Supplementary data

Title: Knockdown of glycine decarboxylase complex (GDC) alters photorespiratory carbon isotope fractionation in *Oryza sativa* leaves

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Supplementary data are available at *JXB* online.

**Protocol S1.** Estimate of the $^{13}$CO$_2$ composition of the growth chamber atmosphere during the light period.

**Protocol S2.** Additional technical information on the system setup to measure online leaf-atmosphere CO$_2$, H$_2$O and $^{13}$CO$_2$ exchange.

**Table S1.** Data used to calculate the fractional contributions of leaf chamber and growth chamber assimilates to $^{13}$C composition of leaf dark evolved CO$_2$.

**Protocol S3.** Estimate of the $^{13}$C signature and total N content in the leaf biomass.

**Protocol S4.** Estimate of the CO$_2$ compensation point in absence of mitochondrial non-photorespiratory respiration.

**Table S2** Values of $^{18}$O based $g_m$.

**Protocol S5.** Estimate of $^\alpha$ and evaluation of the sensitivity of $f$ on $I^*$ and $^\alpha$ (shown in Fig. S1).

**Protocol S6.** Estimate of the fractional contribution of respiratory substrates from leaf chamber and growth chamber assimilates to the $^{13}$C composition of dark evolved CO$_2$.

**Protocol S7.** Estimating of the $^{13}$C composition of dark evolved CO$_2$ for plants grown at atmospheric $^{13}$C composition of $\sim$41.6‰.

**Fig. S2.** Sensitivity of $f$ on $g_m$, $R_l$ and $e^*$.

**Table S3.** Statistics for the model used to fit leaf dark respiration rates.

**Table S4.** Significance for the model used to fit leaf dark respiration rates.
Table S5. Statistics for the model used to fit the $^{13}$C composition of leaf dark respiration rates.

Table S6. Significance for the model used to fit $^{13}$C composition of leaf dark respiration rates.

Protocol S1. Estimate of the $^{13}$CO$_2$ composition of the growth chamber (G$_{ch}$) atmosphere during the light period

Preliminary measurements had shown that the $^{13}$C composition of atmosphere CO$_2$ in the G$_{ch}$ ($\delta^{13}C_{Gch}, \%$) during the light period was dependent on the $^{13}$CO$_2$ composition in the pressurized tank used for enrichment, with no significant deviations of $\delta^{13}C_{Gch}$ at different hours and at different plant growing stages (data not shown). When plants were $\sim$ four week-old, two air samples were taken from the growth chamber around midday and $\delta^{13}C_{Gch}$ was determined with a CO$_2$ isotope analyzer (TDLAS, model TGA 200A). Specifically, each air sampling was performed with an aquarium pump (Air-Tech 2k4, Penn Plax, NY, USA); this was placed inside the G$_{ch}$ half hour before air sampling and connected to a CO$_2$-proof tube that was passed out of the G$_{ch}$ to fill a 5 L Supel$^{\text{TM}}$ Inert multi-layer foil gas sampling bag with a polypropylene screw cap valve (Supelco Analytical, Sigma-Aldrich). Each bag was flushed twice with N$_2$ before inflation and then partially filled with N$_2$ to dilute the CO$_2$ concentration of the air sample to a range (400-600 $\mu$mol mol$^{-1}$) so that it could be correctly measured with the current TDLAS set-up. Each filled bag was connected to the TDLAS to sample the G$_{ch}$ air in the leaf chamber *out* intake and the $\delta^{13}$C was determined for 4-5 cycles.

Protocol S2. Additional technical information on the system set-up to measure online leaf-atmosphere CO$_2$, H$_2$O and $^{13}$CO$_2$ exchange

Plants were placed in a reach-in plant growth chamber (Conviron EF7, Winnipeg, Canada) with fluorescent lamps (F48T12/CW/VHO, Sylvania) set at a PPFD of $\sim$ 250 $\mu$mol photons m$^{-2}$ s$^{-1}$ incident on the plant canopy and $t_{air}$ of 26 °C. Two LI-COR instruments and a vaporization module were placed inside the reach-in plant growth chamber. A dry CO$_2$-free airflow rate of 5.0 L min$^{-1}$ with precise O$_2$ mole fractions (mmol mol$^{-1}$) was generated by blending volumetric ($\sim$ mole) fractions of N$_2$ and O$_2$ from two pressurized gas tanks through two mass flow
controllers (Fm, Aalborg, Orangeburg, NY, USA). For each LI-COR a pressurized CO₂ tank or cartridge was used as CO₂ source that was connected to the LI-COR 6400-01 CO₂ Injector System to control the CO₂ mole fraction (µmol mol⁻¹) in the leaf chamber (Lch). A vaporization module, in line with the main air stream, made it possible to create an input of water vapor for both leaf chambers. This system was composed by two parallel air sub-streams, one of which was passing through a water flask (w) and leaving it saturated with water vapor (d is mixing air flask). A mass flow regulator (Fr, FM-1050 Series, Matheson Tri-Gas, Inc., Montgomeryville, PA, USA) on each of the two sub-streams made it possible to set the proportion of dry and wet mass flow to build a desired H₂O mole fraction (mmol H₂O mol⁻¹ air) entering both leaf chambers. A manual valve in the out air stream from each Lch made it possible to stop the airflow to TDLAS when matching the gas analyzers. TDLAS sampling sequence was air entering (in) and leaving (out) each Lch, and the sampled air passed through a heated Nafion® dryer (Nd; PDT™-200T-12, Perma Pure LLC, Toms River, NJ, USA) before entering the CO₂ isotope analyzer.

In accordance with Ubierna et al. (2013), the TDLAS measured ¹²CO₂ and ¹³CO₂ volume fractions for 20 s on a series of nine inlet air streams (thus measuring each inlet every 180 s), the last four of which were from air flow entering (in) and leaving (out) the two LI-COR leaf chambers. For each cycle, mean values were calculated from the last 10 s of the 20 s interval. The mole fractions of ¹²CO₂ and ¹³CO₂ (µmol CO₂ mol⁻¹ dry air) and the corresponding ¹³C composition (δ³C, ‰) of the in and out Lch air streams were calculated as previous described by Tazoe et al. (2011) and Ubierna et al. (2013). The TDLAS levels of noise (precision) standard deviation for CO₂ (¹²CO₂ +¹³CO₂) mole fraction and for δ³C were ± 0.06 µmol CO₂ mol⁻¹ dry air and ± 0.26‰, respectively. Each LI-COR was programmed to record data every 180 s, synchronized with the TDLAS measurements of the air out of the Lch. During leaf photosynthetic measurements, the two LI-COR gas analyzers were matched after each change in pO₂, while during leaf dark respiration measurements they were matched at the beginning of the dark period and every 15 min thereafter. However, the gas analyzer were matched when the TDLAS was not measuring a Lch air sample.

References
**Tazoe Y, von Caemmerer S, Estavillo GM, Evans JR.** 2011. Using tunable diode laser spectroscopy to measure carbon isotope discrimination and mesophyll conductance to CO\(_2\) diffusion dynamically at different CO\(_2\) concentrations. Plant, Cell and Environment *34*, 580-591. **Ubierna N, Sun W, Kramer DM, Cousins AB.** 2013. The efficiency of C\(_4\) photosynthesis under low light conditions in *Zea mays*, *Miscanthus × giganteus* and *Flaveria bidentis*. Plant, Cell and Environment *36*, 365-381.

**Protocol S3.** Estimate of the \(^{13}\)C signature and total N content in the leaf biomass

One leaf sample from each of the plants used for the combined photosynthetic and \(^{13}\)CO\(_2\) exchange measurements (\(n = 4\) for *gdch*-KD; \(n = 5\) for WT) was dried in a ventilated oven at 55 °C for 48 h. Each sample was ground to a fine powder, and 1-2 mg dry matter was placed in a tin capsule and combusted in an elemental analyzer (Costech ECS 410, Valencia, CA, USA). The resulting CO\(_2\) and N\(_2\) gas were admitted into the inlet of a mass spectrometer (GV Instrument Isoprime, Manchester, UK) to determine the \(^{13}\)C : \(^{12}\)C ratio (\(R_{\text{sample}}, \%\)) and the total N content as fraction (%) of dry matter. The \(^{13}\)C signature (\(\delta^{13}\)C\(_{\text{dm}}, \%\)) was calculated according to Bender *et al.* (1973) as \(\delta^{13}\)C\(_{\text{dm}} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000\), where \(R_{\text{standard}}\) is the \(^{13}\)C : \(^{12}\)C ratio (%) of the V-Pee Dee Belemnite limestone.

**References**

**Bender MM, Rouhani I, Vines HM, Black CC Jr.** 1973. \(^{13}\)C/\(^{12}\)C ratio changes in Crassulacean acid metabolism plants. Plant Physiology *52*, 427-430.

**Protocol S4.** Estimate of the CO\(_2\) compensation point in absence of mitochondrial non-photorespiratory respiration

The photorespiratory CO\(_2\) compensation point (\(\mu^*, T\) mol mol\(^{-1}\)) was modeled for *gdch*-KD and WT plants at 18.4 kPa atmospheric \(pO_2\) and \(t_{\text{leaf}}\) of 25 °C as
\[ I^* = I \left(1 - \frac{R_L}{V_{c,max}}\right) - K_c \left(1 + \frac{O_2}{K_o} \right) \frac{R_L}{V_{c,max}} \]  

Eqn S1

based on von Caemmerer (2000), where \( I^*(\mu\text{mol mol}^{-1}) \) is the CO₂ compensation point (in the presence of \( R_L \)); \( R_L (\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}) \) is the mitochondrial non-photorespiratory CO₂ evolution rate in the light; \( V_{c,max} (\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}) \) is the maximum rate of carboxylation; \( K_c (\mu\text{mol mol}^{-1}) \) and \( K_o (\text{mmol mol}^{-1}) \) are the Michaelis-Menten constants for CO₂ and O₂, respectively; O₂ is the atmospheric O₂ mole fraction of 200 mmol mol⁻¹ (assumed to equal the O₂ mole fraction in the chloroplasts). \( I^* \) was determined for \textit{gdch}-KD and WT plants from the analysis of leaf \( A-C_i \) response curves (see Material and methods, and Table 3 in the text), with mean values of 59.5 ± 0.4 SE (\( n = 3 \)) and 49.3 ± 1.9 SE (\( n = 4 \)) \mu\text{mol mol}^{-1}, respectively. Mean \( R_L \) were 0.98 and 0.82 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} \) for \textit{gdch}-KD and WT plants, respectively (see Results, \textit{Leaf dark respiration responses}). Values of \( K_c \) of 293 \mu\text{mol mol}^{-1} and \( K_o \) of 404 mmol mol⁻¹, determined \textit{in vitro} at 25 °C on WT leaves (Ryan Boyd, unpublished), were applied to both WT and \textit{gdch}-KD plants.

According to von Caemmerer (2000), \( V_{c,max} \) for each plant type was calculated by fitting the leaf \( A-C_c \) response curves acquired at low O₂ mole fraction (20 mmol mol⁻¹; see Material and methods) to the model

\[ A = \frac{(C_c-I^*)V_{c,max}}{(C_c+K_c')} - R_L \]  

Eqn S2

where \( C_c \) is the CO₂ mole fraction in the chloroplast (\mu\text{mol mol}^{-1}), \( A \) is the leaf net photosynthetic rate for \( C_c < 190 \mu\text{mol mol}^{-1}; \) and \( R_L \) is as in Eqn S1. \( C_c \) was calculated based on Fick’s first law using \( g_m (\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}) \) determined based on Evans and von Caemmerer (2013) and \( I^* \mu\text{mol mol}^{-1} \) modeled based on Jordan and Ogren (1984) as

\[ I^* = \frac{\alpha \times O_2 \times 1000}{s} \]  

Eqn S3

where \( \alpha = 0.50 \) are the moles of CO₂ released in the photorespiratory pathway per mole of O₂ reacting with ribulose-1,5-bisphosphate, O₂ was the O₂ mole fraction of 20 mmol mol⁻¹, and
$S$ is the in vitro Rubisco CO$_2$ : O$_2$ specificity factor equal to 2758 mol mol$^{-1}$. The $S$ value was calculated using $K_c$ and $K_o$ as in Eqn S1, and a ratio of maximum rates of carboxylation : oxygenation ($V_{c,max}/V_{o,max}$) of 2.0.

The term $K'_c$ (µmol mol$^{-1}$) is the apparent Michaelis-Menten constant for Rubisco carboxylation (Kubien et al., 2008) estimated according to von Caemmerer et al. (1994) as

$$K'_c = K_c \left(1 + \frac{O_2}{K_o}\right) \quad \text{Eqn S4}$$

where the values of $K_c$ (µmol mol$^{-1}$) and $K_o$ (mmol mol$^{-1}$) are as in Eqn S1, and O$_2$ is the O$_2$ mole fraction of 20 mmol mol$^{-1}$. For each A-C$_c$ response curve, tuning of modeled-observed $A$ was performed by the procedure of minimized sum of squares, which provided the fit of the $V_{c,max}$ term. Mean values of $V_{c,max}$ of 78.9 ± 2.3 SE (n= 3) and 93.4 ± 5.6 SE (n= 4) µmol CO$_2$ m$^{-2}$ s$^{-1}$ for gdch-KD and WT, respectively, were determined and applied in Eqn S1, which lead to modeled mean $I^*$ of 53.3 ± 0.6 SE (n= 3) and 45.0 ± 1.7 SE (n= 4) µmol mol$^{-1}$ for gdch-KD and WT, respectively.

**References**

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**von Caemmerer S, Evans JR, Hudson GS, Andrews TJ.** 1994. The kinetics of ribulose-1,5-bisphosphate carboxylase/oxygenase in vivo inferred from measurements of photosynthesis in leaves of transgenic tobacco. Planta 195, 88-97.

**von Caemmerer S.** 2000. Biochemical models of leaf photosynthesis. No. 2. CSIRO Publishing.
Table S1. Leaf $^{18}$O based $g_m$ determined in gdch-KD and WT plants under atmospheric $pO_2$ of 18.4 kPa. Values are mean ± SE (n= 4). Significance (P< 0.05) was evaluated by a two sample $t$-test.

| Plant-type | $g_m_{18O}$  |
|------------|--------------|
|            | $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$ Pa$^{-1}$ |
| gdch-KD    | 3.04 ± 0.33  |
| WT         | 3.26 ± 0.43  |
| Significance | P= 0.767    |

Protocol S5. Estimate of $\alpha$, and evaluation of the sensitivity of $f$ on $\Gamma^*$ and $\alpha$

Based on Evans and von Caemmerer (2013)

$$f = \frac{\Delta f^* c_a}{\Gamma^*} \frac{(1-t)}{(1+t)}$$  \hspace{1cm} \text{Eqn S5}$$

and the sensitivity of $f$ (‰) to $\Gamma^*$ ($\mu$mol mol$^{-1}$) under photorespiratory conditions was determined and shown in Fig. S1A.

A Rubisco CO$_2$ : O$_2$ specificity factor of 2222 mol mol$^{-1}$ was calculated for WT using Eqn S3, where $\alpha$ was assumed equal to 0.50, O$_2$ was 200 mmol mol$^{-1}$, and $\Gamma^*$ was 45.0 $\mu$mol mol$^{-1}$. Under the assumption that $S$ for gdch-KD plants was the same of WT, the value of $\alpha$ was estimated for the transgenic plants using Eqn S3, where O$_2$ was 200 mmol mol$^{-1}$, and $\Gamma^*$ was 53.3 $\mu$mol mol$^{-1}$.

By merging Eqns S2 and S4, the dependency of $f$ on $\alpha$ was determined as

$$f = \frac{\Delta f^* c_a s}{\alpha * O_2 * 1000} \frac{(1-t)}{(1+t)}$$  \hspace{1cm} \text{Eqn S6}$$

and shown in Fig. S1B.
References

Evans JR, von Caemmerer S. 2013. Temperature response of carbon isotope discrimination and mesophyll conductance in tobacco. Plant, Cell and Environment 36, 745-756.

Fig. S1 (A) Sensitivity of the $f$ parameter to $\Gamma^*$ in Eqn S17 determined with $\Delta f$ of 2.41‰ (mean WT value) and $C_a$ of 300 $\mu$mol mol$^{-1}$. (B) Sensitivity of the $f$ parameter to $\alpha$ in Eqn S6 determined with $\Delta f$ of 2.41‰ and $C_a$ of 300 $\mu$mol mol$^{-1}$, $S$ of 2222 mol mol$^{-1}$, and $O_2$ mole fraction of 200 mmol mol$^{-1}$. The combined plots (A) and (B) visualize the correspondence (dashed lines) among $f$, $\Gamma^*$ and $\alpha$ values; in particular, $\Gamma^*$ of 45.0 $\mu$mol mol$^{-1}$ (mean WT value) corresponds to $\alpha$ of 0.50 and $f$ of 16.2‰; $\Gamma^*$ of 53.3 $\mu$mol mol$^{-1}$ (mean gdch-KD value) corresponds to $\alpha$ of 0.59.

Fig. S2. Sensitivity of the $f$ parameter to (A) $g_m$ (B) $R_L$ and (C) $^{13}$CO$_2$ fractionation associated with $R_L$ ($e'$), based on Evans and von Caemmerer (2013), at $O_2$ mole fraction of 200 mmol mol$^{-1}$ and $C_a$ of 300 $\mu$mol mol$^{-1}$. Mean WT functional values were used; $A$ was equal to 14.3 $\mu$mol
CO₂ m⁻² s⁻¹. (A) \( R_L \) was equal to 0.82 μmol CO₂ m⁻² s⁻¹ and \( e = 0 \); (B) \( g_m \) was equal to 0.38 mol CO₂ m⁻² s⁻¹ and \( e = 0 \); (C) In the present study, it was assumed that no \(^{13}\)CO₂ fractionation occurred in the light during respiration via TCA pathway. Vice-versa, under the assumption of a \(^{13}\)CO₂ fractionation during \( R_L \) via TCA pathway (\( e, \% \)), the \(^{13}\)CO₂ fractionation associated with \( R_L \) (\( e', \% \)) would have been \( e' = e^* + e \) as in Kromdijk et al. (2010) and von Caemmerer et al. (2014), based on Wingate et al. (2007), where \( e^* \) was the difference between \( \delta^{13}C \) of the CO₂ entering the L_ch (\( \delta_m \)) and in the G_ch. In the graph, \( e^* \) was –6.4‰ and \( e \) ranged from 0 to –10‰, \( g_m \) and \( R_L \) were equal to 0.38 and 0.82 μmol CO₂ m⁻² s⁻¹, respectively.

References

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Wingate L, Seibt U, Montcrieff JB, Jarvis PG, Lloyd JOL. 2007. Variations in \(^{13}\)C discrimination during CO₂ exchange by Picea sitchensis branches in the field. Plant, Cell and Environment 30, 600-616.
Table S2. Leaf functional variables and values used to calculate the fractional contributions of leaf chamber (Lch) and growth chamber (Gch) assimilates to $\delta^{13}$C of leaf dark evolved CO$_2$ in gdch-KD and WT plants following leaf photosynthesis under two atmospheric $p$O$_2$. The variables for computations are $\delta^{13}$C of CO$_2$ in the L$_{ch}$ ($\delta_{out}$), leaf $^{13}$C net discrimination in the light ($\Delta_o$), $^{13}$C discrimination associated to $R_L$ ($\Delta_e$), $^{13}$C signature of assimilates produced in the L$_{ch}$ ($\delta^{13}$C$_{Lch,Ph}$), $^{13}$C composition of dark evolved CO$_2$ after 24 h dark ($\delta^{13}$C$_{Lch(24h)}$), and difference between $\delta^{13}$C$_{Lch,Ph}$ and $\delta^{13}$C$_{Lch(24h)}$. For all variables, values are mean (n= 4). The data originate from two batches of plants grown in a G$_{ch}$ with atmospheric $\delta^{13}$C O$_2$ ($\delta^{13}$C$_{Gch}$) of −41.6 and −30.6‰ and from leaf measurements with $\delta^{13}$C in CO$_2$ entering the L$_{ch}$ ($\delta_{in}$) enriched compared to G$_{ch}$. For each plant type, under both $p$O$_2$ experimental conditions, $e^*$ was calculated as ($\delta_{in} - \delta^{13}$C$_{Gch}$). In addition, mean values ± SE for $^{13}$C signature of leaf dry matter ($\delta^{13}$C$_{dm}$; n= 4 for gdch-KD; n= 5 for WT) are shown for plants grown at atmospheric $\delta^{13}$C$_{Gch}$ of −41.6‰, with no significance (P= 0.189) between the two plant types determined by One-way ANOVA (P< 0.05).

| Plant-type | $\delta^{13}$C$_{Gch}$ | $p$O$_2$ | $\delta_{in}$ | $\delta_{out}$ | $\Delta_o$ | $\Delta_e$ | $\delta^{13}$C$_{Lch,Ph}$ | $\delta^{13}$C$_{Lch(24h)}$ | $\delta^{13}$C$_{Lch,Ph} - $\delta^{13}$C$_{Lch(24h)}$ | $\delta^{13}$C$_{dm}$ |
|------------|------------------------|---------|--------------|---------------|-----------|-----------|--------------------------|--------------------------|--------------------------------|------------------|
| gdch-KD    | −41.6                  | 1.84    | −6.1         | 35.5          | −3.8      | 14.9      | −19.8                    | −67.0                    | 47.2                           | −63.4 ± 0.3 |
|            | −30.6                  | 18.4    | −5.5         | 25.1          | −4.4      | 23.7      | −30.5                    | −58.1                    | 27.6                           |
| WT         | −41.6                  | 1.84    | −5.1         | 36.5          | −3.3      | 14.3      | −18.6                    | −67.2                    | 48.6                           | −62.3 ± 0.5 |
|            | −30.6                  | 18.4    | −5.6         | 25.0          | −3.8      | 17.8      | −22.7                    | −58.6                    | 35.9                           |
Protocol S6. Estimate of the fractional contribution of respiratory substrates from leaf chamber and growth chamber assimilates to the \( ^{13} \text{C} \) composition of dark evolved CO\(_2\)

Based on Lehmann et al. (2016), \( R_d \) at light-dark transition \((i= 0 \text{ min})\) was considered totally fueled by recent \( ^{\delta^{13}} \text{C} \) enriched \( \text{L}_{\text{ch}} \) assimilates (mean \( ^{\delta^{13}} \text{C} \) of CO\(_2\) entering the \( \text{L}_{\text{ch}} \) was in the range from \(-6.2 \) to \(-4.8\)‰) and no \( ^{13} \text{CO}_2 \) discrimination by respiration via TCA cycle \((e= 0)\) was assumed at that time. Vice-versa, based on Lehmann et al. (2016), \( R_d \) after 24 h dark was considered fed exclusively by depleted respiratory substrates from leaf carbon fixed in the \( \text{G}_{\text{ch}} \) \( (^{\delta^{13}} \text{C} \) of atmospheric CO\(_2\) in the \( \text{G}_{\text{ch}} \) was \(-41.6 \) or \(-30.6\)‰). These assumptions were supported by the negative hyperbolic distribution of \( ^{\delta^{13}} \text{C} \) over the three h in the dark (Fig. 4), and the \( ^{\delta^{13}} \text{C}_{\text{Rd}(3\text{h})} \) values approaching the \( ^{\delta^{13}} \text{C}_{\text{Rd}(24\text{h})} \) values (Table 3). For \( \text{gdch-KD} \) and WT plants, the \( ^{\delta^{13}} \text{C}_{\text{Rd}} \) at light-dark transition was therefore set equal to the \( ^{\delta^{13}} \text{C} \) of assimilates produced in the \( \text{L}_{\text{ch}} \) \( (^{\delta^{13}} \text{C}_{\text{Lch,Ph}}, \text{‰}) \). Representative \( ^{\delta^{13}} \text{C}_{\text{Lch,Ph}} \) were modeled based on the equation \( ^{\delta^{13}} \text{C}_{\text{Lch,Ph}} = ^{\delta^{13}} \text{C}_{\text{out}} - \Delta_o \), formulated by Farquhar et al. (1982), where \( ^{\delta^{13}} \text{C}_{\text{out}} \) is the \( ^{\delta^{13}} \text{C} \) of CO\(_2\) leaving the \( \text{L}_{\text{ch}} \) \( (%) \) and \( \Delta_o \) is the value of leaf net \( ^{13} \text{CO}_2 \) discrimination \( (%) \); the latter was determined during leaf photosynthesis in the \( \text{L}_{\text{ch}} \) before light-dark transition, with PPFD of 750 and 500 \( \mu \text{mol} \) photons \( \text{m}^{-2} \text{ s}^{-1} \), \( C_a \) of 35.0 Pa, and \( pO_2 \) of 1.84 or 18.4 kPa. Based on the assumption made in the present study, \( R_l \) was fed by respiratory substrates produced in the growth chamber, which had lower atmospheric \( ^{\delta^{13}} \text{C} \) respect to the leaf chamber. The representative \( ^{\delta^{13}} \text{C}_{\text{Lch,Ph}} \) were therefore modeled for \( \text{gdch-KD} \) and WT at both \( O_2 \) experimental levels as \( ^{\delta^{13}} \text{C}_{\text{Lch,Ph}} = ^{\delta^{13}} \text{C}_{\text{out}} - (\Delta_o + \Delta_e) \) to limit the contribution to \( ^{\delta^{13}} \text{C}_{\text{Lch,Ph}} \) of a considerable isotopic disequilibrium of the mitochondrial (non-photorespiratory) respiratory flux (more \( ^{13} \text{C} \) depleted) respect to the photosynthetic flux. In particular, the term \( \Delta_e \) for both plant types at both \( O_2 \) experimental levels was calculated according to Evans and von Caemmerer (2013), with \( e^* \) are as in Table S2. The mean values of \( ^{\delta^{13}} \text{C}_{\text{Lch,Ph}}, ^{\delta^{13}} \text{C}_{\text{out}}, \) and \( ^{\delta^{13}} \text{C}_{\text{Rd}} \) after 24 h dark \( (^{\delta^{13}} \text{C}_{\text{Rd}(24\text{h})}, \text{‰}) \) for both \( \text{gdch-KD} \) and WT plant and for both \( O_2 \) experimental levels are shown in Table S2. The difference between the (enriched) \( ^{\delta^{13}} \text{C}_{\text{Lch,Ph}} \) and the (depleted) \( ^{\delta^{13}} \text{C}_{\text{Rd}(24\text{h})} \) values represents the (absolute) range of \( ^{\delta^{13}} \text{C}_{\text{Rd}} \) \( (%) \) that can be generated over the 24 h in the dark by leaf respiration of substrates produced from carbon fixed in both \( \text{L}_{\text{ch}} \) and \( \text{G}_{\text{ch}} \) (Table S2). For each plant type, under both experimental \( O_2 \) levels, known \( ^{\delta^{13}} \text{C}_{\text{Rd}(i)} \) \( (%) \) as \( ^{\delta^{13}} \text{C} \) of \( R_d \) over 195 min by \( i \) steps of three min from light-dark transition, and
δ[^13]C_Rd(24h) as the value associated with respiration of only substrates from carbon fixed in the G_ch, δ[^13]C_Rd(i) δ[^13]C_Rd(24h) corresponds to the (absolute) δ[^13]C enrichment (‰) of dark evolved CO₂ at time i due to L_ch assimilates. The fractional contributions of L_ch and G_ch assimilates to substrates feeding R_d were then calculated as in Material and Methods Eqn 1 and Eqn 2, respectively.

References

Farquhar GD, O'Leary M, Berry JA. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Functional Plant Biology 9, 121-137.

Lehmann MM, Wegener F, Barthel M, Maurino VG, Siegwolf RT, Buchmann N, Werner RA. 2016. Metabolic fate of the carboxyl groups of malate and pyruvate and their influence on δ[^13]C of leaf-respired CO₂ during light enhanced dark respiration. Frontiers in Plant Science 7, 739.

Protocol S7. Editing of the δ[^13]C composition of dark evolved CO₂ for plants grown at atmospheric δ[^13]C composition of −41.6‰

In the present study, two batches of plants were grown in the G_ch; in particular, the plants used to determine leaf dark respiration after photosynthesis under pO₂ of 1.84 kPa were grown at δ[^13]C_Gch equal to −41.6‰, while the plants used after leaf photosynthesis under ~ current ambient O₂ level were grown at δ[^13]C_Gch equal to −30.6‰. To make it possible a combined analysis of the data collected in the two O₂ experimental conditions, the δ[^13]C_Rd generated from plants grown at the more depleted δ[^13]C_Gch were edited to cancel out the bias in the δ[^13]C_Gch effect on δ[^13]C_Rd respect to the other batch of plants. The δ[^13]C_Rd editing was based on the assumption made to calculate the fractional contribution of δ[^13]C_Gch substr(i) in Eqn 2, i.e. that the leaf respiratory substrates after 24 h in the dark were exclusively from carbon fixed in the G_ch, while no contribution of leaf assimilates produced in the G_ch to respiratory substrates occurred at light-dark transition (see Methods S6). First, the difference between δ[^13]C_Gch of −30.6 versus −41.6‰ was computed (ΔGchδ[^13]C_Rd(24h), ‰) based on data in Table S2. Following
leaf exposure at $pO_2$ of 1.84 kPa, $\delta^{13}C_{Rd}$ during the $i$ min in the dark (from 0 to 195 by steps of three) were edited ($^{13}$C enriched) for $gdch$-KD and WT plants as

$$\text{edited } \delta^{13}C_{Rd(i)} = \delta^{13}C_{Rd(i)} + \delta^{13}C_{ch\_substr(i)} \times \Delta G_{ch} \delta^{13}C_{Rd(24h)}$$

Eqn S7

where $\Delta G_{ch} \delta^{13}C_{Rd(24h)}$ were 8.9 and 8.6‰ for $gdch$-KD and WT, respectively.
Table S3. Statistics for the model used to fit leaf dark respiration rates. Estimates and 95% Confidence Interval (CI) for range (μmol CO₂ m⁻² s⁻¹), slope (μmol CO₂ m⁻² s⁻¹ min⁻¹) and lower asymptote (floor; μmol CO₂ m⁻² s⁻¹) parameters of the non-linear model used to fit $R_d$ after leaf light exposure under $pO_2$ of 1.84 and 18.4 kPa in *gdch*-KD and WT plants (n= 4 replicate curves). CI are determined as CI = parameter estimate ± $t \times SE$ of parameter, where $t$ is the $t$-distribution with a confidence level of 95% and the degrees of freedom from the calculation.

| O₂ level | Plant-type | Estimate (95% CI) |                |                |                |
|----------|------------|-------------------|----------------|----------------|----------------|
|          |            | Range             | Slope          | Lower Asymptote |
| 1.84     | gdch-KD    | 0.54 (0.42, 0.66) | -0.024 (0.012, 0.035) | 0.84 (0.78, 0.89) |
| 1.84     | WT         | 0.58 (0.45, 0.71) | -0.030 (0.017, 0.042) | 0.82 (0.77, 0.86) |
| 18.4     | gdch-KD    | 1.14 (0.98, 1.29) | -0.010 (0.006, 0.015) | 1.18 (0.98, 1.38) |
| 18.4     | WT         | 0.95 (0.80, 1.09) | -0.034 (0.025, 0.043) | 1.12 (1.16, 1.24) |
Table S4. Significance for the model used to fit leaf dark respiration rates. Significance of the comparison of the effects of O₂ level (pO₂ of 1.84 and 18.4 kPa), plant-type (gdch-KD and WT) and plant-type × O₂ level interactions on range, slope and lower asymptote (floor) parameters of the non-linear model fitting Rₙ over ~ three h (n= 4 replicate curves). The Rₙ range was significantly different between O₂ level and plant-type. The slopes were significantly different for plant-type but not O₂ level (P= 0.052); however, the difference between transgenic and WT plant types was driven by the 18.4 pO₂. A significant pO₂ level effect was determined for the lower asymptote, although when looking at plant types separately that difference only achieves strict significance for WT (P < 0.0001).

| Comparisons | Range | Slope | Lower asymptote |
|--------------|-------|-------|-----------------|
| O₂ level     |       |       |                 |
| (1.84 vs. 18.4) | < 0.0001 | 0.052 | < 0.0001        |
| Plant-type (gdch-KD vs. WT) | 0.023 | < 0.0001 | 0.80 |
| 1.84 vs. 18.4 |       |       |                 |
| gdch-KD      | < 0.0001 | 0.059 | 0.059           |
| WT           | <0.001  | 0.67  | <0.0001         |
| gdch-KD vs. WT |       |       |                 |
| 1.84         | 0.65   | 0.59  | 0.67            |
| 18.4         | 0.09   | < 0.0001 | 0.88 |
Table S5. Statistics for the model used to fit the $^{13}$C composition of leaf dark respiration rates. Estimates and 95% Confidence Interval (CI) for range (‰), slope (‰ min$^{-1}$) and lower asymptote (‰) parameters of the non-linear model used to fit $\delta^{13}$C$_{Rd}$ after leaf light exposure under $p$O$_2$ of 1.84 and 18.4 kPa in gdch-KD and WT plants ($n$= 4 replicate curves). CI are calculated as CI = parameter estimate ± $t \times$ SE of parameter, where $t$ is the $t$-distribution with a confidence level of 95% and the degrees of freedom from the calculation.

| O$_2$ level | Plant-type | Range         | Estimate (95% CI) | Lower Asymptote |
|-------------|------------|---------------|-------------------|-----------------|
| 1.84        | gdch-KD    | 15.4 (12.8, 18.1) | $-0.030$ (0.020, 0.040) | $-53.5$ (−54.4, −52.7) |
| 1.84        | WT         | 19.2 (16.1, 22.2)  | $-0.034$ (0.025, 0.043) | $-53.4$ (−54.2, −52.6) |
| 18.4        | gdch-KD    | 11.2 (8.0, 14.5)   | $-0.043$ (0.025, 0.061) | $-54.1$ (−54.7, −53.5) |
| 18.4        | WT         | 10.7 (8.5, 12.8)   | $-0.024$ (0.013, 0.034) | $-53.4$ (−54.5, −52.4) |
Table S6. Significance for the model used to fit the $^{13}$C composition of leaf dark respiration rates. Significance of O$_2$ level ($pO_2$ of 1.84 and 18.4 kPa), plant-type (gdch-KD and WT), plant-type × O$_2$ level interactions on range, slope and lower asymptote parameters of the non-linear model fitting $\delta^{13}C_{RD}$ over ~ three h ($n=4$ replicate curves). Overall, the range is significantly different for the O$_2$ levels, but not for plant-type; the observed differences in O$_2$ levels are significant for WT only ($P<0.0001$). Overall, the slopes for O$_2$ level and plant-type are not significantly different ($P=0.88$ and 0.60, respectively) and none of the pairwise comparisons is significant. Overall, the lower asymptotes for plant-type and O$_2$ levels are not significantly different ($P=0.26$ for both) and none of the pairwise comparisons is significant.

| Comparisons | Range     | Slope | Lower asymptote |
|-------------|-----------|-------|-----------------|
| O$_2$ level |           |       |                 |
| (1.84 vs. 18.4) | $<0.0001$ | 0.88  | 0.26            |
| Plant-type  | 0.52      | 0.60  | 0.26            |
| (gdch-KD vs.WT) |          |       |                 |
| 1.84 vs. 18.4 |          |       |                 |
| gdch-KD     | 0.097     | 0.29  | 0.39            |
| WT          | $<0.0001$ | 0.27  | 0.82            |
| gdch-KD vs.WT |          |       |                 |
| 1.84        | 0.11      | 0.64  | 0.93            |
| 18.4        | 0.79      | 0.12  | 0.37            |