.,* 2014). In order to
322 investigate the effect of diurnal timing, P_N and other physiological traits were quantified for
323 the selected genotypes at different times of the day (Fig. 2c). P_N increased for all the
324 genotypes until mid-day (9.00 h to 12.00 h) and decline thereafter (12.00 h to 15.00 h) (Fig.
325 2c-I). However, the extent of the decline was lesser in wild rice species, indicating that the
326 wild species were able to maintain higher photosynthetic activity throughout the day. The g_s
327 also followed the similar pattern to that of P_N for the selected species at different times of a
328 day (Fig. 2c-II). Interestingly, E was found to be higher for the wild species in the immediate
329 afternoon (13.00-14.00 h), likely due to leaf and stomatal features (Fig. 2c-III).
330 Taken together, all these results showed that the selected wild rice species were
331 photosynthetically more efficient than cultivated rice varieties at a different light intensity,
332 CO_2 concentration, and time points of a day.
333
334 **Leaf developmental traits underpinning photosynthetic variations**
335 Leaves are the prime photosynthetic organs, and expectedly the selected rice
336 accessions exhibited striking differences in overall leaf architecture (Fig. S1). At the
337 morphological level, cultivated and wild rice species showed considerable variation for flag
338 leaf width (FLW) and flag leaf thickness (FLT) (Table S3). Wild rice species, except *O.*
339 *punctata* that showed lower P_N, had thicker and wider leaves. A strong positive association of
340 P_N were found with FLW ($r = 0.829$) and FLT ($r = 0.806$) (Fig.3a).
341 At the anatomical level, remarkable differences in the vascular architecture and
342 mesophyll cell features were observed. Wild rice species, with higher net photosynthesis, had
343 larger and closely spaced veins with fewer number of mesophyll cells between two
344 consecutive veins (Fig. 3b; Table S3-4). Those wild species with higher P_N also had larger
345 mesophyll cell size (Fig. 3c; Table S4). We further quantified mesophyll area in vegetative
346 stage leaves of the selected accessions. More pronounced mesophyll cells were observed,
347 even in the vegetative stage of the wild rice species with higher P_N, as compared to cultivated
348 varieties (Fig. 3d). The considerable variation observed in mesophyll cell features prompted
349 us to investigate the organization of chloroplasts in mesophyll cells of the representative wild
350 and cultivated leaf (Fig. S4). Wild species with higher P_N exhibited larger and numerous

351 chloroplasts, systematically distributed along the mesophyll cell walls. Wild rice with higher
352 leaf photosynthesis also exhibited a higher value of vein height and width (Fig. 3e; Table S3).
353 Pearson correlation coefficient analysis showed a strong positive correlation of P_N with
354 mesophyll area (*r = 0.905*), mesophyll length (*r = 0.874*), mesophyll width (*r = 0.930*), and
355 mesophyll lobbing (*r = 0.771*) (Fig. 3a). The P_N was also strongly correlated with total
356 number of veins (*r = 0.857*), minor veins height (*r = 0.906*), and minor vein width (*r =*
357 *0.758*) (Fig. 3a). However, P_N was poorly correlated with bundle sheath features, number, and
358 area (Fig. 3a). It was interesting to note that among cultivated rice varieties, a similar
359 connection of photosynthesis with developmental traits was also observed. For example,
360 Nipponbare and Swarna that showed relatively higher P_N compared to other cultivated
361 varieties also showed larger vein height and width (Table S3). Swarna, further, had wider leaf
362 and higher vein number.
363
364 **Photochemical differences among the selected rice accessions**
365 We examined the differences in photochemical features of the selected rice accessions
366 that differ in leaf photosynthesis. Most of the accessions with higher photosynthesis also had
367 higher total chlorophyll content (TCC; *r = 0.54*) (Fig. 4a). The *O. australiensis, O. latifolia*
368 and *O. rufipogon* among wild rice species, and *O. glaberrima*, *O. sativa* cv. *Swarna*, and *O.*
369 *sativa* cv. *Nipponbare* among cultivated rice had higher leaf chlorophyll content. The *O.*
370 *punctata*, despite having highest chlorophyll content, showed lower P_N, suggesting
371 developmental and/or biochemical constraints for efficient photosynthesis in the species
372 (Kura-Hotta *et al.,* 1987; Wang *et al.,* 2016). We, then, quantified chlorophyll fluorescence
373 (F_v/F_m) of dark-adapted leaves to determine the differences in quantum photochemical
374 efficiency of Photosystem II (PSII). Significant variations for F_v/F_m, which ranged from 0.73
375 to 0.83, were found across the selected cultivated and wild rice species (Fig. 4b). Wild rice
376 species, *O. australiensis*, and *O. latifolia*, and *O. glaberrima* had higher F_v/F_m, indicating the
377 better quantum yield of PSII chemistry in those species. *O. latifolia* and *O. australiensis*
378 showed a significantly higher value of efficiency of PSII (ϕPSII), photochemical quenching
379 (qP), and electron transport rate (ETR) compared to other accessions (Fig. 4c-e). *O.*
380 *glaberrima*, the African cultivated rice with reasonably higher P_N, also showed relatively
381 higher ϕPSII, qP, and ETR than *O. sativa* cultivated varieties. A strong correlation was
382 observed between P_N with F_v/F_m (*r = 0.789*), ϕPSII (*r =0.981*), qP (*r = 0.980*), and ETR (*r =*
383 *0.981*) (Fig. S5). These results showed that photochemical features of wild rice species, as
384 well as *O. glaberrima*, contribute to the higher leaf photosynthesis in those species.

385 **Expression pattern of genes encoding core photosynthetic and chlorophyll biosynthesis**
386 **enzymes**
387 To envisage any differences in photosynthetic gene expression across the genotype,
388 we checked the expression pattern of representative genes encoding key photosynthetic
389 components, LHC1 (Light harvesting chlorophyll protein complex), Rubisco LS (Large
390 Subunit), Rubisco SS (Small Subunit), and TPT (Triose phosphate translocator) (Table S5).
391 The expression of the selected photosynthetic genes was significantly higher in wild rice
392 species (Fig. 5a), suggesting higher photosynthesis in those species could, at least in part, be
393 due to the higher abundance of these gene products. These genes could possibly increase net
394 photosynthetic efficiency by increasing the efficiency of light capture, CO_2 assimilation, and
395 transport of triose phosphate from chloroplast to cytoplasm. Since wild rice species with
396 higher photosynthesis also showed higher chlorophyll content, we checked the expression
397 level of chlorophyll biosynthesis genes. The expression of chlorophyll biosynthesis genes,
398 such as *GluTR*, *PPOX*, *Mg-CHLD*, *POR*, and *CHLSYN*, were found to be significantly higher
399 in *O. latifolia* and *O. australiensis* (Fig. 5b). *O. rufipogon* also had significantly higher
400 expression of selected photosynthetic and chlorophyll-biosynthetic genes compared to
401 Nipponbare and IR 64.
402 We then investigated the involvement of transcription factors in the regulation of
403 expression of the selected photosynthetic and chlorophyll-biosynthetic genes. A
404 comprehensive list of transcription factors regulating photosynthetic and chlorophyll
405 biosynthetic-genes has been reported in (Sakuraba *et al.,* 2017; Wang *et al.,* 2017; Yuan *et al.,*
406 2017). The differentially expressed photosynthetic and chlorophyll biosynthetic-genes were
407 hierarchically clustered with the expression of potential transcription factors in rice using
408 Genevestigator (https://genevestigator.com/gv/). We found a number of transcription factors
409 co-expressed with the selected differentially expressed genes in flag leaves (Fig. S6). We,
410 then, selected ten such transcription factors and profiled their gene expression at primordia 4
411 (P4) stage of leaf 4 (Table S5). At least six of those ten genes, including *GLK1*, *GLK2*, and
412 *SIGMA* factors, were expressed at higher levels in wild rice with higher leaf photosynthesis
413 (Fig. 5c). *O. glaberrima* and *O. sativa cv. Nipponbare*, with moderately high P_N, also showed
414 higher expression of some of these transcription factors. Differential expression of these
415 transcription factors suggested that differences in the transcriptional module regulating
416 photosynthetic gene expression could be critical for determining leaf photosynthetic
417 efficiency.
418

419 **Rubisco activity differences across the selected rice genotypes**

420 The catalytic properties of Rubisco vary considerably from diverse plant sources,
421 suggesting that differences in the Rubisco activity might contribute to differences in
422 photosynthetic activity (Parry *et al.,* 2003, 2013; Carmo-Silva *et al.,* 2015). To test this
423 hypothesis, we measured the Rubisco activity across selected cultivated and wild rice species.
424 The CO_2-saturated RuBP use and rate of Rubisco carboxylation activity (Vcmax) was found
425 to be significantly higher for wild rice species, *O. latifolia*, *O. australiensis*, and *O. rufipogon*,
426 compared to *Oryza sativa cv.* IR 64 (Fig. 5d,e). Interestingly, *Oryza sativa cv. Nipponbare*, a
427 *japonica* variety, also had higher Rubisco activity compared to *indica* variety IR 64. The
428 photosynthesis rate per unit area, as discussed earlier, was found to be higher in wild rice
429 species as well as Nipponbare compared to IR 64 (Fig. 1a and 5e). Overall, these results
430 showed the existence of variation in Rubisco carboxylation activity within the rice system,
431 cultivated and wild rice, which was also reflected at the level of leaf photosynthetic
432 differences. This possibility of the existence of Rubisco with higher carboxylation activity
433 from the rice system itself could possibly be exploited for increasing CO_2 assimilation.

434

435 **Discussion**

436 The importance of investigating variations in leaf photosynthesis within and across
437 species to be exploited for sustainable increases in crop yield and productivity has been
438 emphasized (Parry *et al.*, 2018; Adachi *et al.*, 2019; van Bezouw *et al.*, 2019). A number of
439 studies have shown the variation in photosynthesis and related traits within species, such as
440 maize (Yabiku & Ueno, 2017), wheat (Driever *et al.*, 2014), rice (Adachi *et al.*, 2014; Qu *et*
441 *al.*, 2017), soybean (Gilbert *et al.*, 2011), and even C_4 species *Gynandropsis gynandra*
442 (Reeves *et al.*, 2018). Extensive comparative genetic and genomic studies have been
443 undertaken to investigate the variation in photosynthetic efficiency across C_3 and C_4 species
444 (Bräutigam *et al.*, 2011; Külahoglu *et al.*, 2014; Wang *et al.*, 2014). Based on these studies,
445 global efforts to engineer C_4 photosynthesis in rice are being made, which is challenging due
446 to extensive genetic, developmental, and biochemical differences between C_4 plants and rice.
447 An alternative and efficient strategy would be exploiting the photosynthetic variations within
448 rice system itself, including cultivated and wild rice. There had been a few studies
449 highlighting the photosynthetic differences within cultivated, and among cultivated and wild
450 rice accessions (Kiran *et al.,* 2013; Kondamudi *et al.*, 2016; Rathore *et al.,* 2016; Haritha *et*
451 *al.,* 2017; Sung *et al.,* 2017). In this study, we not only quantified leaf photosynthetic
452 differences across cultivated and wild rice accessions, but also identified the underlying

453 anatomical, biochemical, and photochemical traits mediating higher leaf photosynthesis in
454 rice. In addition, we evaluated the relationship between photosynthesis and other
455 physiological traits, such as gas exchange, carboxylation efficiency, transpiration, and water
456 use efficiency for the wild and cultivated rice accessions.

457 Wild relatives of rice, *Oryza australiensis*, *Oryza latifolia*, and *Oryza rufipogon*, as
458 well as African domesticated rice *Oryza glaberrima* showed higher leaf photosynthesis per
459 unit area (P_N) compared to cultivated varieties of *Oryza sativa* (Fig. 1a). Stomatal
460 conductance (g_s) is known to be an important factor determining the leaf photosynthesis rate
461 (Kusumi *et al.,* 2012; Gago *et al.,* 2016; Xu *et al.,* 2019). Two wild rice species, *O.*
462 *australiensis* and *O. latifolia*, with highest P_N also had highest values of g_s, which in turn
463 resulted in higher internal CO_2 concentration (Ci) in those species (Fig. 1b,c), supporting a
464 strong correlation between P_N and g_s (Kanemura *et al.,* 2007; Giuliani *et al.*, 2013). *O.*
465 *rufipogon* with reasonably higher P_N, however, had lower g_s and Ci. The higher P_N in *O.*
466 *rufipogon* was likely due to highest carboxylation efficiency (CE) in the species among the
467 selected accessions (Fig. 1f). Further, *O. australiensis* and *O. latifolia* also had reasonably
468 higher CE. The P_N has been shown to be positively associated with CE in rice (Yeo *et al.,*
469 1994; Ding *et al.,* 2015). Indeed, quantification of photosynthesis and related physiological
470 parameters for introgressions from *O. rufipogon* in a cultivated background showed the
471 influence of both CE as well as g_s on higher leaf photosynthesis (Haritha *et al.*, 2017).
472 Furthermore, higher g_s and CE could also explain higher P_N across different PAR in the wild
473 rice (Fig 2a-II, S3a-III). Higher P_N in the wild species at different times of a day, even in the
474 afternoon when stomata tend to close (Condon *et al.*, 2004; Takai *et al.,* 2013), compared to
475 cultivated varieties could also be attributed to relatively higher g_s and CE in those species
476 (Fig. 2c, S3c). Notably higher photosynthetic efficiency in the wild rice species suggests that
477 leaf photosynthesis in cultivated rice has not yet reached the saturation level, and therefore,
478 can possibly be achieved by targeting higher g_s and/or higher CE. Moreover, increments in g_s
479 might also help in optimizing photosynthetic performance under environmental stress
480 conditions (Zheng *et al.*, 2011). *O. australiensis*, interestingly, appeared to be the most water-
481 use efficient among the selected accessions due to high P_N coupled with low transpiration rate
482 (E), making it an ideal genotype to investigate for maintaining higher leaf photosynthesis
483 under drought conditions (Fig. 1a,d,e). Many of the wild rice species, including *O.*
484 *australiensis*, have been marked for their potential usage towards tolerance to heat and
485 drought (Tian *et al.,* 2011; Sanchez *et al.,* 2013; Singh *et al.,* 2015; Menguer *et al.*, 2017).
486 The differences in g_s and E are likely related to differences in the stomatal features or

487 regulation of stomatal opening across the genotypes, as stomatal features are critical for
488 photosynthesis, gas exchange, and water use efficiency (Farquhar & Sharkey, 1982).
489 Kondamudi *et al.* (2016) showed that leaves with larger-sized stomata had higher
490 photosynthesis rate than the leaves with smaller-sized stomata due to higher g_s. Similarly,
491 rice species with broader leaves have been shown to exhibit higher P_N due to increased g_s
492 than species with narrow leaves (Farooq *et al.,* 2010; Giuliani *et al.,* 2013). Differences in
493 internal CO_2 concentration and/or biochemical differences, such as Rubisco activity, may
494 mediate the observed differences in the CE (Brodribb*,* 1996; Eichelmann & Laisk, 2002).
495 Nonetheless, efforts to increase g_s and CE in the cultivated rice varieties comparable to the
496 high photosynthetic wild species may help in increasing photosynthetic efficiency.

497 The strong correlation of leaf developmental traits with P_N is not surprising
498 considering the strong influence of leaf traits on light perception, gas exchange, CO_2
499 assimilation, and downstream photoassimilate partitioning. We observed a strong positive
500 association of P_N with leaf width and thickness (Fig. 3a). Leaf thickness has been suggested
501 to influence photosynthesis through efficient absorption and utilization of light by
502 homogenizing the leaf internal light distribution as well as maintaining leaf temperature
503 (Hanba *et al.*, 2002; Tholen *et al.,* 2012). The association of leaf width with photosynthetic
504 performance and yield in rice has been observed earlier. *WUSCHEL-RELATED*
505 *HOMEOBOX* (*WOX*) genes *NAL1*, *NAL2*, and *NAL3* have been shown to be associated with
506 increased leaf width, which in turn results in increased photosynthetic efficiency and yield
507 (Cho *et al.,* 2013; Fujita *et al.,* 2013; Ishiwata *et al.*, 2013; Jiang *et al.,* 2015). Introgression
508 of a natural variant of *NAL1* from a *japonica* to *indica* cultivar led to thicker and wider leaves
509 with higher leaf photosynthesis rate and yield (Takai *et al.,* 2013).

510 Rice system also displays significant inter-species variation for anatomical traits,
511 suggesting strong genetic control over leaf anatomical features (Chatterjee *et al.*, 2016).
512 Photosynthetically efficient wild rice species have mesophyll cell features that promote both
513 higher access to available CO_2 as well as efficient light harvesting. Larger sized and lobed
514 mesophyll cells in wild rice species likely provides increased surface area to access more
515 internal CO_2 (Fig. 2c-d), whereas the organized pattern and systematic distribution of
516 chloroplasts in the mesophyll cells of those species may promote efficient light capture to
517 enhance photosynthesis (Fig. S4; Fig. 6). Interestingly, *NAL1* (discussed above) promotes
518 leaf width and thickness by increasing mesophyll cell number and area. An increased vein
519 density has been suggested to facilitate higher leaf photosynthesis by promoting efficient
520 export of photosynthates (Amiard *et al.,* 2005; Feldman *et al.*, 2017). Lesser number of

521 mesophyll cells between two consecutive veins in photosynthetically efficient wild species, a
522 feature similar to C_4 plants, should be promoting efficient transport of photoassimilates.
523 Larger vein size in the wild rice species shall, further, be adding to efficient transport, thus
524 increasing the leaf photosynthesis. The larger mesophyll cells with bigger chloroplasts and
525 larger veins in photosynthetically efficient wild rice species are reminiscent of "Proto-kranz
526 anatomy," an evolutionary step towards C_4 photosynthesis and have been suggested to be an
527 essential step towards increasing photosynthetic efficiency (Osborne & Sack, 2012).
528 Photosynthetically efficient wild rice species with desirable anatomical features provide a
529 potential genetic resource towards the identification of genes for desirable manipulation in
530 mesophyll and vein features.

531 Wild species with higher P_N showed significantly higher F_v/F_m compared to cultivated
532 varieties, indicating better quantum efficiency of Photosystem II in those species. The F_v/F_m
533 is used as the most common parameter for monitoring 'stress' in leaves as stress results in
534 inactivation damage of PSII by lowering of F_v/F_m (Murchie & Niyogi, 2011). The
535 comparative higher values for quantum efficiency of PSII (ϕPSII), photochemical quenching
536 (qP), and Electron Transport Rate (ETR) in the wild rice indicate PSII reaction centres are
537 more receptive for electron transport from PSII to PSI, making an efficient link between light
538 and light-independent reactions to increase P_N (Kramer & Evans, 2011). A positive
539 correlation has been reported between chlorophyll fluorescence in leaf and grain yield (von
540 Kroff *et al.,* 2008). The wild species with better photochemical features and higher P_N
541 accumulates more biomass, and not grain yield, likely due to defects in photoassimilate
542 partitioning to the grain. Nonetheless, the desirable photochemical features, at least in part,
543 contribute to higher photosynthetic efficiency of the wild species. Gene expression studies
544 also showed higher expression of *LHC1* gene, which is involved in organization of reaction
545 centre, in photosynthetically efficient wild species.

546 Rubisco is the rate-limiting enzyme for photoassimilation of CO_2, and abundance and
547 activity of Rubisco strongly determines the photosynthetic performance of a plant (Sage,
548 2002; Parry *et al.,* 2013). High photosynthetic wild species had higher expression of genes
549 encoding Rubisco large subunit and small subunit, indicating the higher abundance of
550 Rubisco in those species (Fig. 5a). Those wild species also had higher expression of
551 chlorophyll biosynthetic genes. The photosynthetic process as such, and genes encoding key
552 components of photosynthesis are under strong regulation of transcription factors (Saibo *et*
553 *al.*, 2009; Wang *et al.*, 2017). Differences in the expression of transcription factors, such as
554 *GLKs* involved regulation of chlorophyll biosynthesis and *SIGMA* factors regulating the

chloroplastic gens *Rubisco LS*, suggested the rewiring of transcriptional module regulating photosynthesis as a potential target to increase rice leaf photosynthesis. Moreover, we also observed a higher Rubisco carboxylation activity (Vcmax) in photosynthetically efficient wild species (Fig. 5d-e). The prospects of improving Rubisco catalysis to increase photosynthetic efficiency have been explored (Sharwood, 2017). It has been suggested that transplanting maize Rubisco in rice would improve on CO_2 assimilation (Sharwood *et al.,* 2016). This study suggests a possible existence of an efficient Rubisco in the rice system itself, photosynthetically efficient wild rice. Further studies are needed to characterize the sequence differences in Rubisco subunits across the cultivated and wild rice, and if those sequence differences contribute to Rubisco carboxylation activity.

In summary, we characterized photosynthetically efficient wild rice species for leaf developmental, photochemical, and biochemical differences. Leaf developmental features (larger and lobbed mesophyll cells, larger veins, and lesser number of mesophyll cells between two consecutive veins), photochemical features (quantum efficiency of PSII, Photochemical quenching, and Electron Transport Rate), Rubisco activity contributes to higher leaf photosynthesis in wild rice (Fig. 6). The study highlights the existence of significant exploitable variation in leaf photosynthesis and underlying traits across cultivated and wild rice. Future efforts to dissect the genetic and molecular basis of the underlying developmental, photochemical, and biochemical traits shall be instrumental in tailoring photosynthetically efficient rice.

Acknowledgments

AR acknowledges Ramalingaswamy Re-entry Fellowship and Innovative Young Biotechnologist Award (IYBA) from the Department of Biotechnology, Ministry of Science and Technology, India. JM, AS, and VJ acknowledge their CSIR-JRF, SERB-NPDF, and CSIR-UGC, fellowships, respectively. The core funding from National Institute of Plant Genome Research is duly acknowledged. Seeds of wild rice species and *Oryza glaberrima* were kindly provided by Dr. Kuldeep Singh and Dr. Kumari Neelam, Punjab Agricultural University, Ludhiana, India.

Author contributions

JM and AR conceptualized the study. JM, AS, and AR designed experiments. JM, AS, and VJ performed experiments, analysed data, and compiled figures. AS and AR wrote the manuscript. All authors contributed to and edited the final manuscript.

589 **References**

590 **Adachi S, Baptista LZ, Sueyoshi T, Murata K, Yamamoto T, Ebitani T, Ookawa T,**
591 **Hirasawa T. 2014.** Introgression of two chromosome regions for leaf photosynthesis from an
592 indica rice into the genetic background of a japonica rice. *Journal of Experimental Botany*
593 **65**: 2049–2056.

594 **Adachi S, Yamamoto T, Nakae T, Yamashita M, Uchida M**. **2019**. Genetic architecture of
595 leaf photosynthesis in rice revealed by different types of reciprocal mapping populations.
596 *Journal of Experimental Botany* **doi:10.1093/jxb/erz303.**

597 **Alexandratos. N, Bruinsma J. 2012.** World agriculture towards 2030/2050: the 2012
598 revision Global Perspective Studies Team FAO Agricultural Development Economics
599 Division. *Agricultural Development Economics (ESA) The Food and Agriculture*
600 *Organization of the United Nations Viale delle Terme di Caracalla 00153 Rome, Italy*.

601 **Amiard V, Mueh KE, Demmig-Adams B, Ebbert V, Turgeon R, Adams WW III. 2005.**
602 Anatomical and photosynthetic acclimation to the light environment in species with differing
603 mechanisms of phloem loading. *Proceedings of the National Academy of Sciences USA* **102**:
604 12968–12973.

605 **Atwell BJ, Wang H, Scafaro AP. 2014.** Could abiotic stress tolerance in wild relatives of
606 rice be used to improve *Oryza sativa*? *Plant science* **215–216**: 48–58.

607 **Brodribb T. 1996**. Dynamics of Changing Intercellular CO_2 Concentration (c_i) during
608 Drought and Determination of Minimum Functional c_i. *Plant Physiology* **111**: 179-185.

609 **Brodribb TJ, Feild TS, Jordan GJ**. **2007**. Leaf Maximum Photosynthetic Rate and
610 Venation Are Linked by Hydraulics. *Plant Physiology* **144**: 1890–1898.

611 **Buchanan BB, Holmgren A, Jacquot JP, Scheibe R. 2012.** Fifty years in the thioredoxin
612 field and a bountiful harvest. *Biochimica et Biophysica Acta - General Subjects* **1820**: 1822–
613 1829.

614 **Bräutigam A, Kajala K, Wullenweber J, Sommer M, Gagneul D, Weber KL, Carr KM,**
615 **Gowik U, Mass J, Lercher MJ, *et al.* 2011**. An mRNA blueprint for C_4 photosynthesis
616 derived from comparative transcriptomics of closely related C_3 and C_4 species. *Plant*
617 *Physiology* **155**:142-56.

618 **Cardona T, Shao S, Nixon PJ. 2018.** Enhancing photosynthesis in plants: the light reactions.
619 *Essays In Biochemistry* **62**: 85–94.

620 **Carmo-Silva E, Scales JC, Madgwick PJ, Parry MAJ. 2015.** Optimizing Rubisco and its
621 regulation for greater resource use efficiency. *Plant, Cell and Environment* **38**: 1817–1832.

622 **Chatterjee J, Dionora J, Elmido-Mabilangan A, Wanchana S, Thakur V,**

623 **Bandyopadhyay A, Brar DS, Quick WP. 2016.** The evolutionary basis of naturally diverse
624 rice leaves anatomy. *PLoS ONE* **11**: 1–25.
625 **Cho SH, Yoo SC, Zhang H, Pandeya D, Koh HJ, Hwang J et al. 2013.** The rice *NARROW*
626 *LEAF 2* and *NARROW LEAF 3* loci encode WUSCHEL-related homeobox 3A (OsWOX3A)
627 and function in leaf, spikelet, tiller and lateral root development. *New Phytologist* **198**: 1071–
628 1084.
629 **Condon AG, Richards RA, Rebetzke GJ, Farquhar GD. 2004.** Breeding for high water-
630 use efficiency. *Journal of Experimental Botany* **55**: 2447–2460.
631 **Ding ZS, Sun XF, Huang SH, Zhou BY, Zhao M**. **2015**. Response of photosynthesis to
632 short-term drought stress in rice seedlings overexpressing C_4 phosphoenolpyruvate
633 carboxylase from maize and millet. *Photosynthetica* **53**: 481–488.
634 **Driever SM, Lawson T, Andralojc PJ, Raines CA, Parry MAJ. 2014.** Natural variation in
635 photosynthetic capacity, growth, and yield in 64 field-grown wheat genotypes. *Journal of*
636 *Experimental Botany* **65**: 4959–4973.
637 **Eichelmann H, Laisk A. 2002.** Ribulose-1,5-bisphosphate carboxylase/oxygenase content,
638 assimilatory charge, and mesophyll conductance in leaves. *Plant Physiology* **119**: 179–190.
639 **Evans JR. 2013.** Topical reviews on photosynthesis improvement: Improving photosynthesis.
640 *Plant Physiology* **162**: 1780–1793.
641 **Farooq M, Kobayashi N, Ito O, Wahid A, Serraj R**. **2010**. Broader leaves result in better
642 performance of indica rice under drought stress. *Journal of Plant Physiology* **167**: 1066–1075.
643 **Farquhar GD, Sharkey TD. 1982.** Stomatal conductance and photosynthesis. *Annual*
644 *Review of Plant Physiology* **33**: 317–345.
645 **Feldman AB, Leung H, Baraoidan M, Elmido-mabilangan A. 2017.** Increasing leaf vein
646 density via mutagenesis in rice results in an enhanced rate of photosynthesis, smaller cell
647 sizes and can reduce interveinal mesophyll cell number. *Frontier in Plant Science* **8**: 1–10.
648 **Fermani S, Sparla F, Falini G, Martelli PL, Casadio R, Pupillo P, Ripamonti A, Trost P.**
649 **2007.** Molecular mechanism of thioredoxin regulation in photosynthetic A2B2-
650 glyceraldehyde-3-phosphate dehydrogenase. *Proceedings of the National Academy of*
651 *Sciences USA* **104**: 11109–11114.
652 **Fitter DW, Martin DJ, Copley MJ, Scotland RW, Langdale JA**. **2002**. GLK gene pairs
653 regulate chloroplast development in diverse plant species. *Plant Journal* **31**: 713–727.
654 **Flexas J, Ribas-Carbó M, Diaz-Espejo A, Galmés J, Medrano H**. **2008**. Mesophyll
655 conductance to CO_2: Current knowledge and future prospects. *Plant, Cell and Environment*
656 **31**: 602–621.

Flood J, Harbinson J, Aarts MGM. **2011**. Natural genetic variation in plant photosynthesis. *Trends in Plant Science* **16**: 327–335.

Fujita D, Trijatmiko KR, Tagle AG, Sapasap M V., Koide Y, Sasaki K, Tsakirpaloglou N, Gannaban RB, Nishimura T, Yanagihara S, *et al.* 2013. *NAL1* allele from a rice landrace greatly increases yield in modern indica cultivars. *Proceedings of the National Academy of Sciences USA* **110**: 20431–20436.

Furbank RT, Quick WP, Sirault XRR. **2015**. Improving photosynthesis and yield potential in cereal crops by targeted genetic manipulation: Prospects, progress and challenges. *Field Crops Research* **182**: 19–29.

Gago J, Daloso Dde M, Figueroa CM, Flexas J, Fernie AR, Nikoloski Z. 2016. Relationships of leaf net photosynthesis, stomatal conductance, and mesophyll conductance to primary metabolism: a multispecies meta-analysis approach. *Plant Physiology* **171**: 265–279.

Gilbert ME, Zwieniecki MA, Holbrook NM. 2011. Independent variation in photosynthetic capacity and stomatal conductance leads to differences in intrinsic water use efficiency in 11 soybean genotypes before and during mild drought. *Journal of Experimental Botany* **62**: 2875- 2887.

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.

Gu J, Yin X, Stomph TJ, Struik PC. 2014. Can exploiting natural genetic variation in leaf photosynthesis contribute to increasing rice productivity? A simulation analysis. *Plant, Cell and Environment* **37**: 22–34.

Hanba YT, 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.

Haritha G, Vishnukiran T, Yugandhar P, Sarla N, Subrahmanyam D. **2017**. Introgressions from *Oryza rufipogon* increase photosynthetic efficiency of KMR3 rice lines. *Rice Science* **24**: 85–96.

Häusler RE, Hirsch HJ, Kreuzaler F, Peterhänsel C. **2002**. Overexpression of C_4-cycle enzymes in transgenic C_3 plants: A biotechnological approach to improve C_3-photosynthesis. *Journal of Experimental Botany* **53**: 591–607.

Hirasawa T, Hsiao TC. **1999**. Some characteristics of reduced leaf photosynthesis at midday in maize growing in the field. *Field Crops Research* **62**: 53–62.

691 **Hiyama A, Takemiya A, Munemasa S, Okuma E, Sugiyama N, Tada Y, Murata Y,**
692 **Shimazaki KI. 2017.** Blue light and CO_2 signals converge to regulate light-induced stomatal
693 opening. *Nature Communications* **8**: 1284.
694 **Högy P, Brunnbauer M, Koehler P, Schwadorf K, Breuer J, Franzaring J,**
695 **Zhunusbayeva D, Fangmeier A**. **2013**. Grain quality characteristics of spring wheat
696 (*Triticum aestivum*) as affected by free-air CO_2 enrichment. *Environmental and Experimental*
697 *Botany* **88**: 11–18.
698 **Horton P, Ruban A**. **2005**. Molecular design of the photosystem II light-harvesting antenna:
699 Photosynthesis and photoprotection. *Journal of Experimental Botany* **56**: 365–373.
700 **Huang M, Jiang L, Xia B, Zou Y, Jiang P, Ao H**. **2013**. Yield gap analysis of super hybrid
701 rice between two subtropical environments. *Australian Journal of Crop Science* **7**: 600–608.
702 **Ishiwata A, Ozawa M, Nagasaki H, Kato M, Noda Y, Yamaguchi T, Nosaka M,**
703 **Shimizu-Sato S, Nagasaki A, Maekawa M, *et al.*2013**. Two WUSCHEL-related homeobox
704 genes, *narrow leaf2* and *narrow leaf3*, control leaf width in rice. *Plant and Cell Physiology*
705 **54**: 779–792.
706 **Jansson C, Vogel J, Hazen S, Brutnell T, Mockler T**. **2018**. Climate-smart crops with
707 enhanced photosynthesis. *Journal of Experimental Botany* **69**: 3801–3809.
708 **Jiang D, Fang J, Lou L, Zhao J, Yuan S, Yin L, Sun W, Peng L, Guo B, Li X**. **2015**.
709 Characterization of a null allelic mutant of the rice *NAL1* gene reveals its role in regulating
710 cell division. *PLoS ONE* **10**: 1–19.
711 **Kajala K, Covshoff S, Karki S, Woodfield H, Tolley BJ, Dionora MJA, Mogul RT,**
712 **Mabilangan AE, Danila FR, Hibberd JM, *et al.* 2011**. Strategies for engineering a two-
713 celled C_4 photosynthetic pathway into rice. *Journal of Experimental Botany* **62**: 3001–3010.
714 **Kalaji HM, Jajoo A, Oukarroum A, Brestic M, Zivcak M, Samborska IA, Cetner MD,**
715 **Łukasik I, Goltsev V, Ladle RJ**. **2016**. Chlorophyll a fluorescence as a tool to monitor
716 physiological status of plants under abiotic stress conditions. *Acta Physiologiae Plantarum*
717 **38**: 102.
718 **Kanemura T, Homma K, Ohsumi A, Shiraiwa T, Horie T**. **2007**. Evaluation of genotypic
719 variation in leaf photosynthetic rate and its associated factors by using rice diversity research
720 set of germplasm. *Photosynthesis Research* **94**: 23–30.
721 **Kapralov M V., Smith JAC, Filatov DA. 2012.** Rubisco Evolution in C_4 Eudicots: An
722 Analysis of Amaranthaceae Sensu Lato. *PLoS ONE* **7**: e52974.
723 **Kindgren P, Norén L, Barajas López JDD, Shaikhali J, Strand Å. 2012.** Interplay
724 between HEAT SHOCK PROTEIN 90 and HY5 controls PhANG expression in response to

725 the GUN5 plastid signal. *Molecular Plant* **5**: 901–913.
726 **Kiran T V, Rao Y V, Subrahmanyam D, Rani NS, Bhadana VP, Rao PR. 2013.** Variation
727 in leaf photosynthetic characteristics in wild rice species. *Photosynthetica* **51**: 350–358.
728 **Kobayashi K, Baba S, Obayashi T, Sato M, Toyooka K, Keränen M, Aro E-M, Fukaki**
729 **H, Ohta H, Sugimoto K, *et al.*2012**. Regulation of Root Greening by Light and
730 Auxin/Cytokinin Signaling in Arabidopsis . *The Plant Cell* **24**: 1081–1095.
731 **Kondamudi R, Swamy KN, Rao YV, Kiran TV, Suman K, Rao DS, Rao PR,**
732 **Subrahmanyam D, Sarla N, Ramana Kumari B, *et al.* 2016**. Gas exchange, carbon
733 balance and stomatal traits in wild and cultivated rice (*Oryza sativa L.*) genotypes. *Acta*
734 *Physiologiae Plantarum* **38**: 160.
735 **Koteyeva NK, Voznesenskaya E V., Berry JO, Chuong SDX, Franceschi VR, Edwards**
736 **GE. 2011.** Development of structural and biochemical characteristics of C_4 photosynthesis in
737 two types of Kranz anatomy in genus Suaeda (family Chenopodiaceae). *Journal of*
738 *Experimental Botany* **62**: 3197–3212.
739 **Kramer DM, Evans JR. 2011.** The importance of energy balance in improving
740 photosynthetic productivity. *Plant Physiology* **155**: 70–78.
741 **Kubien DS, Brown CM, Kane HJ. 2011.** Quantifying the amount and activity of rubisco in
742 leaves. *Methods in Molecular Biology* **684**: 349–362.
743 **Külahoglu C, Denton AK, Sommer M, Maß J, Schliesky S, Wrobel TJ, Berckmans B,**
744 **Gongora-Castillo E, Buell CR, Simon R, *et al.* 2014**. Comparative transcriptome atlases
745 reveal altered gene expression modules between two Cleomaceae C_3 and C_4 plant species.
746 *Plant Cell* **26**:3243-60.
747 **Kura-hotta M, Satoh K, Katoh S**. **1987**. Relationship between Photosynthesis and
748 Chlorophyll Content during Leaf Senescence of Rice Seedlings. *Plant and Cell Physiology*
749 **28**: 1321–1329.
750 **Kusumi K, Hirotsuka S, Kumamaru T, Iba K. 2012.** Increased leaf photosynthesis caused
751 by elevated stomatal conductance in a rice mutant deficient in SLAC1, a guard cell anion
752 channel protein. *Journal of Experimental Botany* **63**: 5635– 5644.
753 **Lawson T, Kramer DM, Raines CA. 2012**. Improving yield by exploiting mechanisms
754 underlying natural variation of photosynthesis. *Current Opinion in Biotechnology* **23**: 215–
755 220.
756 **Leong TY, Anderson JM. 1984.** Adaptation of the thylakoid membranes of pea chloroplasts
757 to light intensities. II. Regulation of electron transport capacities, electron carriers, coupling
758 factor (CF1) activity and rates of photosynthesis. *Photosynthesis Research* **5**: 117–128.

759 **Lichtenthaler HK, Wellburn AR**. **1983**. Determinations of total carotenoids and
760 chlorophylls a and b of leaf extracts in different solvents . *Biochemical Society Transactions*
761 **11**: 591–592.
762 **Liu L, Sun G, Ren X, Li C, Sun D**. **2015**. Identification of QTL underlying physiological
763 and morphological traits of flag leaf in barley. *BMC genetics* **16**: 29.
764 **Livak KJ, Schmittgen TD**. **2001**. Analysis of relative gene expression data using real-time
765 quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **25**: 402–408.
766 **Long SP, Zhu X, Naidu SL, Ort DR**. **2006**. Can improvement in photosynthesis increase
767 crop yields ? *Plant, Cell and Environment* **29**: 315–330.
768 **Lux A, Morita S, Abe J, Ito K**. **2005**. An improved method for clearing and staining free-
769 hand sections and whole-mount samples. *Annals of Botany* **96:** 989–996.
770 **Mathan J, Bhattacharya J, Ranjan A. 2016.** Enhancing crop yield by optimizing plant
771 developmental features. *Development* **143**: 3283–3294.
772 **Maxwell K, Johnson GN**. **2000**. Chlorophyll fluorescence — a practical guide. *Journal of*
773 *Experimental Botany* **51**: 659–668.
774 **Menguer PK, Sperotto RA, Ricachenevsky FK**. **2017**. A walk on the wild side: Oryza
775 species as source for rice abiotic stress tolerance. *Genetics and Molecular Biology* **40**: 238–
776 252.
777 **Murchie EH, Chen YZ, Hubbart S, Peng S, Horton P. 1999.** Interactions between
778 senescence and leaf orientation determine in situ patterns of photosynthesis and
779 photoinhibition in field-grown rice. *Plant physiology* **119**: 553–64.
780 **Murchie EH, Niyogi KK. 2011.** Manipulation of Photoprotection to Improve Plant
781 Photosynthesis. *Plant Physiology* **155**: 86–92.
782 **Murchie EH, Lawson T**. **2013**. Chlorophyll fluorescence analysis : a guide to good practice
783 and understanding some new applications. *Journal of Experimental Botany* **64**: 3983–3998.
784 **Ogle K. 2003.** Implications of interveinal distance for quantum yield in C_4 grasses: a
785 modeling and meta-analysis. *Oecologia* **136**: 532–542.
786 **Ort DR, Merchant SS, Alric J, Barkan A, Blankenship RE, Bock R, Croce R, Hanson**
787 **MR, Hibberd JM, Long SP, *et al.* 2015**. Redesigning photosynthesis to sustainably meet
788 global food and bioenergy demand. *Proceedings of the National Academy of Sciences USA*
789 **112**: 8529–8536.
790 **Osborne CP, Sack L. 2012.** Evolution of C_4 plants: A new hypothesis for an interaction of
791 CO_2 and water relations mediated by plant hydraulics. *Philosophical Transactions of the*
792 *Royal Society B: Biological Sciences* **367**: 583–600.

Palmgren MG, Edenbrandt AK, Vedel SE, Andersen MM, Landes X, Østerberg JT, Falhof J, Olsen LI, Christensen SB, Sandøe P, *et al.* 2015. Are we ready for back-to-nature crop breeding? *Trends in plant science* **20**: 155–64.

Parry MAJ, Andralojc PJ, Mitchell RAC, Madgwick PJ, Keys AJ. 2003. Manipulation of Rubisco: The amount, activity, function and regulation. *Journal of Experimental Botany* **54**: 1321–1333.

Parry MAJ, Andralojc PJ, Scales JC, Salvucci ME, Carmo-Silva AE, Alonso H, Whitney SM. 2013. Rubisco activity and regulation as targets for crop improvement. *Journal of Experimental Botany* **64**: 717–730.

Parry MAJ, Reynolds M, Salvucci ME, Raines C, Andralojc PJ, Zhu X, Price GD, Condon AG, Furbank RT. 2018. Raising yield potential of wheat. II. Increasing photosynthetic capacity and efficiency. *Journal of Experimental Botany* **62**: 453–467.

Peng S, Khush GS, Virk P, Tang Q, Zou Y. 2008. Progress in ideotype breeding to increase rice yield potential. *Field Crops Research* **108**: 32–38.

Qu M, Zheng G, Hamdani S, Essemine J, Song Q, Wang H. **2017**. Leaf photosynthetic parameters related to biomass accumulation in a global rice diversity survey. *Plant Physiology* **175**: 248–258.

Rathore M, Singh R, Kumar B, Chauhan BS. **2016**. Characterization of functional trait diversity among Indian cultivated and weedy rice populations. *Scientific Reports* 6:24176 | DOI: 10.1038/srep24176.

Reeves G, Grangé-Guermente MJ, Hibberd JM. 2017. Regulatory gateways for cell-specific gene expression in C_4 leaves with Kranz anatomy. *Journal of Experimental Botany* **68**: 107-116.

Reeves G, Singh P, Rossberg TA, Sogbohossou EOD, Schranz ME, Hibberd JM. **2018**. Natural variation within a species for traits underpinning C_4 photosynthesis. *Plant Physiology* **177**: 504–512.

Reynolds ES. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *The Journal of Cell Biology* **17**: 208–212.

Rogers A, Allen DJ, Davey PA, Morgan PB, Ainsworth EA, Bernacchi CJ, Cornic G, Dermody O, Dohleman FG, Heaton EA, *et al.* 2004. Leaf photosynthesis and carbohydrate dynamics of soybeans grown throughout their life-cycle under Free-Air Carbon dioxide Enrichment. *Plant, Cell and Environment* **27**: 449–458.

Rosenthal DM, Locke AM, Khozaei M, Raines CA, Long SP, Ort DR. 2011. Over-expressing the C_3 photosynthesis cycle enzyme Sedoheptulose-1-7 Bisphosphatase improves

827 photosynthetic carbon gain and yield under fully open air CO_2 fumigation (FACE). *BMC*
828 *Plant Biology* **11**: 123.
829 **Sack L & Scoffoni C. 2013.** Leaf venation: structure, function, development, evolution,
830 ecology and applications in the past, present and future. *New Phytologist* **198**: 983–1000.
831 **Sage RF**. **2002**. Variation in the kcat of Rubisco in C_3 and C_4 plants and some implications
832 for photosynthetic performance at high and low temperature. *Journal of Experimental Botany*
833 **53**: 609–620.
834 **Sage TL, Sage RF**. **2009**.The functional anatomy of rice leaves: implications for refixation
835 of photorespiratory CO_2 and efforts to engineer C_4 photosynthesis into rice. *Plant Cell*
836 *Physiology* **50**: 756–772.
837 **Saibo NJM, Lourenço T, Oliveira MM**. **2009**. Transcription factors and regulation of
838 photosynthetic and related metabolism under environmental stresses. *Annals of Botany* **103**:
839 609–623.
840 **Sakuraba Y, Kim EY, Han SH, Piao W, An G, Todaka D, Yamaguchi-Shinozaki K,**
841 **Paek NC**. **2017**. Rice Phytochrome-Interacting Factor-Like1 (OsPIL1) is involved in the
842 promotion of chlorophyll biosynthesis through feed-forward regulatory loops. *Journal of*
843 *Experimental Botany* **68**: 4103-4114.
844 **Sanchez PL, Wing RA, Brar DS. 2013.** The wild relative of Rice: Genomes and genomics.
845 In: Zhang Q, Wing RA (eds) *Genetics and genomics of rice, plant genetics and genomics:*
846 *crops and models* 5. Springer, New York, 9–25.
847 **Sang T, Ge S. 2013.** Understanding rice domestication and implications for cultivar
848 improvement.*Current opinion in plant biology* **16**: 139–46.
849 **Sasaki H, Hara T, Ito S, Uehara N, Kim HY, Lieffering M, Okada M, Kobayashi K**.
850 **2007**. Effect of free-air CO_2 enrichment on the storage of carbohydrate fixed at different
851 stages in rice (*Oryza sativa L.*). *Field Crops Research* **100**: 24–31.
852 **Schuler ML, Mantegazza O, Weber APM**. **2016**. Engineering C_4 photosynthesis into C_3
853 chassis in the synthetic biology age. *The Plant journal* **87**: 51–65.
854 **Sharwood RE, Ghannoum O, Whitney SM. 2016.** Prospects for improving CO_2 fixation in
855 C_3-crops through understanding C_4-Rubisco biogenesis and catalytic diversity. *Current*
856 *Opinion in Plant Biology* **31**: 135–142.
857 **Sharwood RE**. **2017**. Engineering chloroplasts to improve Rubisco catalysis: prospects for
858 translating improvements into food and fiber crops. *New Phytologist* **213**: 494–510.
859 **Simkin AJ, López-calcagno PE, Raines CA**. **2019**. Feeding the world : improving
860 photosynthetic efficiency for sustainable crop production. *Journal of Experimental Botany*

861 **70**: 1119–1140.
862 **Singh BP, Jayaswal PK, Singh B, Singh PK, Kumar V, Mishra S, Singh N, Panda K and**
863 **Singh NK. 2015.** Natural allelic diversity in *OsDREB1F* gene in the Indian wild rice
864 germplasm led to ascertain its association with drought tolerance. *Plant Cell Reports* **34**:993-
865 1004.
866 **Spurr AR**. **1969**. A low-viscosity epoxy resin embedding medium for electron microscopy.
867 *Journal of Ultrasructure Research* **26**: 31–43.
868 **Sung J, Lee S, Chung JW, Edwards G, Ryu H, Kim T**. **2017**. Photosynthesis, Metabolite
869 Composition and Anatomical Structure of *Oryza sativa* and Two Wild Relatives, *O.*
870 *grandiglumis* and *O. alta*. *Rice Science* **24**: 218–227.
871 **Takai T, Adachi S, Taguchi-shiobara F, Sanoh-arai Y, Iwasawa N, Yoshinaga S, Hirose**
872 **S, Taniguchi Y, Yamanouchi U, Wu J, *et al. 2013*** A natural variant of *NAL1*, selected in
873 high-yield rice breeding programs, pleiotropically increases photosynthesis rate. *Scientific*
874 *Reports* **3**: 1–11.
875 **Tamoi M, Nagaoka M, Miyagawa Y, Shigeoka S**. **2006**. Contribution of fructose-1,6-
876 bisphosphatase and sedoheptulose-1,7- bisphosphatase to the photosynthetic rate and carbon
877 flow in the Calvin cycle in transgenic plants. *Plant and Cell Physiology* **47**: 380–390.
878 **Tholen D, Ethier G, Genty B, Pepin S, Zhu XG. 2012.** Variable mesophyll conductance
879 revisited: theoretical background and experimental implications. *Plant, Cell & Environment*
880 **35**: 2087– 2103.
881 **Tian L, Tan L, Liu F, Cai H and Sun C. 2011.** Identification of quantitative trait loci
882 associated with salt tolerance at seedling stage from *Oryza rufipogon*. *Journal of Genetics*
883 *and Genomics* **38**:593-601
884 **Toledo-Ortiz G, Johansson H, Lee KP, Bou-Torrent J, Stewart K, Steel G, Rodríguez-**
885 **Concepción M, Halliday KJ**. **2014**. The HY5-PIF regulatory module coordinates light and
886 temperature control of photosynthetic gene transcription. *PLoS Genetics* **10**: e1004416
887 **Toth SZ, Nagy V, Puthur JT, Kovacs L, Garab G. 2011.** The physiological role of
888 ascorbate as photosystem II electron donor: Protection against photoinactivation in heat-
889 stressed leaves. *Plant Physiology* **156**: 382–392.
890 **Ueno O, Kawano Y, Wakayama M, Takeda T**. **2006**. Leaf vascular systems in C_3 and C_4
891 grasses: A two-dimensional analysis. *Annals of Botany* **97**: 611–621.
892 **van Bezouw RFHM, Keurentjes JJB, Harbinson J, Aarts MGM. 2019.** Converging
893 phenomics and genomics to study natural variation in plant photosynthetic efficiency. *Plant*
894 *Journal* **97**: 112-133.

895 **von Caemmerer S, Quick WP, Furbank RT. 2012.** The development of C_4 rice: Current
896 progress and future challenges. *Science* **336**: 1671-1672.
897 **van Campen JC, Yaapar MN, Narawatthana S, Lehmeier C, Wanchana S, Thakur V,**
898 **Chater C, Kelly S, Rolfe SA, Quick WP, *et al.* 2016.** Combined chlorophyll fluorescence
899 and transcriptomic analysis identifies the P3/P4 transition as a key stage in rice leaf
900 photosynthetic development. *Plant Physiology* **170**: 1655–1674.
901 **von Korff M, Grando S, This D, Baum M, Ceccarelli S. 2008.** Quantitative trait loci
902 (QTL) associated with agronomic performance of barley under drought. *Theoretical and*
903 *Applied Genetics* **117**: 653–669
904 **Wang M, Kapralov MV, Anisimova M. 2011.** Coevolution of amino acid residues in the
905 key photosynthetic enzyme Rubisco. *BMC Evolutionary Biology* **11**: 1–12.
906 **Wang L, Czedik-Eysenberg A, Mertz RA, Si Y, Tohge T, Nunes-Nesi A, Arrivault S,**
907 **Dedow LK, Bryant DW, Zhou W, *et al.* 2014**. Comparative analyses of C_4 and C_3
908 photosynthesis in developing leaves of maize and rice. *Nature Biotechnology* **32**:1158-65.
909 **Wang J, Lu W, Tong Y, Yang Q**. **2016**. Leaf Morphology, photosynthetic performance,
910 chlorophyll fluorescence, stomatal development of Lettuce (*Lactuca sativa* L.) exposed to
911 different ratios of red light to blue light. *Frontiers in Plant Science* **7**: 1–10.
912 **Wang P, Hendron R, Kelly S**. **2017**. Transcriptional control of photosynthetic capacity :
913 conservation and divergence from Arabidopsis to rice. *New phytologist* **216**: 32–45.
914 **Waters MT, Wang P, Korkaric M, Capper RG, Saunders NJ, Langdale JA. 2009.** GLK
915 transcription factors coordinate expression of the photosynthetic apparatus in Arabidopsis.
916 *The Plant Cell* **21**: 1109–1128.
917 **Wing RA, Purugganan MD, Zhang Q. 2018.** The rice genome revolution: From an ancient
918 grain to Green Super Rice. *Nature Reviews Genetics* **19**:505-517.
919 **Wu A, Hammer GL, Doherty A, von Caemmerer S, Farquhar GD. 2019.** Quantifying
920 impacts of enhancing photosynthesis on crop yield. *Nature plants* **5**: 380-388.
921 **Xu F, Wang K, Yuan W, Xu W, Liu S, Kronzucker HJ, Chen G, Miao R, Zhang M,**
922 **Ding M, *et al.* 2019**. Overexpression of rice aquaporin *OsPIP1;2* improves yield by
923 enhancing mesophyll CO_2 conductance and phloem sucrose transport. *Journal of*
924 *Experimental Botany* **70**: 671–681.
925 **Yabiku T, Ueno O. 2017.** Variations in physiological, biochemical, and structural traits of
926 photosynthesis and resource use efficiency in maize and teosintes (NADP-ME-type C_4).
927 *Plant Production Science* **20**: 448–458.
928 **Yeo ME, Yeo AR, Flowers TJ. 1994.** Photosynthesis and photorespiration in the genus

Oryza. *Journal of Experimental Botany* **45**: 553–560.

Yuan M, Zhao YQ, Zhang ZW, Chen YE, Ding CB, Yuan S. 2017. Light regulates transcription of chlorophyll biosynthetic genes during chloroplast biogenesis. *Critical Reviews in Plant Sciences* **36:** 35-54.

Zhao M, Lafitte HR, Sacks E, Dimayuga G, Acun TLB. 2008. Perennial *O . sativa* × *O . rufipogon* interspecific hybrids : I . Photosynthetic characteristics and their inheritance. *Field Crops Research* **106**: 203–213.

Zhao M, Ding Z, Lafitte R, Sacks E, Dimayuga G, Holt D. 2010. Photosynthetic characteristics in *Oryza* species. *Photosynthetica* **48**: 234–240.

Zheng TC, Zhang XK, Yin GH, Wang LN, Han YL, Chen L, Huang F, Tang JW, Xia XC, He ZH. 2011. Genetic gains in grain yield, net photosynthesis and stomatal conductance achieved in Henan Province of China between 1981 and 2008. *Field Crops Research* **122**: 25–233.

Zhu XG, Long SP, Ort DR. 2010. Improving photosynthetic efficiency for greater yield. *Annual Review of Plant Biology* **61:**235-61.

963 **Figure Legends**

964 **Fig. 1** Variations in leaf photosynthesis and related physiological parameters across
965 cultivated and wild rice species. Quantification of net photosynthesis per unit area of flag leaf
966 surface area (P_N, a), stomatal conductance (g_s, b), intercellular CO_2 concentration (Ci, c),
967 transpiration rate (E, d), water use efficiency (WUE, e), and carboxylation efficiency (CE, f)
968 for six cultivated rice accessions and four wild rice species. Cultivated rice accessions and
969 wild species are marked in blue and green shading, respectively. Asterisks indicate
970 significant differences between genotypes (one-way ANOVA, n = 15, $*P < 0.05$). Different
971 letters indicate significant groupings according to post-hoc Tukey HSD calculation.

972

973

974 **Fig. 2** Effects of PAR intensity, CO_2 concentration, and diurnal time on leaf photosynthesis
975 (P_N), stomatal conductance (g_s), transpiration (E), and water use efficiency (WUE) of
976 different rice genotypes.

977 a) Quantification of P_N (I), g_s (II), E (III), and WUE (IV) in response to increasing PAR from
978 0 to 1500 μmol m^2 s^{-1}.

979 b) Quantification of P_N (I), g_s (II), E (III), and WUE (IV) in response to increasing CO_2
980 concentration from 0 to 1400 μmol mol^{-1}.

981 c) Quantification of P_N (I), g_s (II), E (III), and WUE (IV) at different time points of a day
982 from 9.00 h to 15.00 h

983 Each value represents the mean of 5 data points ± SD.

984

985 **Fig. 3** Leaf anatomical traits effecting photosynthesis rate and related physiological traits of
986 rice.

987 a) Pearson correlation analysis of net photosynthesis rate (P_N) and related physiological traits
988 with leaf developmental features. The level of significance (α) of correlation coefficients at
989 $*P < 0.05$ (± 0.666; degree of freedom (df; n-2): 9-2 = 7).

990 b) Bright-field images of a flag leaf of the selected genotypes showing variations in
991 mesophyll cells and vasculature. Arrows indicate bulliform cells (bc), bundle sheath (bs),
992 epidermis (ep), mesophyll cells (mc), and minor veins (miv). Scale bars represent 100 μm at
993 10X magnification.

994 c) Bright-field microscopy images showing variations in mesophyll cell features, including
995 lobbing. Scale bar represents 100 μm at 100X magnification.

996 d-e) Quantification of mesophyll area (d) and minor vein height (e) of selected cultivated and
997 wild rice species. Cultivated accessions and wild species are shaded in blue and green,
998 respectively. Asterisks indicate significant differences between genotypes (one-way ANOVA,
999 n = 30, *$P < 0.05$). Different letters indicate significant groupings according to post-hoc
1000 Tukey HSD calculation.
1001
1002 **Fig. 4** Quantification of photochemical features of selected cultivated and wild rice species.
1003 Total chlorophyll content (TCC) per gram of fresh weight (a), F_v/F_m in dark-adapted leaves
1004 (b), Efficiency of PSII (ΦPSII, c), Photochemical quenching (qP, d), and Electron transport
1005 rate (ETR, e) of the selected genotypes. Cultivated accessions and wild species are shaded in
1006 blue and green, respectively. All the measurements were performed in fully developed leaves
1007 of controlled growth chambers grown plants. Asterisks indicate significant differences
1008 between genotypes (one-way ANOVA, n = 7, *$P < 0.05$). Different letters indicate
1009 significant groupings according to post-hoc Tukey HSD calculation.
1010
1011 **Fig. 5** Expression analysis of genes and transcription factors regulating photosynthesis, and
1012 differences in Rubisco activity for selected cultivated and wild rice species.
1013 a) Expression levels of genes encoding key component of light harvesting complex, Rubisco,
1014 and Triose phosphate/phosphate translocator.
1015 b) Expression levels of chlorophyll biosynthetic genes in flag leaves.
1016 c) Expression pattern of transcription factors regulating photosynthetic and chlorophyll-
1017 biosynthetic genes in primordia 4 stage (P4) of leaf 4.
1018 d) Continuous measurement of Rubisco activity through RuBP (ribulose-1, 5-bisphosphate)
1019 consumption (n mol s^{-1}) and Vcmax (μmol m^{-2} s^{-1}) demonstrating the conversion of RuBP to
1020 3-PGA (3-phosphoglycerate). The data shows the time course of the decrease in A_{340} nm in
1021 assays to four moles of NADH oxidation per RuBP.
1022 e) Comparison of Vcmax and P_N for the selected genotypes.
1023 Asterisks indicate significant differences between genotypes [one-way ANOVA, n = 6 (two
1024 biological with three technical replications, *$P < 0.05$, **$P < 0.01$]. Different letters indicate
1025 significant groupings according to post-hoc Tukey HSD calculation.
1026 Gene abbreviations: GluTR, *Glutamyl-tRNA reductase*; PPOX, *Protoporphyrinogen oxidase*;
1027 Mg-CHL, *Magnesium chelatase*; CHLSYN, *Chlorophyll synthase*; CAO, *Chlorophyllide a*
1028 *oxygenase*; LHCI, *Light-harvesting chlorophyll protein complex I*; Rubisco SS, *Ribulose*
1029 *bisphosphate carboxylase small subunit*; Rubisco LS, *Ribulose bisphosphate carboxylase*

1030 *large subunit*; TPT, *Triose phosphate/phosphate translocator*; GLK, *Golden like (1* and *2)*;
1031 SIG, *Sigma factors (1, 2, 5,* and *6*); ZML2, *ZINC FINGER PROTEIN 2*; ORE1, ORESARA1*;*
1032 CGA1, *Cytokinin responsive GATA Factor*; GRF5, *Growth regulating factor 5*.
1033
1034 **Fig. 6** Compilation of developmental, photochemical, and biochemical traits contributing
1035 towards leaf photosynthetic differences between two extreme genotypes: *Oryza sativa* ssp.
1036 *indica* cv. IR 64 and *Oryza australiensis*. Wider leaf, larger mesophyll cells, larger veins,
1037 fewer number of mesophyll cells between two consecutive veins, more chloroplasts, efficient
1038 light harvesting and Electron Transport Rate along with more active Rubsico facilitate higher
1039 leaf photosynthesis in wild rice species *Oryza australiensis* compared to cultivated *Oryza*
1040 *sativa* ssp. *indica* cv. IR64.
1041
1042 **Supporting Information**
1043
1044 **Fig. S1** Representative images of cultivated and wild rice species showing remarkable
1045 differences in plant architectural traits, leaf features, and biomass. Shown are the field grown
1046 plants (a), and controlled growth chambers grown plants (b) of the selected genotypes.
1047
1048 **Fig.S2** Pair-wise Pearson correlation coefficient (*r*) analysis among net photosynthetic rate
1049 (P_N) and related physiological traits.
1050
1051 **Fig. S3** Quantification of P_N (max), Ci and CE in response to increasing PAR (a, I-III),
1052 increasing CO_2 concentration (b, I-III), andat different diurnal time (c, I-III) for selected
1053 cultivated and wild rice species. Each value represents the mean of 5 data points ± SD.
1054
1055 **Fig. S4** TEM images of organization of chloroplasts in mesophyll cells. Arrows indicate
1056 chloroplast (ch) and mitochondria (m). Scale bar represents 2.00 µm at 1000X magnification.
1057
1058 **Fig.S5** Pair-wise Pearson correlation coefficient (r) analysis among net photosynthetic rate
1059 (P_N), chlorophyll fluorescence traits, and photochemical features.
1060
1061 **Fig. S6** An *in silico* generated hierarchical clustering dendrogram for the expression pattern
1062 of key photosynthetic and chlorophyll-biosynthetic genes, and transcription factor regulating
1063 photosynthetic machinery across nine anatomical parts of rice plants (seedling, coleoptile,

1064 seedling leaf, stem, vegetative leaf, blade, collar, sheath, flag leaf). The data were collected
1065 and dendrograms was generated using Genevestigator (https://genevestigator.com/gv/).
1066
1067 **Table S1** Details of rice accessions used to investigate the leaf photosynthesis and related
1068 physiological features.
1069
1070 **Table S2** Variation in leaf photosynthesis and related physiological traits among cultivated
1071 and wild rice species under controlled conditions.
1072
1073 **Table S3** Quantitative measurement of flag leaf width (FLW, cm), flag leaf thickness (FLT,
1074 mm), Minor vein width (μm), Minor vein height (μm), and total veins (n) in the fully
1075 expanded flag leaf of cultivated and wild rice species.
1076
1077 **Table S4** Quantitative measurement of mesophyll lobes (n), length (μm), width (μm), and
1078 area (μm^2) and bundle sheath number (n), and area (μm^2) in the fully expanded flag leaf of
1079 cultivated and wild rice species.
1080
1081 **Table S5** Details of genes and transcription factors used for gene expression analysis.
1082
1083 **Table S6** List of primers for qRT-PCR analyses.

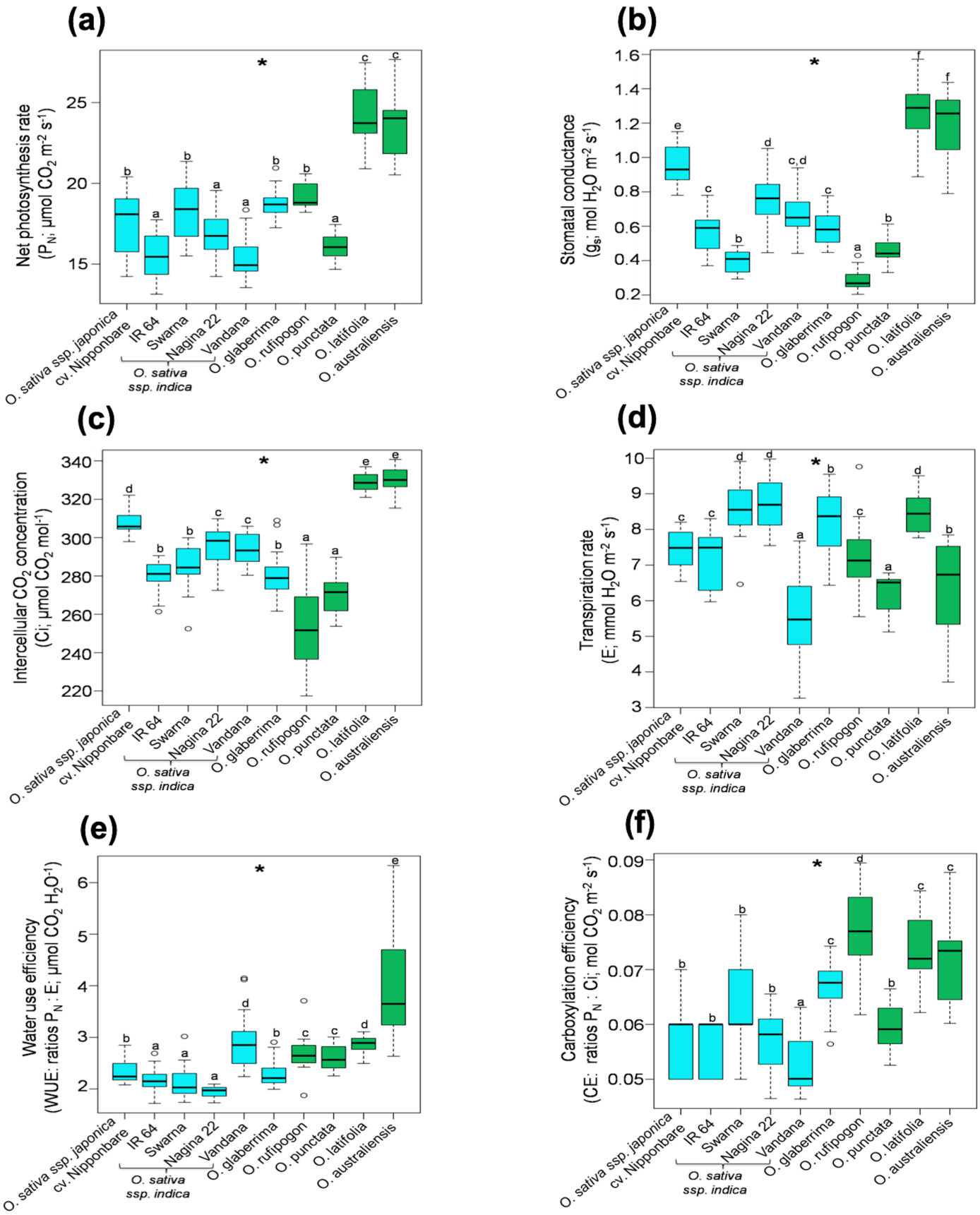

Fig .1 Variations in leaf photosynthesis and related physiological parameters across cultivated and wild rice species. Quantification of net photosynthesis per unit area of flag leaf surface area (P_N, a), stomatal conductance (g_s, b), intercellular CO_2 concentration (Ci, c), transpiration rate (E, d), water use efficiency (WUE, e), and carboxylation efficiency (CE, f) for six cultivated rice accessions and four wild rice species. Cultivated rice accessions and wild species are marked in blue and green shading, respectively. Asterisks indicate significant differences between genotypes (one-way ANOVA, n = 15, $*P < 0.05$). Different letters indicate significant groupings according to post-hoc Tukey HSD calculation.

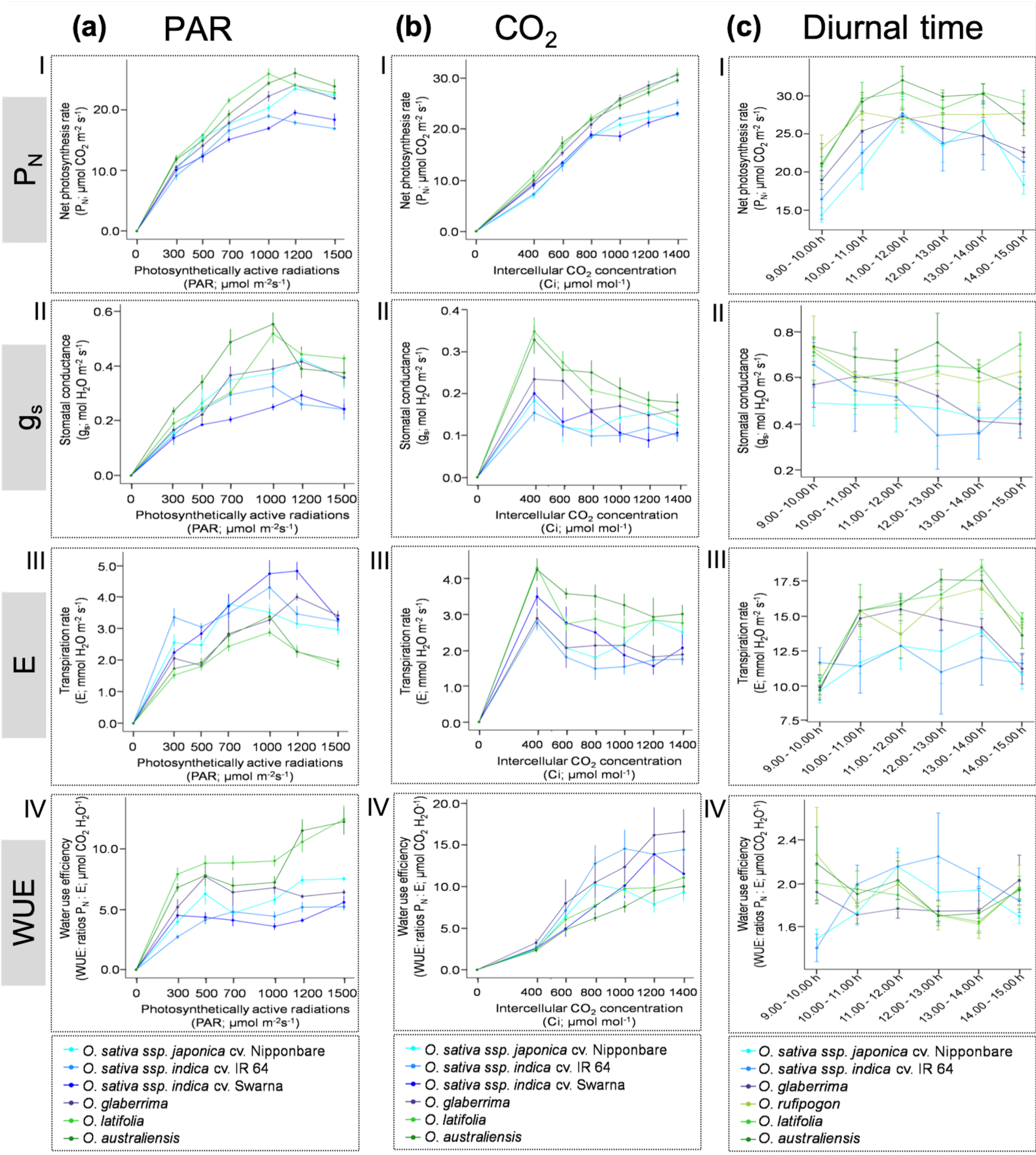

Fig. 2 Effects of PAR intensity, CO_2 concentration, and diurnal time on leaf photosynthesis (P_N), stomatal conductance (g_s), transpiration (E), and water use efficiency (WUE) of different rice genotypes.
a) Quantification of P_N (I), g_s (II), E (III), and WUE (IV) in response to increasing PAR from 0 to 1500 µmol m^2 s^{-1}.
b) Quantification of P_N (I), g_s (II), E (III), and WUE (IV) in response to increasing CO_2 concentration from 0 to 1400 µmol mol^{-1}.
c) Quantification of P_N (I), g_s (II), E (III), and WUE (IV) at different time points of a day from 9.00 h to 15.00 h
Each value represents the mean of 5 data points ± SD.

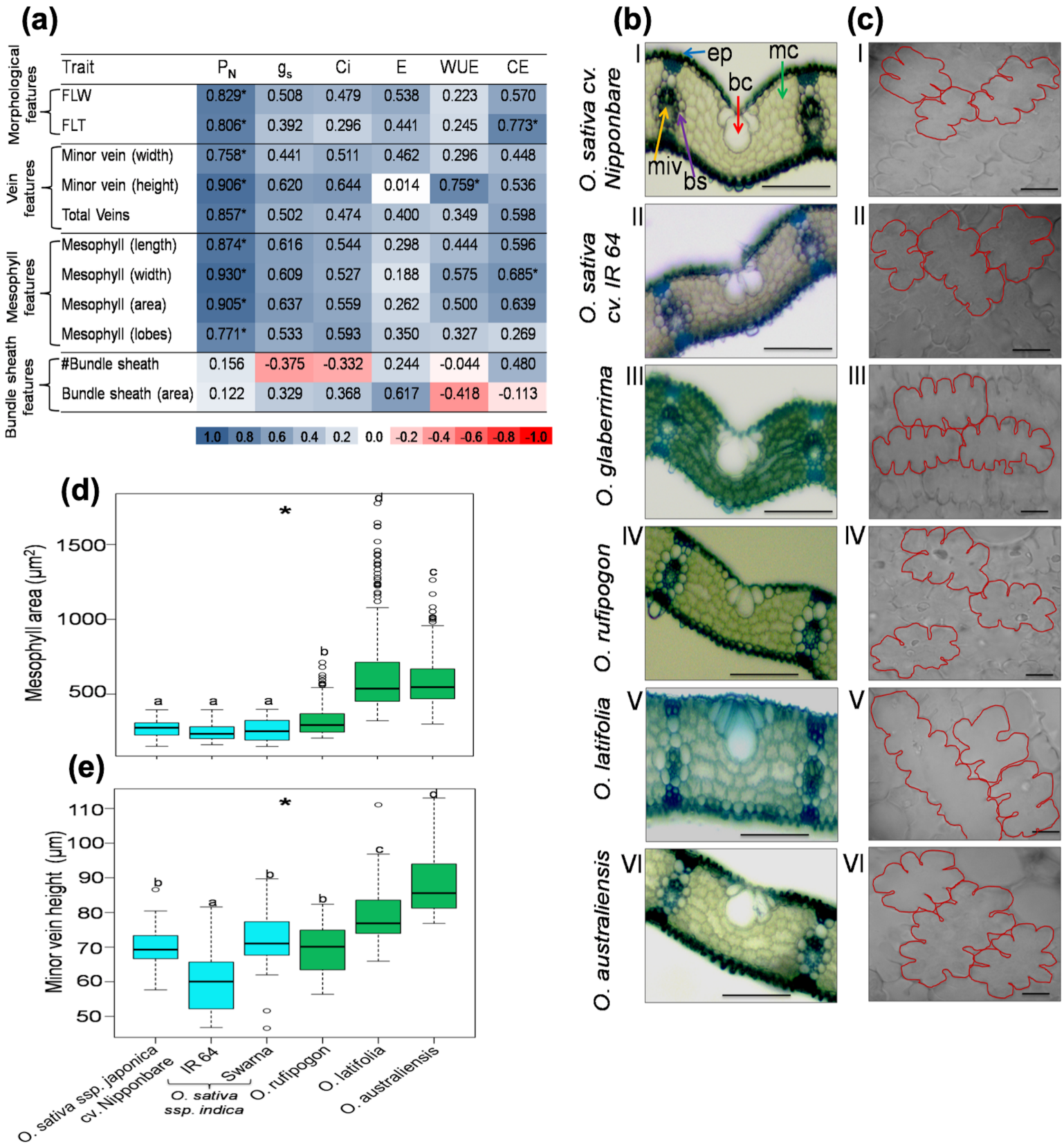

Fig. 3 Leaf anatomical traits effecting photosynthesis rate and related physiological traits of rice.

a) Pearson correlation analysis of net photosynthesis rate (P_N) and related physiological traits with leaf developmental features. The level of significance (α) of correlation coefficients at $*P < 0.05$ (± 0.666; degree of freedom (df; n-2): 9-2 = 7).

b) Bright-field images of a flag leaf of the selected genotypes showing variations in mesophyll cells and vasculature. Arrows indicate bulliform cells (bc), bundle sheath (bs), epidermis (ep), mesophyll cells (mc), and minor veins (miv). Scale bars represent 100 μm at 10X magnification.

c) Bright-field microscopy images showing variations in mesophyll cell features, including lobbing. Scale bar represents 100 μm at 100X magnification.

d-e) Quantification of mesophyll area (d) and minor vein height (e) of selected cultivated and wild rice species. Cultivated accessions and wild species are shaded in blue and green, respectively. Asterisks indicate significant differences between genotypes (one-way ANOVA, n = 30, $*P < 0.05$). Different letters indicate significant groupings according to post-hoc Tukey HSD calculation.

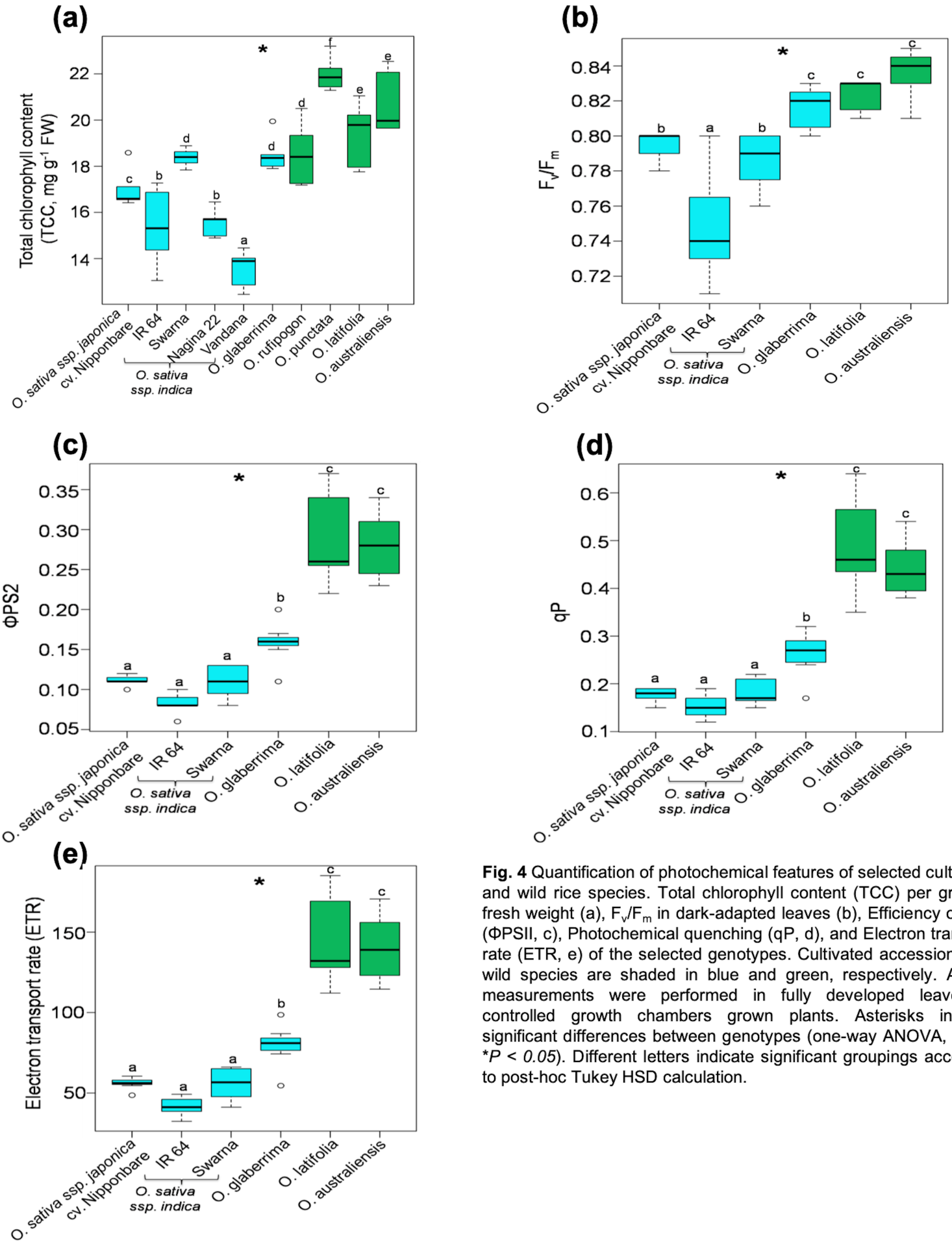

Fig. 4 Quantification of photochemical features of selected cultivated and wild rice species. Total chlorophyll content (TCC) per gram of fresh weight (a), F_v/F_m in dark-adapted leaves (b), Efficiency of PSII (ΦPSII, c), Photochemical quenching (qP, d), and Electron transport rate (ETR, e) of the selected genotypes. Cultivated accessions and wild species are shaded in blue and green, respectively. All the measurements were performed in fully developed leaves of controlled growth chambers grown plants. Asterisks indicate significant differences between genotypes (one-way ANOVA, n = 7, $*P < 0.05$). Different letters indicate significant groupings according to post-hoc Tukey HSD calculation.

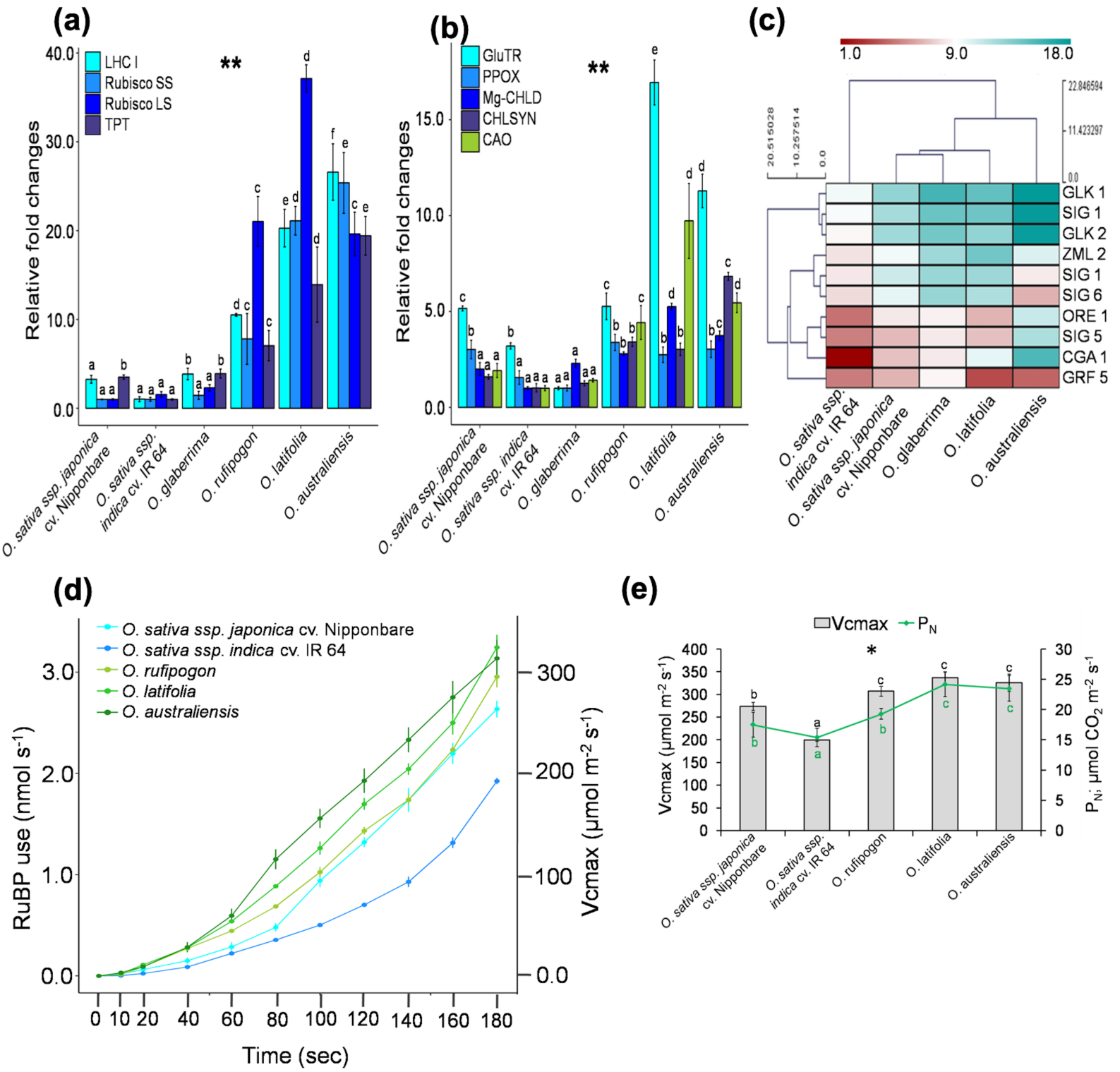

Fig. 5 Expression analysis of genes and transcription factors regulating photosynthesis, and differences in Rubisco activity for selected cultivated and wild rice species.
a) Expression levels of genes encoding key component of light harvesting complex, Rubisco, and Triose phosphate/phosphate translocator.
b) Expression levels of chlorophyll biosynthetic genes in flag leaves.
c) Expression pattern of transcription factors regulating photosynthetic and chlorophyll-biosynthetic genes in primordia 4 stage (P4) of leaf 4.
d) Continuous measurement of Rubisco activity through RuBP (ribulose-1, 5-bisphosphate) consumption (nmol s^{-1}) and Vcmax (µmol m^{-2} s^{-1}) demonstrating the conversion of RuBP to 3-PGA (3-phosphoglycerate). The data shows the time course of the decrease in A_{340} nm in assays to four moles of NADH oxidation per RuBP.
e) Comparison of Vcmax and P_N for the selected genotypes.
Asterisks indicate significant differences between genotypes [one-way ANOVA, n = 6 (two biological with three technical replications, *$P< 0.05$, **$P < 0.01$]. Different letters indicate significant groupings according to post-hoc Tukey HSD calculation.
Gene abbreviations: GluTR, *Glutamyl-tRNA reductase*; PPOX, *Protoporphyrinogen oxidase*; Mg-CHL, *Magnesium chelatase*; CHLSYN, *Chlorophyll synthase*; CAO, *Chlorophyllide a oxygenase*; LHCI, *Light-harvesting chlorophyll protein complex I*; Rubisco SS, *Ribulose bisphosphate carboxylase small subunit*; Rubisco LS, *Ribulose bisphosphate carboxylase large subunit*; TPT, *Triose phosphate/phosphate translocator*; GLK, *Golden like (1* and *2)*; SIG, *Sigma factors (1, 2, 5,* and *6)*; ZML2, *ZINC FINGER PROTEIN 2*; ORE1, *ORESARA1;* CGA1, *Cytokinin responsive GATA Factor*; GRF5, *Growth regulating factor 5*.

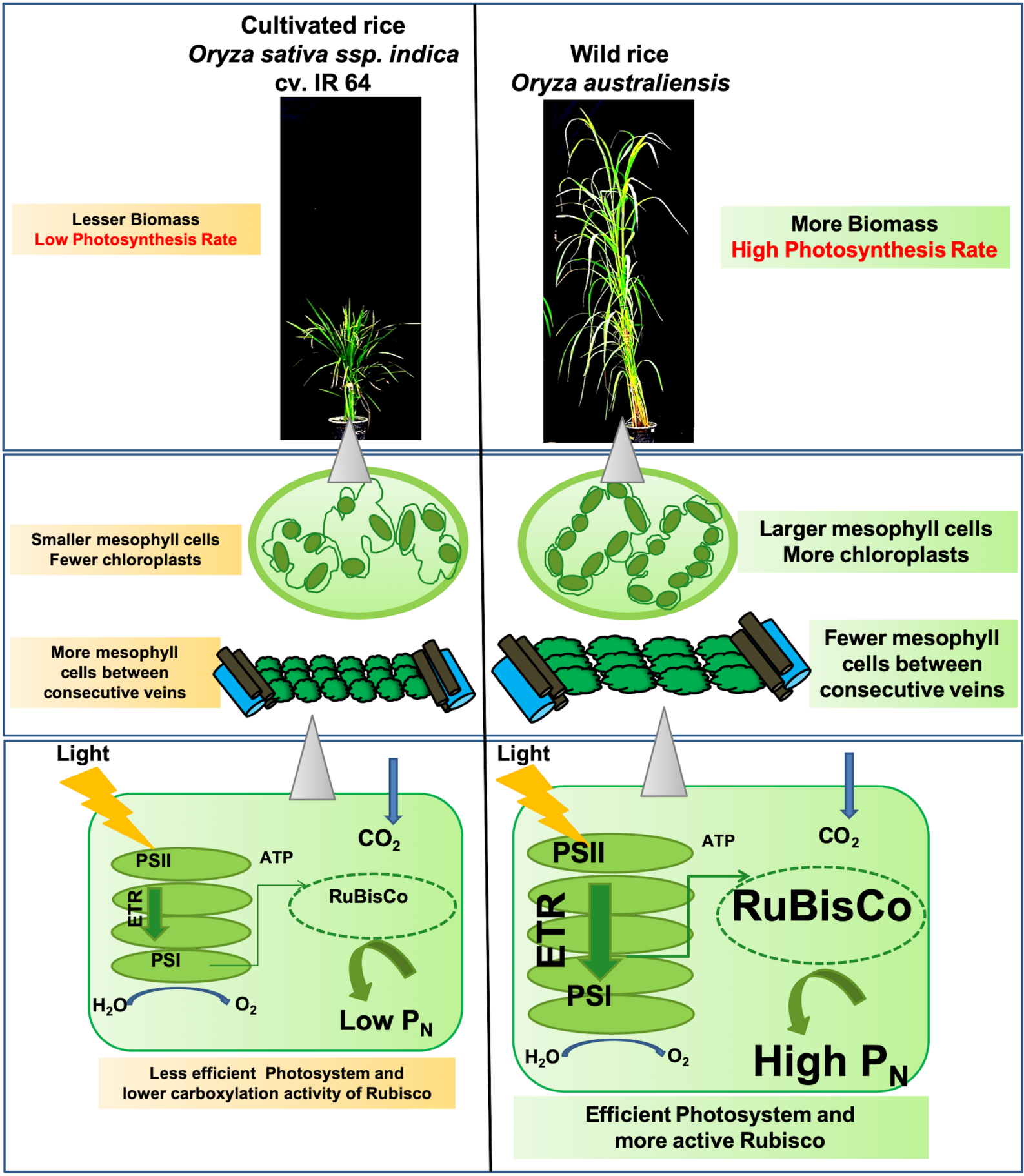

Fig. 6 Compilation of developmental, photochemical, and biochemical traits contributing towards leaf photosynthetic differences between two extreme genotypes: *Oryza sativa* ssp. *indica* cv. IR 64 and *Oryza australiensis*. Wider leaf, larger mesophyll cells, larger veins, fewer number of mesophyll cells between two consecutive veins, more chloroplasts, efficient light harvesting and Electron Transport Rate along with more active Rubsico facilitate higher leaf photosynthesis in wild rice species *Oryza australiensis* compared to cultivated *Oryza sativa* ssp. *indica* cv. IR64.

Supporting Information

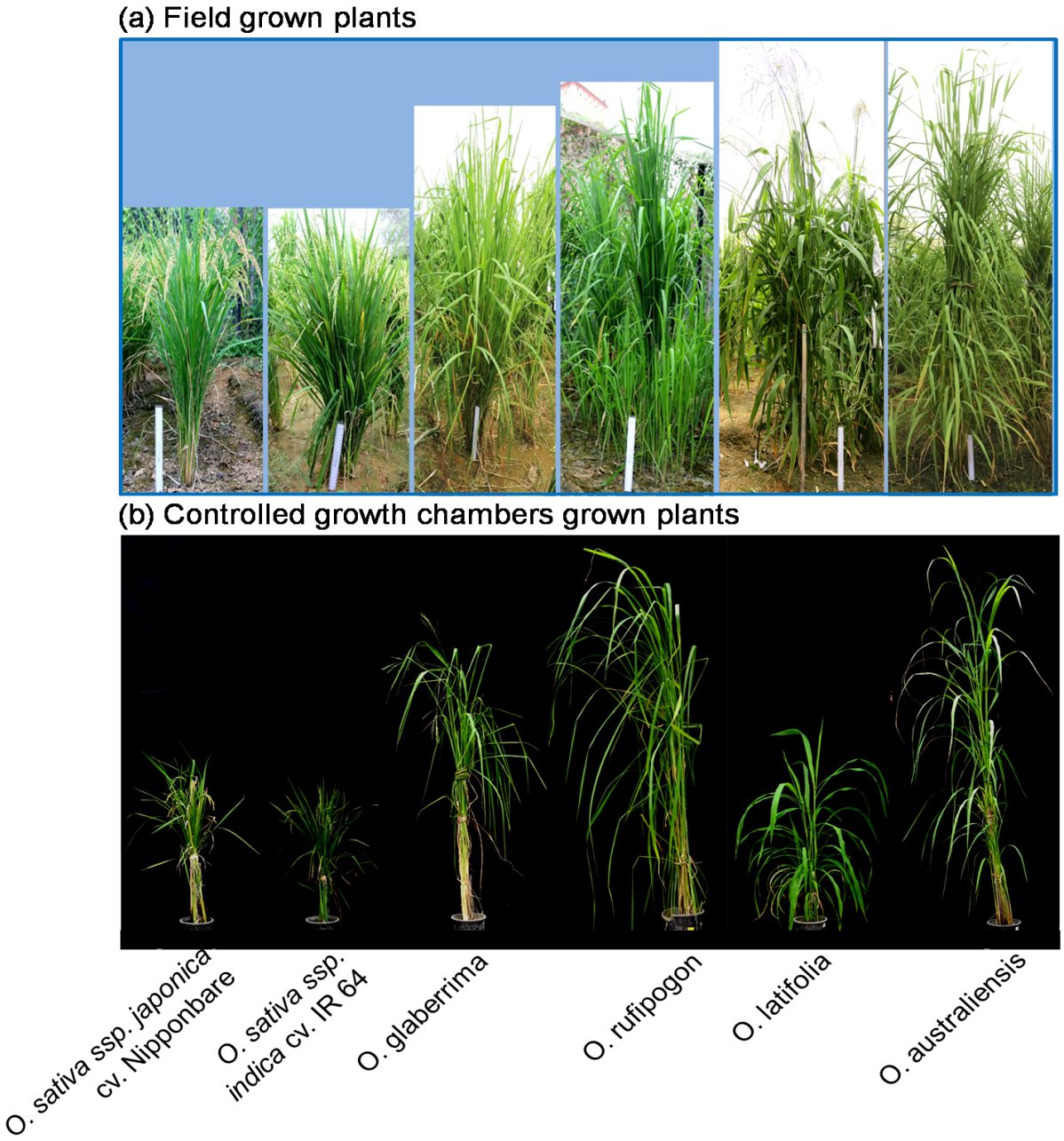

Fig. S1 Representative images of cultivated and wild rice species showing remarkable differences in plant architectural traits, leaf features, and biomass. Shown are the field grown plants (a), and controlled growth chambers grown plants (b) of the selected genotypes.

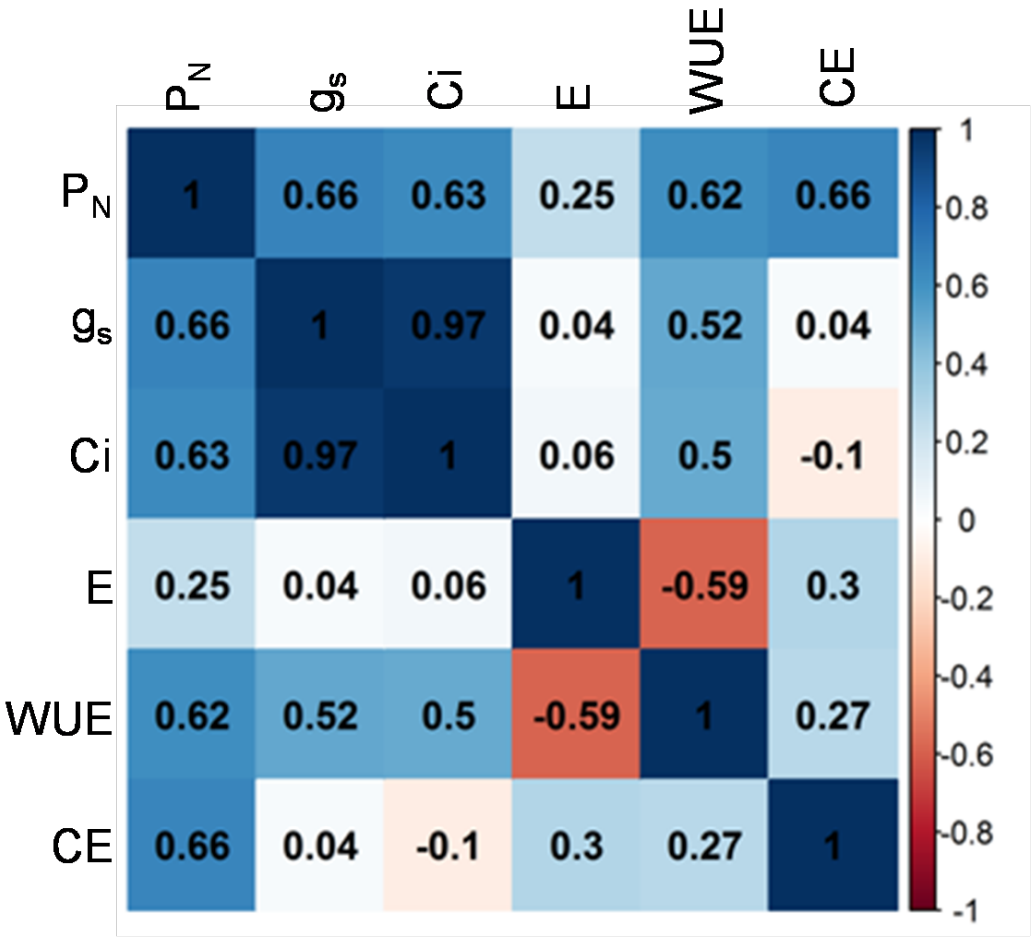

Fig. S2 Pair-wise Pearson correlation coefficient (*r*) analysis among net photosynthetic rate (P_N) and related physiological traits.

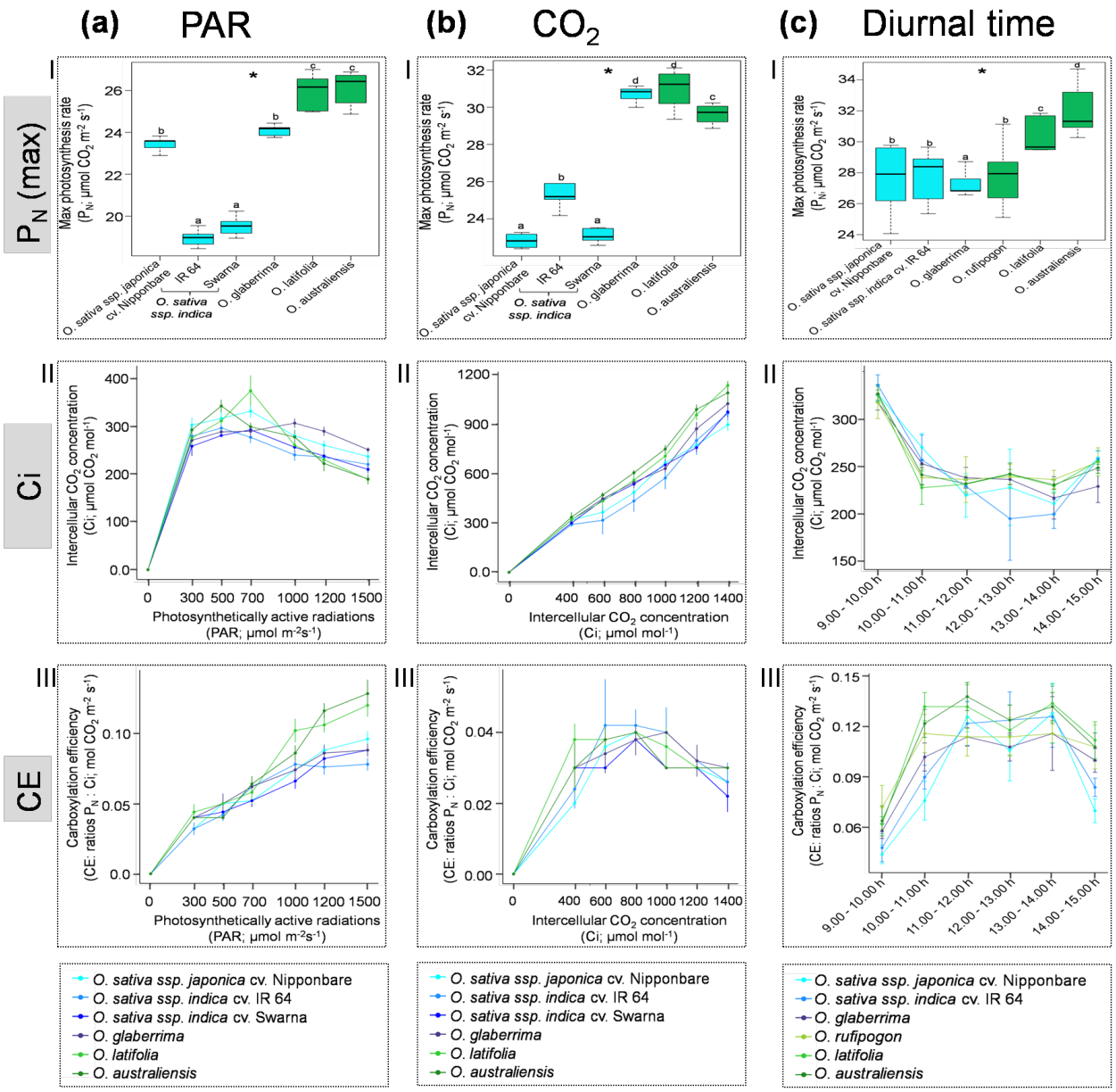

Fig. S3 Quantificationof P_N (max), Ci and CE in response to increasing PAR (a, I-III), increasing CO_2 concentration (b, I-III), and at different diurnal time (c, I-III) for selected cultivated and wild rice species. Each value represents the mean of 5 data points ± SD.

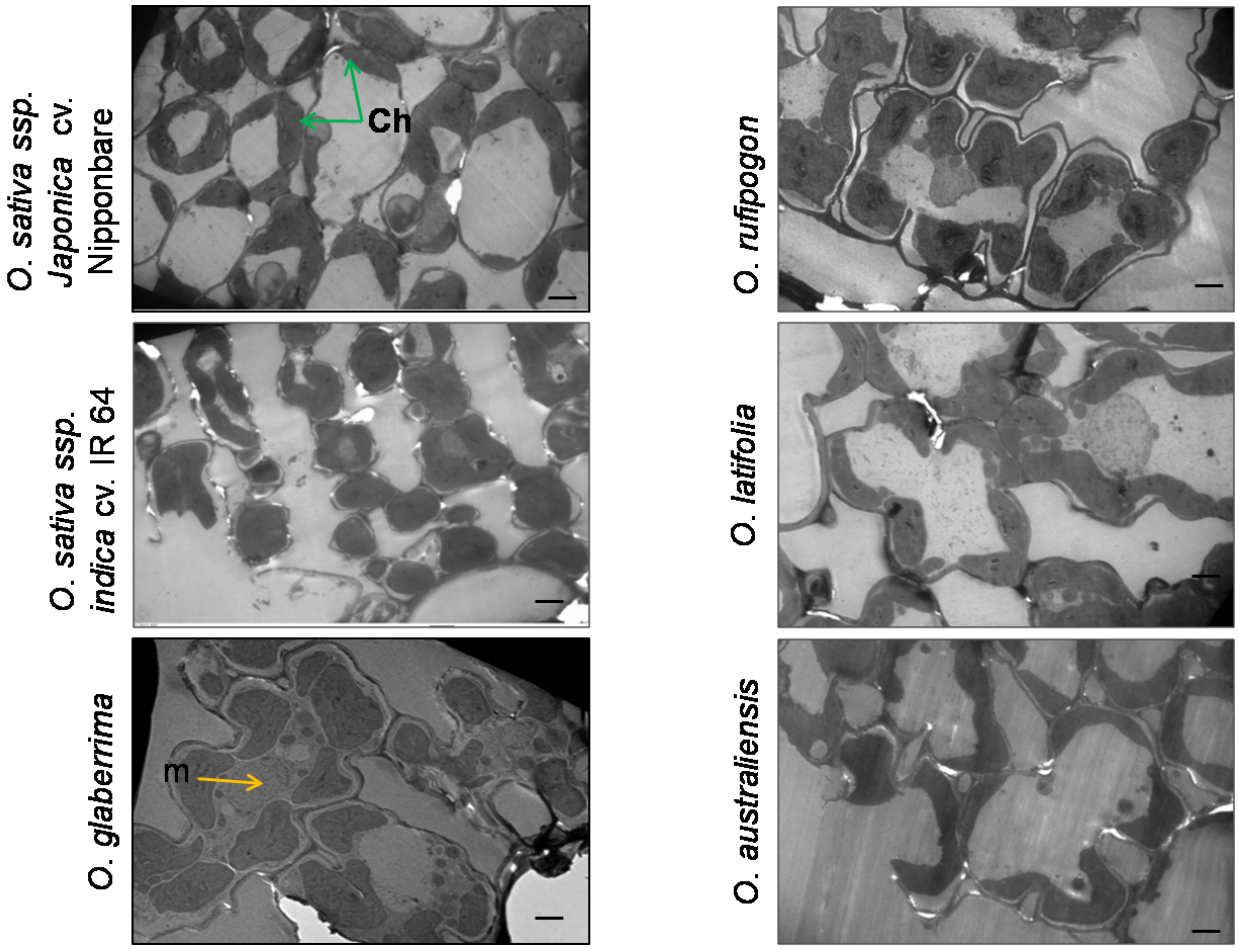

Fig. S4 TEM images of organization of chloroplasts in mesophyll cells. Arrows indicate chloroplast (ch) and mitochondria (m). Scale bar represents 2.00 µm at 1000X magnification.

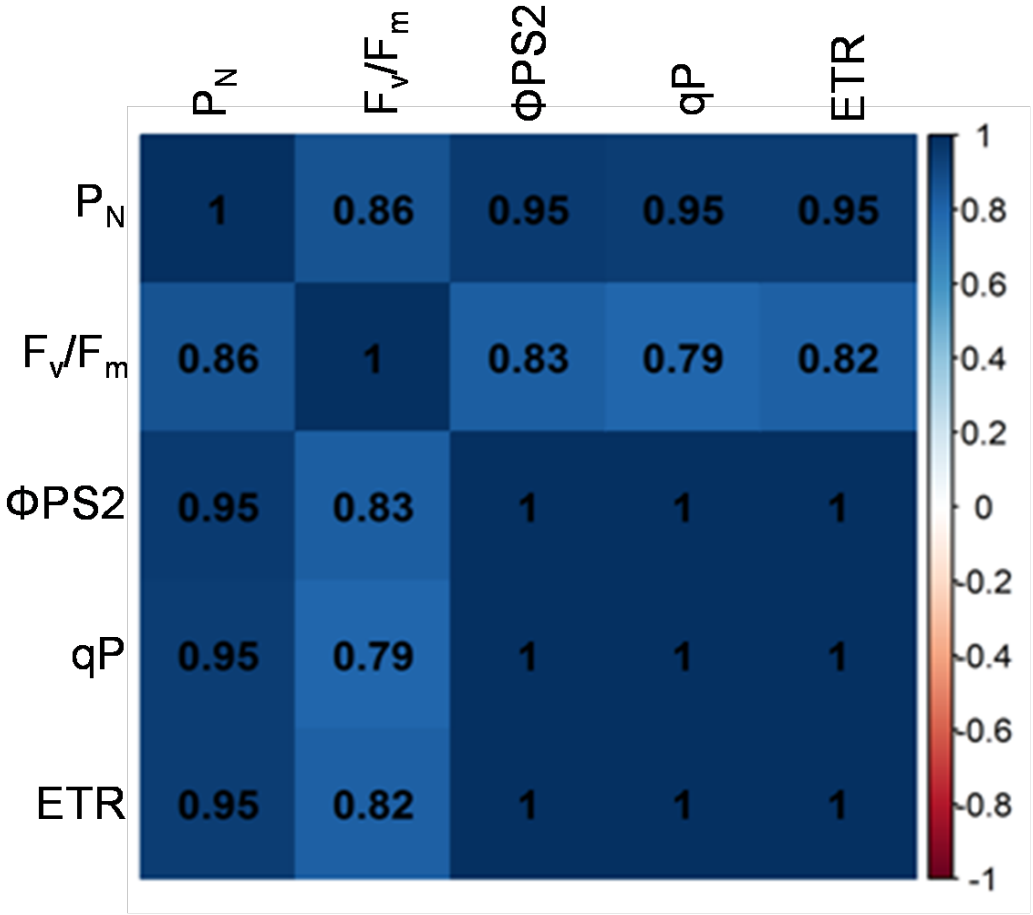

Fig. S5 Pair-wise Pearson correlation coefficient (r) analysis among net photosynthetic rate (P_N),chlorophyll fluorescence traits, and photochemical features.

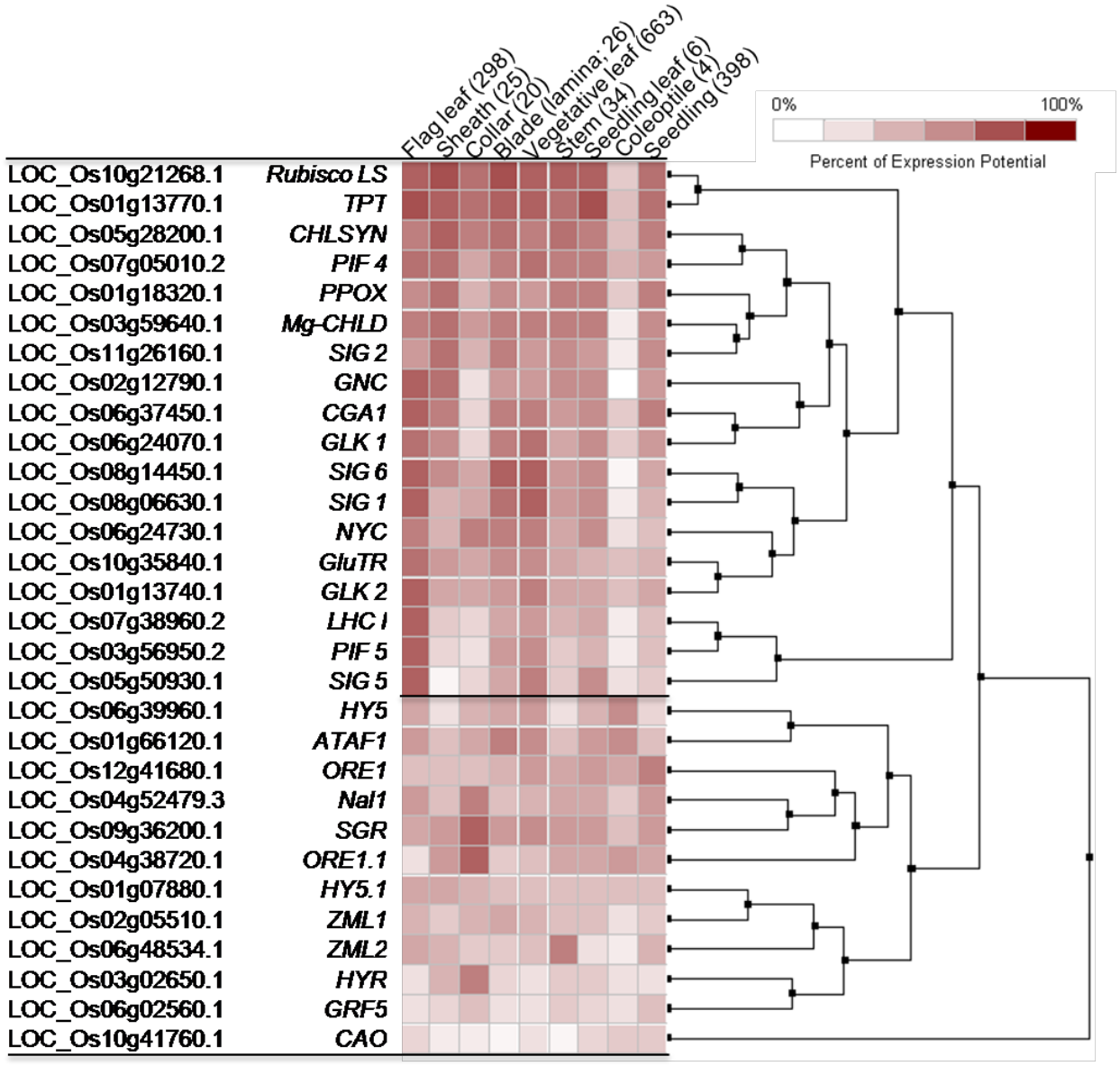

Fig. S6 An *in silico* generated hierarchial clustering dendrogram for the expression pattern of key photosynthetic and chlorophyll-biosynthetic genes, and transcription factor regulating photosynthetic machinery across nine anatomical parts of rice plants (seedling, coleoptile, seedling leaf, stem, vegetative leaf, blade, collar, sheath, flag leaf). The data were collected and dendrogram was generated using Genevestigator (https://genevestigator.com/gv/).

Table S1 Details of rice accessions used to investigate the leaf photosynthesis and related physiological features.

Genome	Genotype	Cultivar/accession number
AA	*Oryza sativa ssp. japonica*	Nipponbare
AA	*Oryza sativa ssp. indica*	IR 64
AA	*Oryza sativa ssp. indica*	Swarna
AA	*Oryza sativa ssp. indica*	Nagina 22
AA	*Oryza sativa ssp. indica*	Vandana
AA	*Oryza glaberrima*	IR 102925
AA	*Oryza rufipogon*	IRGC 99562
BB	*Oryza punctata*	IRGC 105137
CCDD	*Oryza latifolia*	IRGC 99596
EE	*Oryza australiensis*	IRGC 105272

Table S2 Variation in leaf photosynthesis and related physiological traits among cultivated and wild rice species under controlled conditions. Each value represents the mean of 5 data points ± SD. One-way ANOVA analyses followed by LSD calculation by Fisher's test (α = 0.05). Degree of freedom corresponding to numerator and denominator and F values calculating *p values*. Asterisks represent the significance of differences among genotypes. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

Genotype(s)	**2018-Controlled conditions**					
	P_N	**g_s**	**Ci**	**E**	**WUE**	**CE**
O. sativa cv. Nipponbare	9.25 ± 0.48	0.10 ± 0.03	192.06 ± 48.11	5.46 ± 0.94	1.76 ± 0.47	0.05 ± 0.02
O. sativa cv. IR 64	7.98 ± 0.47	0.08 ± 0.02	180.63 ± 44.63	4.00 ± 0.69	2.00 ± 0.4	0.05 ± 0.01
O. sativa cv. Swarna	8.20 ± 0.64	0.09 ± 0.02	188.91 ± 38.66	4.90 ± 0.52	1.68 ± 0.19	0.04 ± 0.01
O. sativa cv. Nagina 22	7.87 ± 0.57	0.08 ± 0.01	196.93 ± 11.22	4.11 ± 0.54	1.93 ± 0.15	0.04 ± 0.00
O. sativa cv. Vandana	7.85 ± 0.46	0.09 ± 0.02	203.66 ± 31.00	4.78 ± 0.73	1.68 ± 0.33	0.04 ± 0.01
O. glaberrima	10.74 ± 0.42	0.11 ± 0.02	191.79 ± 19.28	5.77 ± 0.66	1.88 ± 0.19	0.06 ± 0.01
O. rufipogon	13.07 ± 0.75	0.18 ± 0.01	244.81 ± 8.24	4.72 ± 0.23	2.77 ± 0.12	0.05 ± 0.00
O. punctata	7.65 ± 0.42	0.06 ± 0.01	141.67 ± 31.40	3.52 ± 0.57	2.21 ± 0.31	0.06 ± 0.01
O. latifolia	12.59 ± 1.10	0.13 ± 0.03	190.43 ± 35.71	6.28 ± 1.20	2.05 ± 0.31	0.07 ± 0.01
O. australiensis	11.73 ± 0.86	0.10 ± 0.02	169.06 ± 22.43	5.40 ± 0.52	2.18 ± 0.16	0.07 ± 0.01
Mean ± SD	9.69 ± 0.62	0.1 ± 0.02	189.99 ± 29.07	4.89 ± 0.66	2.02 ± 0.26	0.05 ± 0.01
LSD	0.83	0.027	40.383	0.897	0.364	0.013
df	9, 40	9, 40	9, 40	9, 40	9, 40	9, 40
F value	53.911	12.951	3.364	7.368	6.433	5.595
p values	0.000***	0.000***	0.004**	0.000***	0.000***	0.000***

P_N – net photosynthesis rate (μmol CO_2 m^{-2} s^{-1}); g_s– stomatal conductance to water (mol H_2O m^{-2} s^{-1}); Ci – intercellular CO_2 concentration (μmol CO_2 mol^{-1}); E – transpiration rate (mmol H_2O m^{-2} s^{-1}); WUE – Water use efficiency (ratios P_N : E); CE – Carboxylation efficiency (ratios P_N : Ci).

Table S3 Quantitative measurement of flag leaf width (FLW, cm), flag leaf thickness (FLT, mm), Minor vein width (µm), Minor vein height (µm), and total veins (n) in the fully expanded flag leaf of cultivated and wild rice species. Each value represents the mean ± SD. One-way ANOVA analysesfollowed by LSD calculation by Fisher's test (α = 0.05). Degree of freedom corresponding to numerator and denominator and F values calculating *p values*. Asterisks represent the significance of differences among genotypes. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

Rice genotypes	**FLW**	**FLT**	**Minor vein width**	**Minor vein height**	**Total Veins**
O. sativa cv. Nipponbare	1.55 ± 0.11	0.36 ± 0.06	55.54 ± 6.81	69.93 ± 6.24	77.33 ± 1.15
O. sativa cv. IR 64	1.52 ± 0.08	0.33 ± 0.09	48.38 ± 8.80	59.71 ± 8.82	73.33 ± 1.53
O. sativa cv. Swarna	2.17 ± 0.16	0.36 ± 0.07	62.24 ± 6.39	71.50 ± 9.16	96.33 ± 3.21
O. sativa cv. Nagina 22	2.01 ± 0.09	0.32 ± 0.05	49.64 ± 7.67	56.66 ± 8.41	80.00 ± 3.00
O. sativa cv. Vandana	1.51 ± 0.13	0.26 ± 0.05	41.95 ± 14.14	62.32 ± 9.20	82.00 ± 2.00
O. glaberrima	2.32 ± 0.19	0.63 ± 0.07	NA	NA	NA
O. rufipogon	2.40 ± 0.16	0.54 ± 0.11	50.94 ± 7.56	69.28 ± 7.40	111.00 ± 5.20
O. punctata	1.14 ± 0.10	0.27 ± 0.07	50.29 ± 6.34	60.66 ± 8.12	61.00 ± 2.00
O. latifolia	4.08 ± 0.43	0.68 ± 0.09	59.67 ± 8.72	79.47 ± 8.97	163.00 ± 7.81
O. australiensis	2.24 ± 0.18	0.41 ± 0.07	62.36 ± 8.19	88.29 ± 9.07	105.33 ± 5.13
Mean ± SD	2.09 ± 0.16	0.42 ± 0.07	53.45 ± 6.57	68.65 ± 9.66	94.37 ± 28.51
LSD	0.17	0.07	4.38	4.29	6.75
df	9, 90	9, 90	8, 261	8, 261	8, 18
F value	187.49	40.10	19.74	44.36	169.91
p values	0.000***	0.000***	0.000***	0.000***	0.000***

NA – Not available

Table S4 Quantitative measurement of mesophyll lobes (n), length (µm), width (µm), and area (µm^2) and bundle sheath number (n), and area (µm^2) in the fully expanded flag leaf of cultivated and wild rice species. Each value represents the mean ± SD. One-way ANOVA analyses followed by LSD calculation by Fisher's test (α = 0.05). Degree of freedom corresponding to numerator and denominator and F values calculating *p values*. Asterisks represent the significance of differences among genotypes. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

Rice genotypes	**Mesophyll (lobes)**	**Mesophyll (length)**	**Mesophyll (width)**	**Mesophyll (area)**	**#Bundle sheath**	**Bundle sheath (area)**
O. sativa cv. Nipponbare	7.63 ± 0.85	551.86 ± 92.69	351.57 ± 41.34	2605.73 ± 313.04	22.78 ± 1.22	5479.19 ± 762.54
O. sativa cv. IR 64	7.03 ± 1.16	542.53 ± 130.35	336.62 ± 51.81	2468.26 ± 591.24	18.94 ± 0.89	4840.1 ± 792.46
O. sativa cv. Swarna	8.23 ± 1.36	571.61 ± 151.67	354.24 ± 43.33	2554.70 ± 393.38	24.33 ± 1.26	5145.77 ± 682.59
O. sativa cv. Nagina 22	7.3 ± 0.82	634.61 ± 100.33	356.84 ± 41.66	2853.24 ± 259.29	19.25 ± 1.00	4950.23 ± 2361.76
O. sativa cv. Vandana	7.73 ± 0.87	600.67 ± 111.46	352.44 ± 40.27	2688.79 ± 387.26	18.31 ± 1.09	4773.96 ± 786.02
O. glaberrima	NA	NA	NA	NA	NA	NA
O. rufipogon	7.57 ± 1.52	748.65 ± 146.21	473.50 ± 63.53	3639.71 ± 664.23	24.28 ± 0.97	4745.59 ± 837.37
O. punctata	7.33 ± 1.32	514.53 ± 94.45	355.37 ± 41.81	2297.97 ± 372.73	20.67 ± 0.76	4592.8 ± 967.88
O. latifolia	9.10 ± 1.81	1099.00 ± 316.53	648.13 ± 85.05	5414.77 ± 890.14	19.14 ± 0.96	5191.85 ± 795.21
O. australiensis	7.93 ± 1.05	783.99 ± 177.64	519.88 ± 71.76	3958.76 ± 660.07	21.36 ± 0.96	4656.88 ± 539.59
Mean ± SD	7.76 ± 0.58	671.94 ± 174.26	416.51 ± 101.9	3164.66 ± 952.38	21.01 ± 2.19	4930.71 ± 273.23
LSD	0.64	83.92	28.78	283.49	0.48	499.92
df	8, 241	8, 241	8, 241	8, 241	8, 315	8, 315
F value	6.72	37.52	106.50	97.71	185.67	2.61
p values	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***

NA – Not available

Table S5 Details of genes and transcription factors used for gene expression analysis.

Gene	Acronym	Locus ID	Function	[1]Pathway
Glutamyl-tRNA reductase	GluTR	LOC_Os10g35840.1	Synthesis of 5-Aminolevulinic acid (ALA)	CB
Protoporphyrinogen oxidase	PPOX	LOC_Os01g18320.1	Involved in the production of heme molecule	
Magnesium chelatase subunit ChlD	Mg-CHLD	LOC_Os03g59640.1	Insertion of Mg into protoporphyrin IX in an ATP-dependant manner	
Chlorophyll synthase	CHLSYN	LOC_Os05g28200.1	Conversion of chlorophyllide to chlorophyll a	
Chlorophyllide a oxygenase	CAO	LOC_Os10g41760.1	Oxidation of chlorophyll a to chlorophyll b	
Light-harvesting chlorophyll protein complex	LHC I	LOC_Os07g38960	Functions as a light receptor	P_N
Ribulose bisphosphate carboxylase	[2]Rubisco SS [3]Rubisco LS	LOC_Os12g17600.1 LOC_Os10g21268.1	Catalyzes the rate-limiting step of CO2 fixation in photosynthesis	
Triose phosphate/phosphate translocator	TPT	LOC_Os01g13770.1	Mediates the export of stromal triose-P into the cytosol in a counter exchange for cytosolic Pi	
Cytokinin responsive GATA Factor	CGA1	LOC_Os06g37450.1	Activates photosynthesis gene expression via promoting plastid development	TF
GATA Nitrate-inducible Carbon Metabolism	GNC	LOC_Os02g12790.1	Activates photosynthesis gene expression promoting plastid development	
Growth regulating factor 5	GRF5	LOC_Os06g02560.1	Positively regulates chloroplast division, causing an increase in the number of chloroplasts per cell	
ZINC FINGER PROTEIN	ZML2	LOC_Os06g48534.1	Promotes the expression of photo-protective genes and maintenance of chlorophyll content	
ELONGATED HYPOCOTYL 5	HY5	LOC_Os06g39960.1	bZIP transcription factor domain containing protein, promotes photosynthesis gene expression.	
PHYTOCHROME INTERACTING FACTOR 4	PIF 4 PIF 5	LOC_Os07g05010.2 LOC_Os03g56950.2	H-L-H DNA-binding domain protein: negatively regulates the expression of photosynthesis genes.	
Golden like	GLK1 GLK2	LOC_Os06g24070.1 LOC_Os01g13740.1	MYB-like DNA-binding protein, promote the expression of LHC proteins and chlorophyll biosynthetic pathway genes.	
ORESARA1	ORE1	LOC_Os12g41680.1	NAC domain containing protein inhibiting the expression of GLK genes	
Plastid sigma factors	SIG 1 SIG 2 SIG 5 SIG 6	LOC_Os08g06630.1 LOC_Os11g26160.1 LOC_Os05g50930.1 LOC_Os08g14450.1	Mediate transcription initiation for plastid-encoded RNA polymerase (PEP)	

[1]Pathways: CB – Chlorophyll biosynthesis; P_N – Photosynthesis; TF – Transcription factor
[2]Rubisco SS– Small subunit, (nuclear)
[3]Rubisco LS– Large subunit, (plastidial)

Table S6 List of primers for qRT-PCR analyses.

Acronym	Primer pairs (5'-3')	
	Forward	**Reverse**
GluTR	AGGGAGCATCTATTCATGTTG	TGCATCCTTGAACATCCTATC
PPOX	CTGAAAGTGAGCTGGTAGAAG	CGAGGAACAATCCATCATAAC
Mg-CHLD	ATGCAATTAAAACTGCTCTGCTG	AGCATCATATTGAACTTGGTTAG
CHLSYN	GCATTATTTGGAACCCTTACTC	GTCAATCGCTCCTACACATATC
CAO	CGGTGACCTGAAAGATGATAC	CATTGGATTCTACCCTCACTAAC
LHC I	CATCTTTCCCCAACAACAAGTTC	GTGAAGGCCTGGAAAATGGTG
Rubisco SS	ACTCCAGCTTCGGCAACGTCAGCA	ATACGGACGAATGCATCAGGGTAC
Rubisco LS	ACACTGATATCTTGGCAGCATTCCGAG	GTAGAGCGCGTAGGGCTTTGAAAC
TPT	TGGAGAAGTACCCTGCCTTGATCA	AGTGTGGGCAAACGAGACTGCAAC
CGA1	GATGATGGCCTCCGGACTAC	CGTCGGCTCTCTTCTCCTTC
GRF5	TTACAAGTACCTCGTCGCCG	GTGGTGGTGGTGGTAGAAGG
ZML2	CAAGGGGATGTTGAGGGACC	TTCTTGCTCGGTGGTAGGTG
GLK1	CCATTGACTCCACCAGCACT	AACATCACCGATAGCTGCGT
GLK2	CGCCATGTTGCAGAGGTTTC	GGCAGCGAGTTTATTCCCCT
ORE1	GCACTTGTGGGGATGAGGAA	GCACACCCTCCATCCTGTAC
SIG 1	ACGATCAAGGATGCACGAGT	AGCCAACTCAACACCAAGGT
SIG 2	TTGTGGAAGATGACGGGTCG	GGCCACCATTGTACTCTGCA
SIG 5	GACGGACTACAACGAGGTGG	CTTCCGCTTGGTCTGTTTGC
SIG 6	AGCTGCAGTCCTCTATTCGC	AACCCACTGCATCCATCCTG
Actin	GAAGTGCGACGTGGATATTAG	CAGACACTGTACTTCCTTTCAG