Physiological evidence for plasticity in glycolate/glycerate transport during photorespiration

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Abstract Photorespiration recycles fixed carbon following the oxygenation reaction of Ribulose, 1–5, carboxylase oxygenase (Rubisco). The recycling of photorespiratory C2 to C3 intermediates is not perfectly efficient and reduces photosynthesis in C3 plants. Recently, a plastidic glycolate/glycerate transporter (PLGG1) in photorespiration was identified in Arabidopsis thaliana, but it is not known how critical this transporter is for maintaining photorespiratory efficiency. We examined a mutant deficient in PLGG1 (plgg1-1) using modeling, gas exchange, and Rubisco biochemistry. Under low light (under 65 μmol m−2 s−1 PAR), there was no difference in the quantum efficiency of CO2 assimilation or in the photorespiratory CO2 compensation point of plgg1-1, indicating that photorespiration proceeded with wild-type efficiency under sub-saturating light irradiances. Under saturating light irradiance (1200 μmol m−2 s−1 PAR), plgg1-1 showed decreased CO2 assimilation that was explained by decreases in the maximum rate of Rubisco carboxylation and photosynthetic linear electron transport. Decreased rates of Rubisco carboxylation resulted from probable decreases in the Rubisco activation state. These results suggest that glycolate/glycerate transport during photorespiration can proceed in moderate rates through an alternative transport process with wild-type efficiencies. These findings also suggest that decreases in net CO2 assimilation that occur due to disruption to photorespiration can occur by decreases in Rubisco activity and not necessarily decreases in the recycling efficiency of photorespiration.

Keywords Photorespiration · Photosynthesis · Quantum efficiency · Abiotic stress · Central metabolism · Photosynthetic models

Introduction

Gross CO2 assimilation during photosynthesis is diminished by CO2 loss from photorespiration in C3 plants. This CO2 loss is dependent on temperature and CO2 partial pressures and comprises the largest single loss of carbon to an illuminated C3 leaf, resulting in an annual decrease of 322 trillion dietary calories in the Midwestern United States alone (Walker et al. 2016b; Sharkey 1988). Photorespiration is initiated when Ribulose 1–5 bisphosphate carboxylase oxygenase (Rubisco) reacts with O2 instead of CO2 and produces 3-phosphoglycerate and 2-phosphoglycerate. Photorespiration recycles phosphoglycolate into the C3 intermediate 3-phosphoglycerate in reactions involving
the chloroplast, peroxisome, and mitochondrion (Foyer et al. 2009; Bauwe et al. 2010). According to current understanding, this photorespiratory recycling is not perfectly efficient on a carbon basis in that for every four carbons that enter photorespiration as two phosphoglycolate, one is lost as CO₂, while the remaining three form 3-phosphoglycerate.

There is evidence that photorespiratory carbon efficiency can decrease further when photorespiration is disrupted genetically. Photorespiratory mutants typically have decreased net CO₂ assimilation rates, which could be driven by decreases to photorespiratory efficiency (Somerville and Ogren 1979, 1980a, b; Timm et al. 2012; Pick et al. 2013). Decreases in photorespiratory efficiency increase the stoichiometry of CO₂ release per Rubisco oxygenation (α) above the normally assumed value of 0.5. ¹⁸O₂ and ¹⁰O₂ exchange experiments indicate that α increases in Arabidopsis thaliana missing the photorespiratory genes (Pick et al. 2013, supporting Fig. 5 therein). Decreases in photorespiratory efficiency in hpr, pmdh1pmdh2, and hprpmdh1pmdh2 suggest the presence of alternative metabolic fates for downstream products of glycolate metabolism and highlight the plasticity of photorespiration. For this work, we define plasticity as the ability for photorespiration to channel metabolites through pathways or protein-mediated processes (such as transport) that are not currently included in dogmatic portrayal of photorespiration. While this decrease in photorespiratory recycling efficiency has been characterized explicitly for mutants deficient in hydroxypyruvate reduction, it has not yet been examined in other photorespiratory mutants.

In this work, we have defined decreases in photorespiratory efficiency as increases in the amount of CO₂ released per Rubisco oxygenation (α), although we recognize it could be also considered on an energy basis. Decreases in photorespiratory efficiency are reflected in increases to the CO₂ compensation (Γ) and photorespiratory CO₂ compensation point (Γ*), which scale with α according to the relationship.

\[ \Gamma = \frac{2O}{S_{c/o}} \]  

and

\[ \Gamma^* = \frac{K_c(1 + O/K_o)R_d/V_{cmax}}{1 - R_d/V_{cmax}} \]  

where O, S_{c/o}, K_c, K_o, R_d, and V_{cmax} represent the partial pressure of oxygen, specificity of Rubisco for reaction with CO₂ relative to oxygen, Km for CO₂, Km for oxygen, rates of day respiration, and the maximum rate of Rubisco carboxylation, respectively (von Caemmerer 2000; Cousins et al. 2008, 2011; Timm et al. 2008, 2012). Decreases in photorespiratory efficiency should also decrease the quantum efficiency of net CO₂ assimilation (ΦCO₂), since more CO₂ would be lost through photorespiration per photon of light absorbed; however, we are unaware of any modeling framework quantifying the impact of changes in α on ΦCO₂.

Somewhat recently a key photorespiratory plastidic glycolate glycerate antiporter gene (PLGG1) was identified (Pick et al. 2013), over 30 years after the transporter had been extensively characterized biochemically (Howitz and McCarty 1982, 1986, 1991; Howitz and McCarty 1985). PLGG1 is present in only one copy in Arabidopsis thaliana (plgg1) and is thought to be the key plastidic transporter of photorespiratory carbon skeletons by exchanging glycolate generated following the oxidation by Rubisco for glycerate produced in the peroxisome. plgg1-1 shows several hallmarks of a photorespiratory mutant such as visible leaf damage following 7 days transition to ambient from high CO₂ growth conditions and decreased net CO₂ assimilation at ambient compared to high CO₂; however, the visual phenotype is not as severe when compared side-by-side with a mutant lacking serine hydroxymethyltransferase (Pick et al. 2013, supporting Fig. 5 therein).

It is also interesting that given its central role to photorespiration and the presence of only a single copy in A. thaliana, plgg1 managed to evade discovery despite several decades of photorespiratory screens using a variety of detection methods (Somerville 1986; Badger et al. 2009; Timm and Bauwe 2013). Additionally, while the biochemical characterization of PLGG1 reveals it to be an antiporter of glycolate and glycerate with a 1:1 stoichiometry, the stoichiometry would need to be 2:1 to explain carbon transport in the current schema of photorespiration (Howitz and McCarty 1982, 1986, 1991; Ogren 1984; Howitz and McCarty 1985). That PLGG1 evaded detection for so long could have been the result plasticity in glycolate/glycerate exchange across the chloroplast envelope membrane and/or plasticity within the photorespiratory metabolic pathway itself (Timm et al. 2012).

The purpose of this study is to investigate the plasticity of glycolate/glycerate transport during photorespiration through measurements of gas exchange of plgg1-1 under photorespiratory and non-photorespiratory conditions. We also developed a model to determine the impact of increases in α to ΦCO₂, and compared this model to measurements under photorespiratory and non-photorespiratory conditions. We parameterize this model for plgg1-1 based on the α value from hprpmdh1pmdh2 hypothesizing that a full blockage of glycerate return to the chloroplast would have a similar metabolic phenotype as a mutant defective in the immediately downstream reaction forming the
glycerate, assuming that chloroplastic export of glycolate can occur via simple diffusion. This work revealed that PLGG1 does not appear essential to maintain photorespiratory efficiency on a CO2 exchange basis under low irradiance (under 65 μmol m−2 s−1 PAR) and that decreases in net CO2 assimilation instead are driven mainly by decreases in the activation state of Rubisco and capacity for electron transport. These findings indicate that photorespiration is plastic in transport processes and suggest a mechanism for the regulation of photosynthesis by photorespiration.

**Materials and methods**

**Growth conditions and transgenic confirmation**

Seeds for pgg1-1 (SALK line SALK_053469C) were obtained from the Arabidopsis Biological Resource Center. T-DNA insertional interruption and homozygosity were confirmed by PCR on pgg1-1 using the primers and methods reported previously (Pick et al. 2013). Wild-type Arabidopsis thaliana (Col-0) and plgg1-1 were stratified in distilled water for 2–3 days at 4 °C and sown directly on soil. Plants were grown in a climate-controlled cabinet (Conviron, Winnipeg, Manitoba, Canada) with day/night cycles of 8/16 h and 23/18 °C under an irradiance of 250 μmol m−2 s−1. CO2 was maintained at ~200 Pa and periodically monitored using an infra-red gas analyzer (SBA-5, PP systems, Amesbury, MA, USA) and datalogger (CR1000, Campbell Scientific, Logan, UT, USA). Identical chambers with no CO2 enrichment (~40 Pa) were used for ambient treatments. Plants were watered as needed and fertilized weekly (Peters 20-20-20, J.R. Peters, Allentown, PA, USA).

**Gas exchange and leaf-level chlorophyll fluorescence**

The youngest fully expanded leaves of 30–40 days old plants were used for gas exchange and subsequent analysis. The plants where measured during the end of the principle growth stage 3 (Boyes et al. 2001) and the youngest fully expanded leaf was defined as the youngest leaf that had begun petiolar elongation and was expanded to an area larger than ~3 cm2. Gas exchange was performed using a LI-COR 6400 XT with 2 cm2 fluorescence measuring head (LI-COR Biosciences, Lincoln, NE, USA) with gasket leaks corrected as outlined in the manual. The multiphase flash protocol was employed for leaf-level chlorophyll fluorescence with appropriate optimizations of flash intensity and kinetics (Loriaux et al. 2013). The operational quantum efficiency of PSII (ΦPSII), a unitless indicator of the proportion of light energy absorbed by PS II that is put towards plastoquinone reduction, was determined according to standard PAM fluorescence equations (Genty et al. 1989). Chloroplastic CO2 was determined from intercellular CO2 assuming a mesophyll conductance of 3 mol m−2 s−1 MPa−1 as determined previously in Arabidopsis grown under elevated CO2 and similar conditions (Walker et al. 2013).

Light response curves were measured by acclimating a clamped leaf under 1200 μmol m−2 s−1 PAR and then decreasing the irradiance stepwise to 380, 120, 65, 40, 30, 27, 18, and 10 μmol m−2 s−1 at both high and low intercellular CO2 (~10 and 100 Pa with 21 kPa oxygen) and low oxygen (2 kPa and ~25 Pa intercellular CO2). Following the light response curve, leaf absorbance was determined using an integrating sphere (Jaz Spectroclip, Ocean Optics, Dunedin, FL, USA) and used to determine absorbed irradiance. The quantum efficiency of net CO2 fixation (ΦCO2) was determined as the slope of the response of net CO2 assimilation to increasing absorbed irradiance up to 30 μmol m−2 s−1. Low oxygen (2 kPa) was provided using mass flow controllers regulating oxygen and nitrogen flow using a custom-built Raspberry-Pi controller.

The photorespiratory CO2 compensation point (Γ+) and R4 were determined from the common intersection of the linear portions of photosynthetic CO2 response curves measured at sub-saturating irradiances using slope-intercept regression and assuming a single mesophyll conductance (Laisk 1977; von Caemmerer et al. 1994; Walker and Ort 2015; Walker et al. 2016a). CO2 assimilation was measured stepwise from 10 to 1.5 Pa CO2 using a LI-COR 6400 XT modified to reach low CO2 concentrations at irradiances of 250, 150, 75, and 50 μmol m−2 s−1. Standard errors on all Γ+ measurements where smaller when slope-intercept regression was applied compared to standard common intercept averaging (Walker et al. 2016a).

Full photosynthetic CO2 response curves were measured using CO2 partial pressures in the following order: 40, 25, 15, 5, 10, 40, 120, 200, 160, 80, and 40 Pa CO2, under saturating irradiance (1200 μmol m−2 s−1) and fitted to determine Vcmax and Jmax using standard biochemical models of photosynthesis and Arabidopsis–specific Rubisco kinetics (Sharkey et al. 2007; von Caemmerer and Farquhar 1981; Walker et al. 2013).

**Modeling the quantum efficiency of CO2 fixation and compensation point**

To determine the modeled impact of an increase to the CO2 release per Rubisco oxygenation (z), ΦCO2 is defined as

$$\Phi_{CO_2} = \frac{GA}{PAR_{abs}},$$

where GA and PARabs represent gross CO2 assimilation and absorbed irradiance. According to the standard model
for leaf photosynthesis, GA accounting for CO₂ fixation and photorespired loss is represented by

\[
GA = V_c - \alpha V_o,
\]

where \(V_c\) and \(V_o\) are rates of Rubisco carboxylation and oxygenation and \(V_c/V_o\) is determined as

\[
\frac{V_o}{V_c} = \frac{O}{CS_{c/o}},
\]

where \(O\), \(C\), and \(S_{c/o}\) represent the oxygen partial pressure, CO₂ partial pressure, and Rubisco specificity (von Caemmerer 2000). By re-arrangement of Eqs. 4 and 5,

\[
GA = V_c \left(1 - \frac{\alpha O}{CS_{c/o}}\right).
\]

To model the relationship between PAR abs and GA, assume that the rate of NADPH produced from photochemistry (\(V_{NADPH}\)) is defined as

\[
2V_{NADPH} = 0.5 \times \text{PAR abs} \times \phi_{PSII}
\]

and

\[
V_{NADPH} = 2V_c + 2V_o
\]

according to Ruuska et al. (2000a). By combining Eqs. 5, 7, and 8,

\[
\text{PAR abs} = \frac{4V_c \left(2 + \frac{\alpha O}{CS_{c/o}}\right)}{\phi_{PSII}}
\]

which is combined with Eqs. 3 and 6 to produce

\[
\phi_{CO₂} = \frac{\phi_{PSII} (CS_{c/o} - \alpha O)}{8(CS_{c/o} + O)}.
\]

Equation 10 modeled \(\phi_{CO₂}\) using the average \(\phi_{PSII}\) values from the lowest irradiances of the measured light response curves for each CO₂ as presented in Table 3 and an assumed \(S_{c/o}\) of 2599 calculated on a partial pressure basis. This model provides a novel framework to examine the impact of changes in photorespiratory efficiency to net CO₂ gas exchange. It is interesting to note that rates of day respiration (\(R_d\)) are not needed when measuring or modeling the \(\phi_{CO₂}\) as long as \(R_d\) is assumed constant. This is because \(\phi_{CO₂}\) is defined as the increase of net CO₂ fixation per quantum of light absorbed or the initial slope of a light response curve. Changes in \(R_d\) only impact the y-intercept of this relationship and not the slope.

**Response of plgg1-1 to ambient CO₂**

Wild type and plgg1-1 were removed from high CO₂ growth conditions and dark adapted for at least 20 min before measurement of \(F_v/F_m\) in a chlorophyll fluorescence imager (CF imager, Technologica Ltd, Colchester, UK). Using this image, youngest fully expanded leaves with homogenous \(F_v/F_m\) values were selected for steady-state gas exchange at 40 Pa CO₂. The measurement was repeated on the same plants following 2, 4, and 6 days at ambient CO₂. **Rubisco content, activation state, protein content, and chlorophyll content of plgg1-1**

Rubisco binding sites were determined on flash frozen leaf disks extracted immediately following photosynthetic CO₂ response curve measurements (Walker et al. 2013). Following the last CO₂ response curve measurement at 40 Pa CO₂ and 1200 µmol m⁻² s⁻¹ PAR, an ~1.5 cm² leaf punch was made from the leaf from where it had been enclosed in the gas exchange chamber. The leaf disk was transferred into a micro-centrifuge tube and dropped into a container of liquid nitrogen. This process took less than 3 s for each sample. Following storage at ~80 °C, frozen disks were disrupted in a glass homogenizer in ice cold buffer (50 mM HEPES–NaOH (pH 7.8), 1% polyvinylpolypyrrolidone, 1 mM EDTA, 10 mM DTT, 0.1% Triton, and 1X Sigma protease inhibitor cocktail), centrifuged at 17,000 × g relative centrifugal force for 5 min at 4 °C and activated in 15 mM MgCl₂ and 15 mM NaHCO₃ for 30 min at room temperature and placed on ice. Rubisco content was determined from the stoichiometric binding of radiolabeled ¹⁴C-carboxy-arabinitol-bis-phosphate (¹⁴CABP) (Ruuska et al. 1998). Protein content was determined using the Bradford method (Bio-Rad Protein Assay, Bio-Rad, Hercules, CA, USA).

Activation state was determined in a separate set of plants flash frozen following 20-min acclimation in conditions identical to the A–Ci curve measurements by measuring initial and chemically activated Rubisco activity in raw extracts. Initial Rubisco activity was assayed following rapid extraction at 4 °C (50 mM HEPES–NaOH, pH 7.8, 1 % polyvinylpolypyrrolidone, 1 mM EDTA, 10 mM DTT, 5 mM MgCl₂, 0.1% Triton, and 1X Sigma Aldrich plant protease inhibitor cocktail) using a 2-mL tissue grinder. Following homogenization, the extract was centrifuged at 21,100 × g for 20 s at 4 °C and the supernatant assayed for initial activity spectrophotometrically from the enzymatically coupled conversion of NADH to NAD + (100 mM EPPS-NaOH, pH 8.0, 10 mM MgCl₂, 1 mM EDTA, 1 mM ATP, 5 mM phosphocreatine, 20 mM NaHCO₃, 0.2 mM NADH, and 0.5 mM RuBP with coupling enzymes: 25 U mL⁻¹ creatine phosphokinase, 250 U mL⁻¹ carbonic anhydrase, 25 U mL⁻¹ 3-phosphoglycerate kinase, 20 U mL⁻¹ glyceraldehyde-3-phosphate dehydrogenase, 20 U mL⁻¹ glycerol-3-phosphate dehydrogenase, and at least 55 U mL⁻¹ triosephosphate isomerase) (Ruuska et al. 2000; Yamori and von Caemmerer 2009; Carmo-Silva and
between plgg1 and wild type when only one treatment and time where compared (Tables 2, 4; Fig. 1). A two-way (genotype by day) repeated measures ANOVA was used to test significance \( p < 0.05 \) of experiments involving the same plants with multiple sampling times (Fig. 3). A one-way ANOVA was used to test remaining significance \( p < 0.05 \). All ANOVA were followed with a Tukey’s post hoc test and determined using statistical software (OriginPro 9.0, OriginLab, Northhampton, MA, USA).

Results

Decreases in photorespiratory efficiency decrease modeled \( \Phi_{CO_2} \) under photorespiratory conditions

A modeling approach was used to predict the impact of increases in \( \alpha \) on \( \Phi_{CO_2} \) under high (10 Pa CO\textsubscript{2} and 20 kPa O\textsubscript{2}) and low photorespiratory conditions (90 Pa CO\textsubscript{2} and 20 kPa O\textsubscript{2}, 25 Pa CO\textsubscript{2} and 2 kPa O\textsubscript{2}, Table 1). Values of \( \alpha \) were assumed to be 0.5 for wild-type photorespiration as predicted from the dogmatic scheme of photorespiration and 0.8 based on previous analysis of \( hrrpmdh1pmdh2 \), which showed increases in \( \alpha \) (von Caemmerer 2000; Cousins et al. 2008, 2011). This simulation revealed that the decreases in photorespiratory efficiency of carbon recycling found in \( hrrpmdh1pmdh2 \) would be expected to decrease \( \Phi_{CO_2} \) by \( \sim 30\% \) under photorespiratory conditions (10 Pa CO\textsubscript{2} and 20 kPa O\textsubscript{2}) but have little impact on \( \Phi_{CO_2} \) when photorespiration is suppressed by high CO\textsubscript{2} (3 % decrease) or low O\textsubscript{2} (no difference). The high and low photorespiratory conditions in the model produced meaningful controls for comparison to measurements since the predictions produce situations where \( \alpha \) is and is not expected to impact \( \Phi_{CO_2} \). These modeled values were next compared to measured observations to determine if there was support for an increase in \( \alpha \) by examining \( \Phi_{CO_2} \).

Measurements of \( \Gamma^* \) and \( \Phi_{CO_2} \) reveal no large change in photorespiratory recycling efficiency in plgg1-1

We next measured \( \Gamma^* \) to determine if there was gas exchange evidence for decreases in the efficiency of carbon recycling during photorespiration in plgg1-1 to compared to modeled values (Table 2). While plgg1-1 had a larger \( \Gamma^* \) (\( \sim 25\% \)), this difference was not quite significant as determined by a Student’s \( t \) test \( (p = 0.06) \). Rates of day respiration \( (R_d) \) where identical between genotypes, but \( \Gamma \) was significantly larger in plgg1-1.

To test the carbon recycling efficiency of impaired glyceraldehyde/glycolate transport in photorespiration, \( \Phi_{CO_2} \) was determined from light response curves measured in plgg1-1

### Table 1 Modeled impact of changes in CO\textsubscript{2} release per Rubisco oxygenation on maximum quantum efficiency of CO\textsubscript{2} fixation

| \( C_c \) (Pa) | \( O_2 \) (kPa) | \( V_o/V_c \) | \( \Phi_{PSII} \) | \( \alpha \) | \( \Phi_{CO_2} \times 100 \) |
|---|---|---|---|---|---|
| 10 | 20 | 0.769 | 0.72 | 0.50 | 3.1 |
| 10 | 20 | 0.769 | 0.72 | 0.80 | 2.0 |
| 90 | 20 | 0.085 | 0.66 | 0.50 | 7.3 |
| 90 | 20 | 0.085 | 0.66 | 0.80 | 7.1 |
| 25 | 2 | 0.031 | 0.67 | 0.50 | 7.9 |
| 25 | 2 | 0.031 | 0.67 | 0.80 | 7.9 |

The maximum quantum efficiency of CO\textsubscript{2} fixation \( (\Phi_{CO_2}) \) was modeled in response to changes in the amount of CO\textsubscript{2} released per Rubisco oxygenation \( (\alpha) \). The chloroplastic CO\textsubscript{2} partial pressure \( (C_c) \) and quantum efficiency of PSII \( (\Phi_{PSII}) \) values used were averaged from the \( \Phi_{CO_2} \) measurements presented in Fig. 1. Rubisco specificity was assumed to be 2888 Pa/Pa as determined from wild-type \( \Gamma^* \) measurements from Table 2 and Eq. 2.
under high photorespiratory conditions (10 Pa CO₂ and 20 kPa O₂) and low photorespiratory conditions (90 Pa CO₂ and 20 kPa O₂, 2 kPa O₂ and 25 Pa CO₂, Fig. 1). As expected, \( U_{\text{CO}_2} \) was lower under low CO₂ and increased under high CO₂ generally. There was no statistical difference between plgg1-1 and wild type under conditions of low CO₂, neither were differences found under high CO₂. The absolute values of \( U_{\text{CO}_2} \) agreed well with our modeled values that assumed \( \alpha \) was equal to 0.5 under low and high CO₂. This suggests that photorespiration was able to maintain efficiency despite genetic disruption in plgg1-1 under conditions of high photorespiration relative to CO₂ assimilation (low CO₂).

Since \( \Phi_{\text{CO}_2} \) is measured under very low irradiances (<100 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)) from the initial close-to-linear portion of the light response curve, we next examined the full light response curve to understand the impact of disruption of PLGG1 to net photosynthesis under increasing irradiances and gross photorespiratory flux. The full light response curves from these measurements reveal that wild type and plgg1-1 show similar responses to increasing irradiances when photorespiration is suppressed through high CO₂ or low O₂ (Fig. 2). plgg1-1 shows a decrease in net photosynthetic CO₂ assimilation under photorespiratory conditions.

**CO₂ response curves reveal that decreased assimilation in plgg1-1 results from decreased photosynthetic biochemistry**

The response of net CO₂ assimilation to CO₂ (A–Cₜ curves) was measured under saturating irradiance to determine at what Cₜ plgg1-1 starts showing decreased photosynthetic rates under constant illumination as the rate of photorespiration is varied. A–Cₜ curves where measured on plants taken directly from a high CO₂ growth condition (~200 Pa, day 0) and following 48 h under ambient CO₂ (~40 Pa, day 2). On day 0 with measurement CO₂ concentrations close to ambient (40 Pa), plgg1-1 showed a ~40 % decrease in assimilation compared to wild type (Table 3, S2). High CO₂ (200 Pa) partially rescued plgg1-1 (to 30 % of wild type). Photosynthetic biochemical parameters derived from these A–Cₜ curves were lower in plgg1-1. The maximum rate of Rubisco carboxylation (\( V_{\text{cmax}} \)) was reduced by 60 % and maximum rates of electron transport (\( J_{\text{max}} \)) was reduced by 30 % in the null mutant. These decreases in plgg1-1 became more severe following 2 days growth at ambient CO₂. Assimilation rates at 40 and 200 Pa CO₂ decreased to 60 and 50 % of

|                | Wild type | plgg1-1 |
|----------------|-----------|---------|
| \( \Gamma^* \) (Pa) | 3.6 ± 0.3a | 4.5 ± 0.1a |
| \( \Gamma \) (Pa)   | 4.6 ± 0.1a | 5.9 ± 0.1b |
| \( R_d \) (\( \mu \text{mol m}^{-2} \text{s}^{-1} \)) | 0.5 ± 0.0a | 0.6 ± 0.0a |

Measurements of the photorespiratory CO₂ compensation point (\( \Gamma^* \)), CO₂ compensation point (\( \Gamma \)), and day respiration (\( R_d \)) where made on wild type and plgg1-1 using slope-intercept regression on CO₂ response curves measured under sub-saturating irradiances. The presented \( \Gamma \) values are from measurements made at 1200 PAR. Means of \( n = 3–4 \) are shown with ± standard error. Significant differences within a measurement are indicated with different letters according to a Student’s \( t \) test with \( p < 0.05 \).
wild type, while $V_{\text{cmax}}$ and $J_{\text{max}}$ decreased by 70 and 50 % of wild type.

Decreases in $V_{\text{cmax}}$ in plgg1-I were explained by a reduction in the Rubisco activation state. Wild type and plgg1-I had identical Rubisco content expressed on a protein and leaf area basis (Table 4). While the Rubisco content was similar, plgg1-I had a significantly lower activation state as determined from in vitro activities. Chlorophyll content was similar between wild type and plgg1-I immediately following and after 2 days at ambient CO$_2$ (Table 4).

plgg1-I has decreased photosynthetic activity in old, but not young, leaves following transition to ambient CO$_2$

Additional gas exchange and chlorophyll fluorescence imaging further highlighted the plasticity in the ability of plgg1-I to compensate for impaired glycolate/glycerate transport. Dark adapted $F_v/F_m$ values did not respond to transitions to ambient CO$_2$ in wild type, but decreased by 10 % in plgg1-I after 2 days in ambient CO$_2$ (Fig. 3a, b). In plgg1-I, $F_v/F_m$ decreased more in older leaves as compared to developing leaves (Fig. 3a) following exposure to ambient CO$_2$ and did not increase following 2 days (Fig. 3b). This decrease in $F_v/F_m$ occurred in a patchy manner, with some regions of the older leaves showing a greater decrease than others making absolute quantification difficult. These areas of decreased $F_v/F_m$ in older leaves developed chlorotic lesions following 4 days of exposure to ambient CO$_2$. Interestingly, young leaves continued to develop and plants set seeds under longer periods of exposure to ambient CO$_2$. Initial experiments also indicated that plgg1-I could complete its lifecycle under ambient CO$_2$ and produce viable seed under low light conditions. Net CO$_2$ assimilation decreased in plgg1-I by 36 % on day 0 to 63 % on day 6 compared to wild type (Fig. 3c).

Discussion

Despite lacking the key glycolate/glycerate exchange transporter of photorespiration, plgg1-I showed surprising physiological plasticity in maintaining photorespiratory flux in terms of photorespiratory recycling efficiency. Despite a modeled decrease in $\varphi_{\text{CO}_2}$ with decreased photorespiratory efficiency, plgg1-I showed a similar $\varphi_{\text{CO}_2}$ to wild type, even when measured near the CO$_2$ compensation point for plgg1-I where rates of Rubisco oxygenation approach those of carboxylation (Fig. 1; Tables 1, 2). Furthermore, there was no significant increase in $\Gamma^\star$, suggesting that $\alpha$ is maintained in plgg1-I (Table 2; Eq. 1).

When light is saturating, a classic, all-be-it weak (i.e., plgg1-I plants can survive and grow slowly in ambient [CO$_2$]), photorespiratory phenotype is found in plgg1-I with reduced photosynthetic rates that are partially rescued under low photorespiratory conditions in both light and A–C$_1$ curves (Table 3; Fig. 1). Timm and Bauwe (2013) provide a framework for classifying the range of photorespiratory mutant phenotypes. Class I mutants are not rescued even under high (~2 kPa) CO$_2$ partial pressures.

**Table 3** Biochemical characteristics of wild type and plgg1-I

| Day 0 | Day 2 |
|-------|-------|
| **Wild type** | **plgg1-I** | **Wild type** | **plgg1-I** |
| $A_{40}$ (µmol m$^{-2}$ s$^{-1}$) | 13.0 ± 0.8$^a$ | 7.6 ± 1.0$^b$ | 13.3 ± 0.6$^a$ | 5.5 ± 0.6$^b$ |
| $A_{200}$ (µmol m$^{-2}$ s$^{-1}$) | 22.4 ± 1.6$^a$ | 14.7 ± 1.8$^b$ | 22.9 ± 1.5$^a$ | 11.8 ± 0.8$^b$ |
| $V_{\text{cmax}}$ (µmol m$^{-2}$ s$^{-1}$) | 65.3 ± 4.8$^a$ | 29.0 ± 4.4$^b$ | 72.8 ± 7.1$^a$ | 19.9 ± 2.5$^b$ |
| $J_{\text{max}}$ (µmol m$^{-2}$ s$^{-1}$) | 93.8 ± 7.2$^a$ | 62.3 ± 8.8$^b$ | 97.5 ± 6.4$^a$ | 46.9 ± 4.1$^b$ |
| Chlorophyll content (mg Chl m$^{-2}$) | 103.4 ± 1.3$^a$ | 102.4 ± 0.5$^a$ | 98.9 ± 0.6$^a$ | 99.2 ± 0.3$^a$ |

Measurements were made in plants grown under high (200 Pa CO$_2$) and measured immediately (Day 0) or after 2 days in ambient CO$_2$ (Day 2) under 1200 µmol PAR. Rates of CO$_2$ exchange at reference intercellular CO$_2$ concentration of ~200 Pa (A$_{200}$) or ~40 Pa (A$_{40}$), and maximum rates of Rubisco carboxylation ($V_{\text{cmax}}$) and electron transport ($J_{\text{max}}$) were calculated from a photosynthetic CO$_2$ response curve. Means of $n = 4$ are shown with ± standard error. Significant differences within a measurement are indicated with different letters according to a two-way ANOVA with $p < 0.05$. 

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and require supplemental sucrose for growth. Class II mutants display the so-called “classic” photorespiratory phenotype and show a conditional lethality to ambient CO₂ conditions. Class III mutants show retarded but viable growth at ambient CO₂ that is compensated under elevated CO₂ partial pressures. Decreased Rubisco activity would also explain why plgg1-l has a higher compensation point but similar Γ⁺ and Rₐ, since the compensation point is sensitive to the ratio of day respiration to maximum Rubisco carboxylation rates (Eq. 2; Tables 2, 3). Similar decreases in Rubisco activity are found in hrpmdh1,pmdh2 and in rice plants inducibly expressing an antisense glycolate oxidase gene, suggesting that photorespiration may inhibit the C3 cycle via feedback mechanisms on Rubisco (Xu et al. 2009; Cousins et al. 2011). This observation is also an important consideration when interpreting changes to the compensation point in photorespiratory mutants, since increases can be explained by decreases in the maximum Rubisco carboxylation rate and not necessarily changes to photorespiratory efficiency (Timm et al. 2012).

The mechanism of photorespiratory-mediated deactivation of Rubisco is not clear, but could be mediated by photorespiratory metabolites directly. Early work examining isolated chloroplasts indicate that glycolate and glycinate builds up in the absence of peroxisomes and mitochondria (i.e., fully functioning photorespiratory cycle, Kearney and Tolbert 1962). It has been shown that Rubisco activation correlates with glycolate concentrations in vivo and in vitro (Chastain and Ogren 1989) and in intact, lysed and reconstituted chloroplasts (Campbell and Ogren 1990). Additionally, excess glycolate was shown to correlate with net photosynthesis in rice plants expressing down-regulated glycolate oxidase, although it is unclear why plants exhibiting decreased glycolate oxidase activity showed increases in glycolate concentrations (Lu et al. 2014). Interestingly, glycolate concentrations are lower in plgg1-l plants as compared to wild type immediately following multiple days under ambient CO₂.

Table 4 Rubisco content of wild type and plgg1-l

|                       | Wild type | plgg1-l |
|-----------------------|-----------|---------|
| Rubisco (µmol sites m⁻²) | 8.8 ± 0.5⁺ | 8.4 ± 0.5⁺ |
| Total protein (µg m⁻²)   | 6.6 ± 0.1⁺ | 7.2 ± 0.6⁺ |
| Rubisco:protein (µmol sites µg⁻¹) | 1.3 ± 0.1⁺ | 1.2 ± 0.1⁺ |
| Rubisco activation state (%) | 82 ± 2.0⁺ | 69 ± 2.4⁺ |
| Rubisco initial activity (µmol CO₂ m⁻² s⁻¹) | 21.3 ± 0.1⁺ | 21.0 ± 1.7⁺ |
| Rubisco final activity (µmol CO₂ m⁻² s⁻¹)  | 26.1 ± 0.8⁺ | 29.8 ± 1.9⁺ |

Rubisco content was measured in raw leaf extracts from the binding of ¹⁴CABP and total protein content quantified using the Bradford assay. Means of n = 3–7 are shown with ± standard error. Rubisco quantification, Rubisco activity assays, and chlorophyll extractions were performed on separate generations of plants. Significant differences between genotypes are indicated with different letters according to a Student’s t test with p < 0.05.

are sufficient to attribute at least some the decrease in V_cmax to decreases in Rubisco activation state.

This possible decrease in Rubisco activation state may have been due to decreased Rubisco activation due to insufficient Rubisco activase activity in plgg1-l, which would explain the lowered CO₂ assimilation at lower CO₂ partial pressures. Decreased Rubisco activity would also explain why plgg1-l has a higher compensation point but similar Γ⁺ and Rₐ, since the compensation point is sensitive to the ratio of day respiration to maximum Rubisco carboxylation rates (Eq. 2; Tables 2, 3). Similar decreases in Rubisco activity are found in hrpmdh1,pmdh2 and in rice plants inducibly expressing an antisense glycolate oxidase gene, suggesting that photorespiration may inhibit the C3 cycle via feedback mechanisms on Rubisco (Xu et al. 2009; Cousins et al. 2011). This observation is also an important consideration when interpreting changes to the compensation point in photorespiratory mutants, since increases can be explained by decreases in the maximum Rubisco carboxylation rate and not necessarily changes to photorespiratory efficiency (Timm et al. 2012).

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following transfer to ambient CO₂, although glyoxylate increases somewhat (although insignificantly) in plgg1-1 following 2 and 5 days at ambient CO₂ (Pick et al. 2013). These seemingly contradictory findings suggest that the impact of glyoxylate on Rubisco activation state may be secondary, although clearly more work is needed to directly resolve and confirm this hypothesis. Glycolate is also greatly increased in plgg1-1 (Pick et al. 2013), perhaps indicating it could exert a feedback control on Rubisco.

A similar decrease in $J_{\text{max}}$ observed in plgg1-1 (Table 3) is found in hprpmdh1pmdh2, which limits the maximum rate of net CO₂ assimilation (Cousins et al. 2011). This decrease in the maximum rate of electron transport could result from increased photoinhibition as indicated by the decrease in dark adapted $F_{v}/F_{m}$ following exposure to ambient CO₂, assuming that photosystem II centers were completely repaired during the dark adaptation period (Fig. 3a, b, Baker and Oxborough 2004). Increases in photoinhibition are found in other photorespiratory mutants and are attributed to the impaired repair of photoinhibition, but without more in-depth analysis, we cannot speculate further on the mechanism of our decreased $J_{\text{max}}$ (Takahashi et al. 2007; Badger et al. 2009; Timm et al. 2012).

Decreases in $F_{v}/F_{m}$ were observed on the older leaves of the rosette, but not younger, expanding leaves (Fig. 3a). A similar pattern of decreased $F_{v}/F_{m}$ is observed in plants lacking the primary mitochondrial serine hydroxymethyltransferase and may indicate an acclimation response to ambient CO₂ provided by a gene with overlapping function (Timm et al. 2012: Fig. 3b therein). Increased expression of a gene with overlapping transport activity could explain the plasticity of the plgg1-1 phenotype in young but not older leaves, but more work would be needed to clarify this. The chlorophyll content of plgg1-1 was similar to wild type (Table 3). This finding of similar chlorophyll contents differs somewhat from the finding that overexpression of a PLGG1 homolog in tomato, where overexpression of a PLGG1 homolog resulted in decreased chlorophyll biosynthesis (Solanum lycopersicum, Kang et al. 2016).

Since the photorespiratory efficiencies of plgg1-1 were unaffected under low rates of oxygenation, it is possible that the mutation is leaky, allowing normal photorespiratory operation compensated when fluxes were low. However, the plgg1-1 line results from a T-DNA insertion in the first intron of plgg1 and shows no PLGG1 expression as determined from transcript abundance, and plgg1 has no homologs in Arabidopsis (Pick et al. 2013), confirming that these mutants were complete knockouts. This suggests that either other genes, potentially un-described transporters, and/or glycolate diffusion across the chloroplast envelope are adequate to support moderate rates of photorespiration when PLGG1 is absent.

Longer periods of exposure to ambient CO₂ led to decreased $F_{v}/F_{m}$ and chlorotic lesions in older leaves, similar to that observed previously (Pick et al. 2013). This observation indicates that the plasticity of photorespiration in plgg1-1 cannot fully protect against leaf damage under these conditions, at least in mature leaves. It is possible that this leaf damage is secondary to the compensatory mechanism that preserves the efficiency of photorespiration immediately following transfer to ambient CO₂, but not following longer term exposure. It is interesting to note that plants continued to develop under low CO₂ and could even complete an entire life cycle under ambient CO₂. These
observations further suggest an alternative plastic mechanism for glycolate and glyc erate transport in photorespiration. This mechanism could be an alternative low-affinity alternative transporter, pore, or simple diffusion through the chloroplast membrane. The plgg1-1 shows increased glycolate and glyc erate pools that increase with the number of days at ambient CO₂ (Pick et al. 2013). While this metabolic evidence (and the additional transport activities) demonstrates plgg1-1 is a glycolate/glycerate transporter, they do not exclude the possibility that alternative transport processes participate in photorespiration when substrate concentrations of glycolate and glyc erate become high enough. The research presented here demonstrates that despite these elevated pools, the efficiency of photorespiration appears unaffected, although the absolute rate is changed based on changes in Rubisco activation state.

Conclusions

Taken together, these observations lead us to hypothesize that plgg1-1 compensates for impaired glycolate/glycerate transport by additional transport processes including undescribed transporters and/or passive diffusion of glycolate across the chloroplast envelope as suggested previously (Pick et al. 2013). These alternative transport processes are adequate to maintain wild-type rates of net assimilation when net photorespiration is low as found under sub-saturating irradiance. As photorespiratory rates increase, net assimilation is decreased not by compromised photorespiratory efficiency, but by decreased Rubisco activity and electron transport. This mechanism may be specific to plgg1-1, but may also apply to other photorespiratory mutants or possibly when photorespiration is compromised in wild-type plants due to environmental factors such as increases in temperature, which could put similar pressures on photorespiratory metabolism.

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Author contributions B. J. Walker, P. F. South, and D. R. Ort planned and designed the research. B. J. Walker performed experiments and B. J. Walker wrote the manuscript.

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