

The Benefits of Photorespiratory Bypasses: How Can They Work?¹[OPEN]

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Bypassing the photorespiratory pathway is regarded as a way to increase carbon assimilation and, correspondingly, biomass production in C_3 crops. Here, the benefits of three published photorespiratory bypass strategies are systemically explored using a systems-modeling approach. Our analysis shows that full decarboxylation of glycolate during photorespiration would decrease photosynthesis, because a large amount of the released CO_2 escapes back to the atmosphere. Furthermore, we show that photosynthesis can be enhanced by lowering the energy demands of photorespiration and by relocating photorespiratory CO_2 release into the chloroplasts. The conductance of the chloroplast membranes to CO_2 is a key feature determining the benefit of the relocation of photorespiratory CO_2 release. Although our results indicate that the benefit of photorespiratory bypasses can be improved by increasing sedoheptulose biphosphatase activity and/or increasing the flux through the bypass, the effectiveness of such approaches depends on the complex regulation between photorespiration and other metabolic pathways.

In C_3 plants, the first step of photosynthesis is the fixation of CO_2 by ribulose biphosphate (RuBP). For every molecule of CO_2 fixed, this reaction produces two molecules of a three-carbon acid, i.e., 3-phosphoglycerate (PGA), and is catalyzed by the Rubisco enzyme. A small portion of the carbon in PGA is used for the production of Suc and starch, whereas the remainder (i.e. five-sixths) is used for the regeneration of RuBP (Fig. 1). The regeneration of the Rubisco substrate RuBP in the Calvin-Benson-Bassham (CBB) cycle ensures that ample RuBP is available for carbon fixation (Bassham, 1964; Wood, 1966; Beck and Hopf, 1982). Rubisco is a bifunctional enzyme that catalyzes not only RuBP carboxylation but also RuBP oxygenation (Spreitzer and Salvucci, 2002). RuBP oxygenation generates only one molecule of PGA and one molecule of 2-phosphoglycolate (P-Gly; Ogren, 1984). The photorespiratory pathway converts this P-Gly back to RuBP in order to maintain the CBB cycle.

In higher plants, P-Gly is dephosphorylated to glycolate, which is transferred into the peroxisomes, where

it is oxidized to hydrogen peroxide and glyoxylate. Then, glyoxylate is aminated to produce Gly, which is subsequently transferred to the mitochondria. There, two molecules of Gly are converted into one Ser plus one CO_2 and one NH_3 (Ogren, 1984; Peterhansel et al., 2010). The Ser is ultimately converted back to PGA (Tolbert, 1997). CO_2 and NH_3 are gasses that can escape to the atmosphere (Sharkey, 1988; Kumagai et al., 2011), and the loss of carbon and nitrogen essential for biomass accumulation will decrease the efficiency of photosynthesis and plant growth (Zhu et al., 2010). Fortunately, both substances are partially reassimilated in the chloroplast, but this results in decreased photosynthetic energy efficiency. At 25°C and current atmospheric CO_2 concentrations, approximately 30% of the carbon fixed in C_3 photosynthesis may be lost via photorespiration and the size of this loss increases with temperature (Sharkey, 1988; Zhu et al., 2010). As a result, photorespiration has been regarded as a pathway that could be altered to improve photosynthetic efficiency (Zelitch and Day, 1973; Oliver, 1978; Ogren, 1984; Zhu et al., 2008, 2010).

There are several approaches that may be used to alter photorespiration to improve photosynthetic efficiency. First, it might be possible to increase the specificity of Rubisco to CO_2 versus oxygen ($S_{c/o}$; Dhingra et al., 2004; Spreitzer et al., 2005; Whitney and Sharwood, 2007). However, previous studies have shown that there is an inverse correlation between $S_{c/o}$ and the maximum carboxylation rate of Rubisco (Jordan and Ogren, 1983; Zhu et al., 2004), and there are some indications that the $S_{c/o}$ of different organisms may be close to optimal for their respective environments (Tcherkez et al., 2006; Savir et al., 2010).

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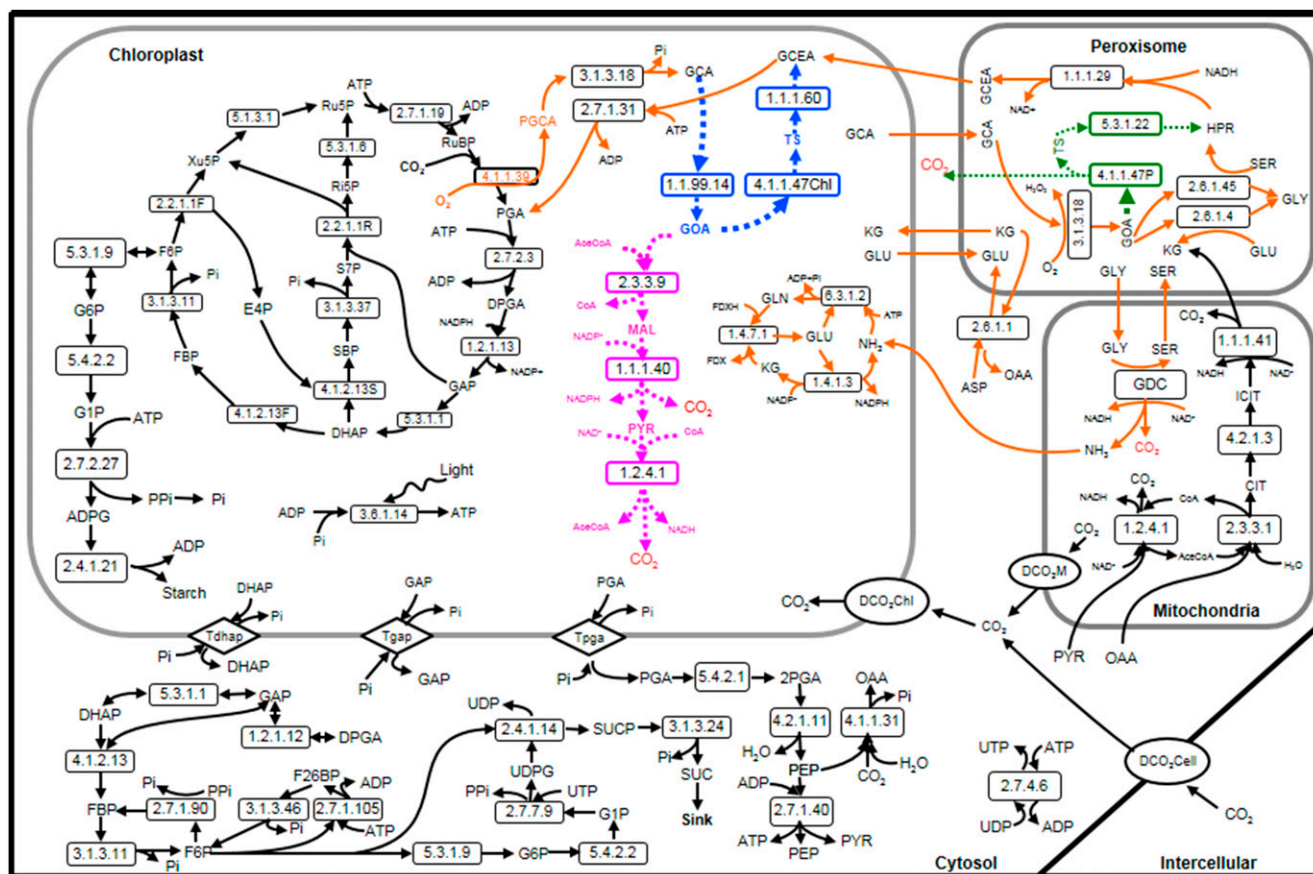


Figure 1. Schematic representation of the C_3 photosynthesis kinetic model with three different photorespiratory bypass pathways. The bypass described by Kebeish et al. (2007) is indicated in blue, the bypass described by Maier et al. (2012) in pink, and the bypass described by Carvalho et al. (2011) in green. The original photorespiratory pathway is marked in orange, and CO_2 released from photorespiration (including the original pathway and bypass pathways) is indicated in red. 2PGA, 2-Phosphoglyceric acid; ASP, Asp; CIT, citrate; ICIT, isocitrate; PGA, 3-phosphoglycerate; DPGA, glycerate-1,3-bisphosphate; GAP, glyceraldehyde 3-phosphate; DHAP, dihydroxyacetone phosphate; SBP, sedoheptulose-1,7-bisphosphate; S7P, sedoheptulose-7-phosphate; Ri5P, ribose-5-phosphate; Ru5P, ribulose-5-phosphate; FBP, fructose-1,6-bisphosphatase; F6P, fructose 6-phosphate; Xu5P, xylulose-5-phosphate; G6P, glucose-6-phosphate; G1P, glucose-1-phosphate; ADPG, ADP-glucose; F26BP, fructose-2,6-bisphosphate; UDPG, uridine diphosphate glucose; SUCP, sucrose-6P-phosphate; SUC, Suc; PEP, phosphoenolpyruvate; OAA, oxaloacetate; PGCA, phosphoglycolate; GCA, glycolate; GOA, glyoxylate; GCEA, glycerate; GOA, glyoxylate; GCEA, glycerate; GLU, glutamate; KG, α -ketoglutarate; GLN, Gln; HPR, hydroxyypyruvate; RuBP, ribulose bisphosphate; SER, Ser; GLY, Gly; TS, tartronic semialdehyde.

Second, a CO_2 -concentrating mechanism could be engineered into C_3 plants. For example, introducing cyanobacterial bicarbonate transporters (Price et al., 2011) or introducing C_4 metabolism could be used to concentrate CO_2 in the vicinity of Rubisco and, thereby, suppress the oxygenation reaction of Rubisco (Furbank and Hatch, 1987; Mitchell and Sheehy, 2006). Past efforts to introduce a C_4 pathway into C_3 plants have focused on biochemical reactions related to C_4 photosynthesis without taking into account the anatomical differences between C_3 and C_4 plants, which may have been responsible for the limited success of such endeavors (Fukayama et al., 2003). Recently, there has been renewed interest in engineering C_4 photosynthetic pathways into C_3 plants, with efforts focusing on understanding and engineering the

genetic regulatory network related to the control of both the anatomical and biochemical properties related to C_4 photosynthesis (Mitchell and Sheehy, 2006; Langdale, 2011).

Transgenic approaches have been used to knock down or knock out enzymes in the photorespiratory pathway. Unfortunately, the inhibition of photorespiration by the deletion or down-regulation of enzymes in the photorespiratory pathway resulted in a conditional lethal phenotype (i.e. such plants cannot survive under ambient oxygen and CO_2 concentrations but may be rescued by growing them under low-oxygen or high- CO_2 conditions; for review, see Somerville and Ogren, 1982; Somerville, 2001). Another approach to reduce photorespiration is to block (or inhibit) enzymes in this pathway using chemical

inhibitors. Zelitch (1966, 1974, 1979) reported that net photosynthesis increased by inhibiting glycolate oxidase or glycolate synthesis. However, other groups showed that the inhibition of glycolate oxidase or Gly decarboxylation led to the inhibition of photosynthesis (Chollet, 1976; Kumarasinghe et al., 1977; Servaites and Ogren, 1977; Baumann et al., 1981). It turns out that plants cannot efficiently metabolize photorespiratory intermediates without a photorespiratory pathway, and suppression of this pathway inhibits the recycling of carbon back toward RuBP, which is necessary for maintaining the CBB cycle (Peterhansel et al., 2010; Peterhansel and Maurino, 2011). Moreover, the accumulation of toxic metabolic intermediates (e.g. P-Gly) can strongly inhibit photosynthesis (Anderson, 1971; Kelly and Latzko, 1976; Chastain and Ogren, 1989; Campbell and Ogren, 1990). This may explain why earlier attempts to block or reduce photorespiration have failed to improve carbon gain.

Instead of reducing photorespiration directly, a promising idea is to engineer a photorespiratory bypass pathway. Such a pathway would metabolize P-Gly produced by RuBP oxygenation but minimize carbon, nitrogen, and energy losses and avoid the accumulation of photorespiratory intermediates. Kebeish et al. (2007) introduced the glycolate catabolic pathway from *Escherichia coli* into *Arabidopsis* (*Arabidopsis thaliana*); we will subsequently call this type of bypass the Kebeish bypass. In such transgenic plants, glycolate is converted to glycerate in the chloroplasts without ammonia release (Fig. 1). Previous studies suggested that this pathway theoretically requires less energy and shifts CO₂ release from mitochondria to chloroplasts (Peterhansel and Maurino, 2011; Peterhansel et al., 2013); experimental results indicated that the bypass allowed for increased net photosynthesis and biomass production in *Arabidopsis* (Kebeish et al., 2007). There are reports of two other photorespiratory bypass pathways in the literature (Carvalho, 2005; Carvalho et al., 2011; Maier et al., 2012). In the Carvalho bypass (Carvalho, 2005; Carvalho et al., 2011), glyoxylate is converted to hydroxypyruvate in the peroxisome. Similar to the Kebeish bypass, the ammonia release is abolished, one-quarter of the carbon from glycolate is released as CO₂ in the peroxisomes, and three-quarters of the carbon from glycolate is converted back to PGA. However, this pathway has only been partially realized in tobacco (*Nicotiana tabacum*); that is, the enzyme of the second reaction of this pathway was not detectable in the transgenic plants, and plants expressing this pathway showed stunted growth when grown in ambient air (Carvalho et al., 2011). The Maier bypass (Maier et al., 2012) is characterized by complete oxidation of glycolate in the chloroplasts. Initial results suggested that the photosynthesis and biomass of transgenic *Arabidopsis* with this pathway were enhanced (Maier et al., 2012).

Recently, the design and benefits of the three bypass pathways were reviewed (Peterhansel et al., 2013), and it was suggested that a photorespiratory bypass can contribute to an enhanced photosynthetic CO₂ uptake rate by lowering energy costs and minimizing carbon

and nitrogen losses. However, a systematic and quantitative analysis of the potential contributions of these different factors to photosynthesis improvement has not yet been conducted. Systems modeling can help to design new metabolic pathways and improve our understanding of biochemical mechanisms (McNeil et al., 2000; Wendisch, 2005; Zhu et al., 2007; Bar-Even et al., 2010; Basler et al., 2012). Such models have been used successfully to gain insight into the photosynthetic metabolism (Laisk et al., 1989, 2006; Laisk and Edwards, 2000; Zhu et al., 2007, 2013; Wang et al., 2014). In this study, we use an extended kinetic model of C₃ photosynthesis based on earlier work by Zhu et al. (2007) to systematically analyze the potential of three photorespiratory bypass pathways for improving photosynthetic efficiency (Supplemental Model S1). In addition, we determined under what conditions such bypass pathways may lead to increased photosynthesis and biomass production in C₃ plants and how to further improve the photosynthesis of plants with such a bypass. Our analysis suggests that the benefit of a photorespiratory bypass varies dramatically if it is engineered into different crops.

RESULTS

We first extended the kinetic model of C₃ photosynthesis (Zhu et al., 2007) by incorporating the ATP cost of NH₃ refixation, CO₂ diffusion in the mesophyll, and part of the tricarboxylic acid cycle (Fig. 1). This model can predict the response of photosynthetic CO₂ uptake rate to intercellular CO₂ concentration (C_i) and to light intensity (Fig. 2). Using default model parameters (Supplemental Tables S1–S3), the predicted rate of CO₂ release by photorespiration at ambient CO₂ partial pressures (38.5 Pa; C_i = 27 Pa, which corresponds to a CO₂ concentration of 9 μM in the liquid phase) is about 38% of the net CO₂ assimilation (Supplemental Table S4), which is comparable to previous reports (Gerbaud and Andre, 1987; Sharkey, 1988).

We further modified the C₃ primary metabolism model to evaluate the effects of three photorespiratory bypass pathways on the net photosynthetic rate. To this end, we

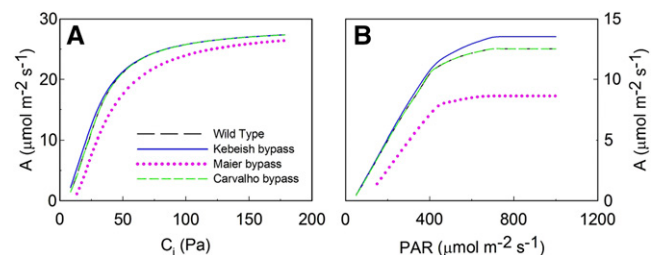


Figure 2. Simulated CO₂ response curves (A; where A = photosynthetic CO₂ uptake rate) under saturating light conditions (photosynthetically active radiation [PAR] = 1,000 μmol m⁻² s⁻¹) and light response curves (B) under ambient CO₂ conditions (C_i = 27 Pa; 9 μM in the liquid phase) for wild-type and bypass plants.

added three different bypass pathways to the model. In Figure 1, the normal C_3 metabolism and the three photorespiratory bypass pathways are shown.

The responses of photosynthesis to different intercellular CO_2 concentrations and light intensities were estimated using the kinetic model for wild-type and bypass plants. The results suggested that the Kebeish bypass pathway could enhance photosynthesis (Fig. 2; Supplemental Fig. S1), whereas the Maier bypass pathway decreased the photosynthetic rate. The Carvalho bypass pathway did not affect the photosynthetic rate under the tested conditions (Fig. 2; Supplemental Fig. S1).

Specifically, our model indicated that, compared with wild-type controls, chloroplast CO_2 concentrations and the amount of photorespired CO_2 that is refixed by Rubisco are increased in plants expressing the Kebeish or Maier bypass (Fig. 3). At saturating light and at ambient CO_2 levels, the results using the default parameterization (Supplemental Tables S1–S3) suggested that the photosynthetic rate of plants expressing the Kebeish bypass is about 8% higher than that of wild-type plants (Fig. 3A). However, the photosynthetic rate predicted for plants expressing the Maier bypass was 31% lower than that of the wild type (Fig. 3A). The photosynthetic rate of plants with the Carvalho bypass was indistinguishable from that of the wild type (Fig. 3A).

To explore why the photosynthetic rate was not predicted to increase in plants expressing the Carvalho bypass, or why it even decreased in plants with the Maier bypass, we examined the fluxes through the bypasses and normal photorespiratory pathways in detail. In contrast to

the situation in the wild type and in plants expressing the Kebeish bypass, where only part of the carbon (25%) in glyoxylate is released as CO_2 , plants expressing the Maier bypass release all carbons of glyoxylate as CO_2 . Indeed, the flux of CO_2 that escapes to the atmosphere relative to the total CO_2 fixation rate dramatically increased in the model with a Maier bypass, while it was decreased in the Kebeish bypass (Fig. 3D). Mainly as a result of the high K_m for glyoxylate of glyoxylate carboligase (EC 4.1.1.47), the flux through the Carvalho bypass pathway was extremely low (Supplemental Table S5).

To understand why plants expressing the Kebeish bypass have higher rates of photosynthesis, we separately examined the contributions of avoiding ammonium loss and of relocating photorespiratory CO_2 release from mitochondria to chloroplast. Models representing three different hypothetical scenarios were constructed. The first scenario was similar to that with a Kebeish bypass, in that photorespiratory CO_2 release was relocated from mitochondria to chloroplasts and no ammonium was released by photorespiration (i.e. no ATP is needed for the refixation of released ammonia). However, in contrast to the plants expressing the Kebeish bypass, where part of the photorespiratory flux still goes through the normal photorespiratory pathway, these effects were applied to 100% of the flux (Fig. 4B). In the second scenario, all release of photorespiratory CO_2 was relocated to the chloroplasts, but ammonium was still released by photorespiration and refixed as in the wild type (Fig. 4C). For the third scenario, CO_2 was released

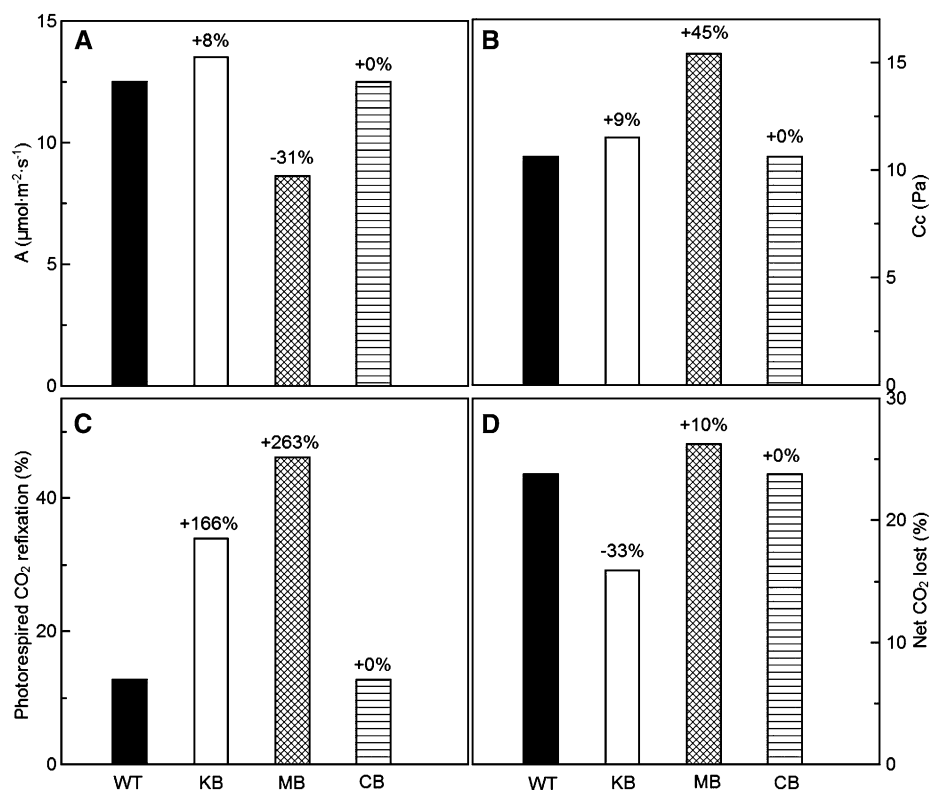
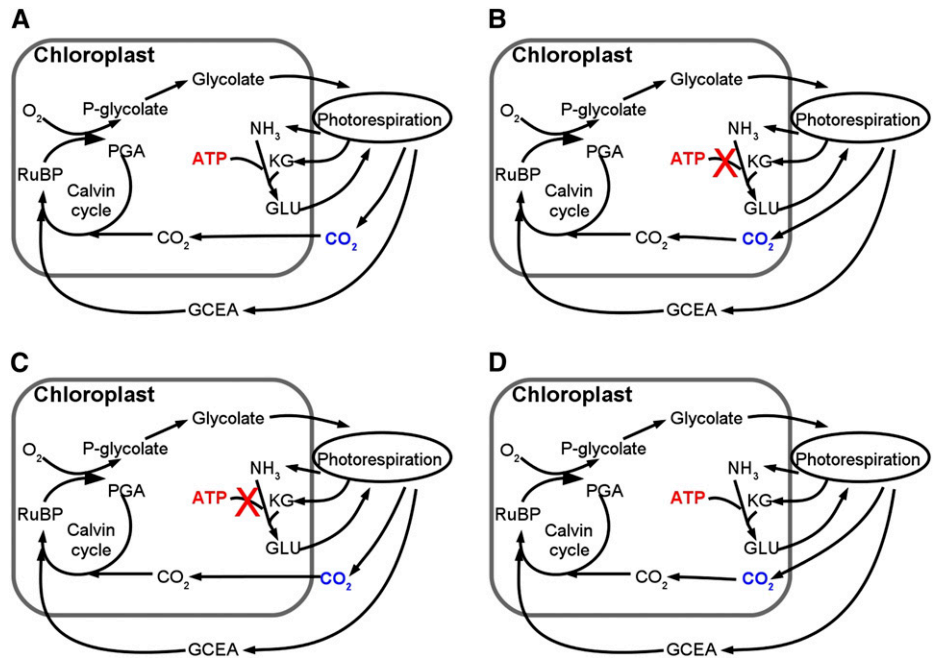


Figure 3. A, Simulated photosynthetic rates (where A = photosynthetic CO_2 uptake rate). B, Partial pressure of CO_2 inside the chloroplasts (C_c). C, Percentage of (photo)respired CO_2 that is refixed. D, Flux of CO_2 lost to the atmosphere relative to the total CO_2 fixation rate of wild-type (WT) and bypass plants with the default parameters. CB, Carvalho bypass; KB, Kebeish bypass; MB, Maier bypass. C_i = 27 Pa (corresponds to 9 μM in the liquid phase), and PAR = 1,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Figure 4. A, The wild-type pathway. B, A photorespiratory pathway that does not use ATP for the refixation of ammonia and relocates CO₂ release into the chloroplasts. C, A photorespiratory pathway that does not use ATP for the refixation of ammonia. D, A scenario where only the CO₂ release has been relocated from mitochondria to chloroplasts. GCEA, Glycerate; KG, α -ketoglutarate.



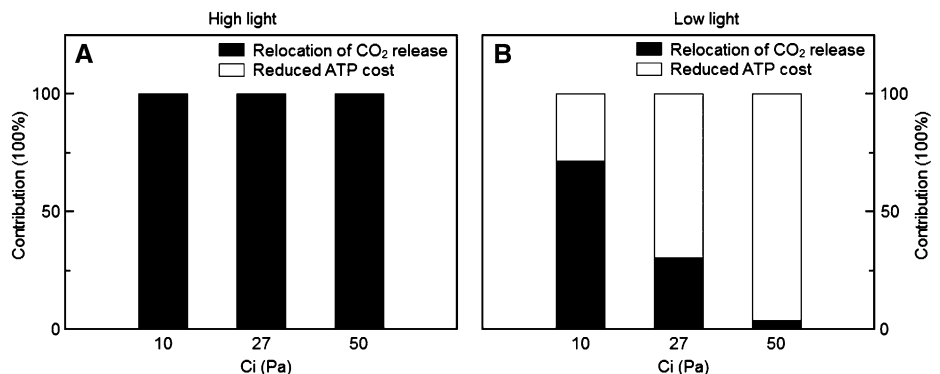
in the mitochondria as in the wild type but no ammonium was released by photorespiration (Fig. 4D). Again, for all three scenarios, we assumed that the flux through the normal photorespiration pathway was zero. Simulations show that, under high light, the increase of the photosynthetic rate can be fully attributed to the relocation of CO₂ release from mitochondria to chloroplasts (Fig. 5A). Under low light, these lower ATP costs contributed to an enhanced photosynthesis, especially at higher CO₂ concentrations (Fig. 5B). Therefore, under low light and ambient or high CO₂ concentrations, the enhanced photosynthesis of bypass plants can be mainly attributed to the lower ATP costs of the bypass plant compared with a plant with the normal photorespiratory pathway (Fig. 5). However, the benefit of lower ATP costs was rather small (Supplemental Table S6).

The conductance of CO₂ between the cytosol and the site of fixation in the chloroplast stroma (g_{chl}) may influence the effect of relocating the site of CO₂ release from mitochondria to chloroplasts. Therefore, we tested the effect of changing g_{chl} on photosynthetic rates of the wild type and the bypass. In bypass and wild-type plants,

increasing g_{chl} would also increase the overall conductance of CO₂ between the atmosphere and the Rubisco enzyme, resulting in higher photosynthetic rates (Fig. 6A). In addition, in wild-type plants, such an increase in g_{chl} would also allow a greater amount of (photo)respired CO₂ to be refixed by Rubisco (Fig. 6B). By contrast, in bypass plants, where CO₂ is released inside the chloroplast stroma, increasing g_{chl} would allow for more CO₂ to escape from the stroma and, thus, lower the amount of photorespiratory CO₂ that can be refixed. This would result in a much greater effect of the bypass on photosynthesis in plants with a low g_{chl} compared with plants with a high g_{chl} (Fig. 6). For example, doubling the default g_{chl} increased the photosynthetic rate of the wild type by 34%, but at the same time it decreased the benefit of the bypass to photosynthesis to only 1.8% (Fig. 6A).

We further explored whether the effect of the Kebeish bypass on photosynthetic rate depends on enzyme activities used in the model. To this end, we systematically increased and decreased each enzyme's capacity (V_{max}) by 10% and simulated the corresponding photosynthetic rates. All of these simulations were conducted with the

Figure 5. The relative contribution of abolishing the energy cost for ammonia refixation (white bars) and relocating photorespiratory CO₂ release into chloroplasts (black bars) on the change in the rate of photosynthesis in bypass under different light intensities and CO₂ partial pressures when all the flux goes through the bypass. For high light (A), PAR = 1,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$; for low light (B), PAR = 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$.



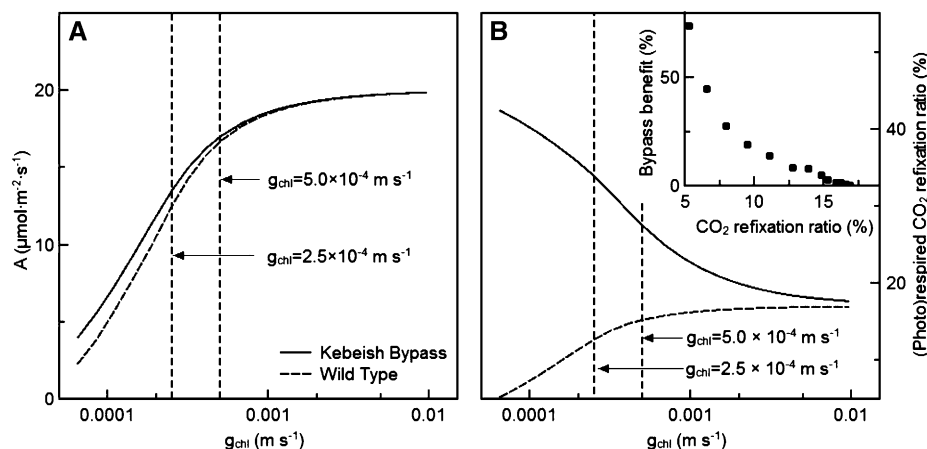


Figure 6. The predicted effects of g_{chl} on photosynthesis (A; where A = photosynthetic CO₂ uptake rate) and on the percentage of (photo)respired CO₂ that is refixed (B) for wild-type plants (dashed lines) and plants expressing a Kebeish bypass (continuous lines). The relationship between the predicted CO₂ refixation ratio and bypass benefit is shown in the inset in B. $C_i = 27 \text{ Pa}$ ($9 \mu\text{M}$ in the liquid phase), and $\text{PAR} = 1,000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

default parameters listed in Supplemental Tables S1 to S3. The results show that increasing Rubisco carboxylation capacity decreased the effect of the bypass, whereas increasing Rubisco oxygenation, sedoheptulose biphosphatase (SBPase), or glycolate dehydrogenase (GDH) capacity increased the benefit effect of the bypass (Fig. 7). Increasing GDH capacity led to an increased flux through the bypass pathway, which enhanced the benefit effect of the bypass (Fig. 8; Supplemental Fig. S2). Under ambient conditions, if all photorespiratory flux was forced through the Kebeish bypass (by blocking the flux through the normal photorespiration pathway), the photosynthetic rate could be enhanced by 15% (Fig. 8) under the default conditions listed in Supplemental Tables S1 to S3. The capacities of all the other enzymes in our model had no significant effect on the benefit of the Kebeish bypass (Supplemental Table S7).

DISCUSSION

Engineering a bypass for photorespiration is regarded as a promising approach to increase photosynthesis and plant productivity (Peterhansel and Maurino, 2011; Peterhansel et al., 2013). So far, there are only a few experimental studies suggesting that the photosynthetic rate may be increased as a result of the introduction of photorespiratory bypasses (Kebeish et al., 2007; Maier et al., 2012). This study systematically evaluated different photorespiratory bypass strategies using a systems and synthetic biology approach. Our results indicated that under certain conditions, a photorespiratory bypass can indeed increase photosynthesis by up to 8%. Such an effect may seem small, but it will, as a result of exponential growth rates, result in large differences in biomass over time and significantly improve a plant's competitive ability (Givnish, 1986; Kirschbaum, 2011). We showed that reduced energy cost by avoiding ammonium refixation and an increase in the refixation of photorespiratory CO₂ as a result of releasing CO₂ inside the chloroplasts contributed to the enhancement of photosynthesis. We further demonstrated that the permeability of the chloroplast envelopes and the activities

of key enzymes can influence the potential benefit of the photorespiratory bypass pathway.

The Benefit of Different Bypass Strategies to Photosynthesis

Similar to the normal photorespiration pathway, the Kebeish-pathway releases 0.5 mol of CO₂ for every 1 mol of glyoxylate produced. However, in the bypass, CO₂ is released in the chloroplast stroma instead of in mitochondria (Kebeish et al., 2007). This shift in the site of CO₂ release can potentially increase the CO₂ concentration in the chloroplast stroma, resulting in a reduced RuBP oxygenation rate. In addition, the relocation of the CO₂ release to the chloroplast stroma also improves the refixation of photorespiratory CO₂, improving the CO₂ fixation rate (Peterhansel et al., 2013). The release and refixation of NH₃, which occurs during normal photorespiration, are avoided in this bypass pathway. Thus, this bypass also has the potential benefit of reducing ATP costs associated with carbon assimilation

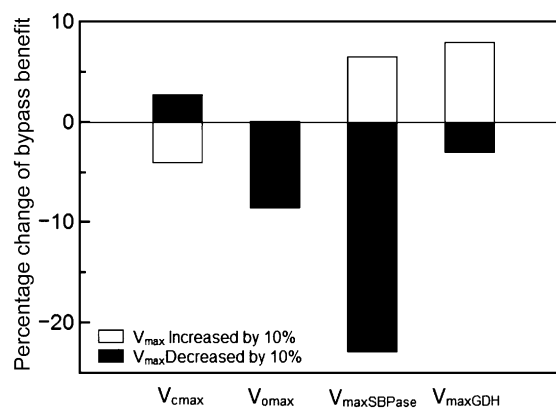


Figure 7. The effects of changing the maximal activity (V_{max}) of different enzymes on the estimated benefit of the Kebeish bypass to photosynthesis. V_{cmax} is the maximum carboxylation capacity of Rubisco, V_{omax} is the maximum oxygenation capacity of Rubisco, $V_{\text{maxSBPase}}$ is the maximum capacity of SBPase, and V_{maxGDH} is the maximum capacity of GDH. $C_i = 27 \text{ Pa}$ ($9 \mu\text{M}$ in the liquid phase), and $\text{PAR} = 1,000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

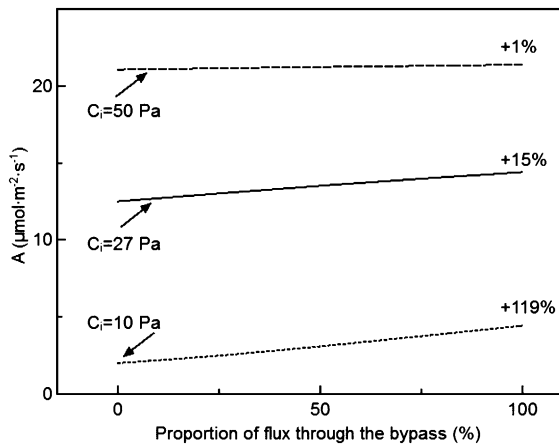


Figure 8. The predicted benefit to photosynthesis of the proportion of the photorespiratory flux through the Kebeish bypass relative to the total oxygenation rate at three different CO₂ levels. PAR = 1,000 μmol m⁻² s⁻¹. A, Photosynthetic CO₂ uptake rate.

(Kebeish et al., 2007; Maurino and Peterhansel, 2010; Peterhansel et al., 2010, 2013). Since all these features could enhance photosynthesis, we evaluated their relative magnitude and dependence on physiological characteristics and environmental conditions. Our simulations suggested that, under high light, the enhanced photosynthesis in bypass plants can be completely attributed to the relocation of the CO₂ release from mitochondria to chloroplasts. By contrast, under low light, the reduced ATP cost as a result of the absence of ammonium refixation also contributed to an improved photosynthetic efficiency.

In agreement with experimental observations (Kebeish et al., 2007), our simulations showed that, compared with the wild type, the Kebeish bypass results in higher photosynthetic rates under a range of CO₂ and light conditions (Fig. 2). The chloroplast CO₂ concentration and the amount of photorespiratory CO₂ that is refixed by Rubisco also were predicted to be increased in plants containing such a bypass (Fig. 3). However, in contrast to experimental observations suggesting that transgenic plants only show an enhanced biomass under low-light growth conditions (Kebeish et al., 2007), our modeled results indicated that photosynthetic rates in bypass plants also could be enhanced, even to a greater extent, under high light (Fig. 2B). This discrepancy between experiment and simulation may be caused by model simplifications of the photosynthetic pathway. In particular, the effect of redox signaling (Foyer et al., 2009; Foyer and Noctor, 2009) and the inhibition of glyoxylate on Rubisco activation (Campbell and Ogren, 1990) are not included in the current model. In this respect, it is worthwhile to note that both the redox state and the chloroplast glyoxylate concentrations of bypass plants are different from those of wild-type controls (Kebeish et al., 2007; Maurino and Peterhansel, 2010; Peterhansel and Maurino, 2011). Under high light, such effects would be enhanced as a result of increased photorespiration

rates and may explain the difference between modeled and experimental results.

The bypass pathway described by Maier et al. (2012) also shifts the photorespiratory CO₂ release from mitochondria into chloroplasts, and it was suggested that chloroplast CO₂ concentrations increased (Peterhansel et al., 2010, 2013). In contrast to experimental observations that such transgenic plants show an enhanced photosynthetic rate (Maier et al., 2012), our simulation predicted a reduced photosynthetic rate in plants with the Maier bypass (Fig. 3A). This may be explained by the fact that 2 mol of CO₂ is released per 1 mol of glyoxylate in the Maier bypass (an 8-fold increase compared with the release in a normal photorespiratory pathway; for review, see Peterhansel and Maurino, 2011). Our results indicated that the amount of photorespiration that is lost to the atmosphere relative to the CO₂ fixation rate is increased dramatically in Maier bypass plants (Fig. 3D), suggesting that the benefit of an increased chloroplast CO₂ concentration cannot fully compensate for the loss of CO₂ in this bypass pathway.

In the pathway described by Carvalho (2005) and Carvalho et al. (2011), a bypass was implemented in the peroxisome and NH₃ release is avoided; hence, the energy necessary for NH₃ refixation is saved. The lower energy cost in such a bypass has potential benefits for photosynthesis (for review, see Peterhansel et al., 2013). Our simulation results suggested that lowering ATP costs during photorespiration may enhance the photosynthesis under low light intensity (Fig. 5; Supplemental Table S6). However, simulations of the Carvalho bypass indicated that the photosynthetic rate in plants with this bypass was not substantially different from that of controls even under low-light conditions (Fig. 2B), in agreement with experimental results (Carvalho et al., 2011). Further analysis suggested that the lack of an effect was the result of an extremely low reaction flux through this bypass pathway (Supplemental Table S5). The reaction flux of this bypass pathway was limited by the glyoxylate carboligase activity, especially by the high K_m for glyoxylate (Supplemental Table S5). Increasing the proportion of photorespiratory fluxes through the bypass by modifying the kinetic parameters of glyoxylate carboligase in this pathway slightly increased photosynthetic rates of bypass plants under low light intensity (Supplemental Table S5). This was expected because completely abolishing ATP cost for NH₃ fixation resulted in an enhancement of photosynthesis of only 4.6% under low-light conditions (Supplemental Table S6). Since experimental results have indicated that the expression of some of the bypass enzymes was low or absent (Carvalho et al., 2011), a low flux through this bypass may explain the lack of a significant effect on photosynthesis in the transgenic plants.

Mechanisms Underlying Enhanced Photosynthesis in Plants Expressing the Kebeish Bypass

As mentioned in the previous section, plants with a Kebeish bypass benefit from a lower ATP cost for

ammonia refixation and from shifting the site of photorespiratory CO₂ release into the chloroplasts. The relative contribution of these factors to the improvement of photosynthesis is still unknown (Peterhansel and Maurino, 2011; Peterhansel et al., 2013).

Peterhansel et al. (2013) suggested that the benefit of the relocation of photorespiratory CO₂ release in the bypass plant may strongly depend on the amount of photorespiratory CO₂ that is released and subsequently refixed. If photorespiratory CO₂ release by the mitochondria results in a relatively large flux of CO₂ escaping to the atmosphere instead of being refixed by Rubisco, the introduction of a bypass that shifts CO₂ release into the chloroplasts may increase refixation and, therefore, photosynthesis. The amount of CO₂ that escapes from the leaf to the atmosphere depends on the resistance between the site of CO₂ release and the atmosphere and on the resistance between the site of CO₂ release and the site of CO₂ fixation. The most important factor that controls this last resistance is the permeability of the chloroplast envelopes to CO₂ (Evans et al., 2009). Most C₃ photosynthetic models implicitly assumed that the chloroplast envelopes offer no significant resistance to diffusion and that the only barrier to refixation is the relatively slow turnover rate of Rubisco itself (Tholen et al., 2012b). This ignores the fact that current experimental estimates of the permeability of isolated chloroplast envelopes in *Arabidopsis* put it at much lower values ($2 \times 10^{-5} \text{ m s}^{-1}$; Uehlein et al., 2008), although it must be emphasized that considerable uncertainty surrounds these values (i.e. about 4 orders of magnitude; Evans et al., 2009; Kaldenhoff et al., 2014). As explained by Tholen et al. (2012a, 2012b), estimates of the effective in vivo resistances are not only a result of the membrane permeability itself but also take into account the structural arrangement of the organelles in the cell. For example, in rice (*Oryza sativa*), more than 95% of the cell periphery is covered by chloroplast or chloroplast extrusions, forcing CO₂ to exit the cells via chloroplasts. Such a cellular anatomy will increase the effective g_{chl} and this may explain the relatively large amount of photorespiratory CO₂ (up to 38% at a CO₂ concentration of 20 Pa, which corresponds to a CO₂ concentration of 6.6 μM in the liquid phase) that can be refixed in such leaves (Busch et al., 2013).

Because the presence of the Kebeish bypass resulted in increased CO₂ concentrations inside the chloroplast stroma (Fig. 3B), the g_{chl} was expected to affect the leakage of photorespired CO₂ from chloroplast to cytosol and, correspondingly, the potential enhancement to photosynthesis by the photorespiratory bypass. In our simulations, we used a value for g_{chl} of $2.5 \times 10^{-4} \text{ m s}^{-1}$, which is between the estimate by Uehlein et al. (2008; $1.85 \times 10^{-5} \text{ m s}^{-1}$) and that by Evans et al. (2009; $3.5 \times 10^{-3} \text{ m s}^{-1}$; Fig. 6A). To account for the uncertainty in these values, we tested how our model predictions depended on the magnitude of g_{chl} . The results indicated that, although the photosynthetic rate of both wild-type and bypass plants increase with an increase in g_{chl} the advantage of the bypass over wild-type plants decreased with an increase

in g_{chl} (Fig. 6A). If g_{chl} is doubled ($5 \times 10^{-4} \text{ m s}^{-1}$), the photosynthesis of the wild type increased dramatically; however, the positive effect of the bypass on photosynthesis was decreased by a factor of 4. Nevertheless, even with such a large g_{chl} the bypass may still be beneficial to photosynthesis under low-CO₂ conditions (Supplemental Fig. S2).

We further examined the effects of g_{chl} on the amount of photorespired CO₂ that was refixed by Rubisco in wild-type and bypass plants. Our analysis shows that in wild-type plants, the refixation ratio increased with an increase in g_{chl} whereas the refixation ratio of bypass plants gradually decreased with an increase in g_{chl} (Fig. 6B). The benefit of the photorespiratory bypass to photosynthesis gradually decreased with an increase in the CO₂ refixation ratio of wild-type plants. When about 30% of the photorespired CO₂ can be refixed, as is the case under current atmospheric conditions in species like wheat (*Triticum aestivum*) and rice (Busch et al., 2013), the effect of the bypass was negligible (Fig. 6). Given these results, it seems unlikely that introducing a photorespiratory bypass would be beneficial for enhancing the rate of photosynthesis in such species.

How Can the Effect of a Photorespiratory Bypass Be Increased?

The simulations using our systems model suggested that, under ambient conditions, the bypass only has significant benefits when g_{chl} is relatively low (Fig. 6). In fact, such a low g_{chl} would be somewhat suboptimal for photosynthesis, and engineering plants with a higher g_{chl} may allow for a greater enhancement of photosynthesis compared with introducing a bypass. However, it is possible that the biochemical composition of the chloroplast envelope prevents such highly permeable chloroplast envelopes (Kaldenhoff et al., 2014). Our findings highlight the need to obtain more reliable estimates for the permeability of chloroplast membranes to CO₂.

We analyzed whether the photosynthesis of bypass plants could be further enhanced by altering the activities of enzymes in the photosynthetic metabolism. Our simulations showed that changing the maximal activity of GDH, SBPase, and Rubisco influenced the benefit of the Kebeish bypass for photosynthesis (Fig. 7), while changing the capacity of other enzymes in our model has virtually no effect (Supplemental Table S7). A number of earlier reports suggested that increasing SBPase concentration can improve photosynthetic energy conversion efficiency (Lefebvre et al., 2005; Zhu et al., 2007; Rosenthal et al., 2011). If the relative activities of these different enzymes used in the model were not altered in the photorespiratory bypass plant, then the overexpression of SBPase will, in theory, also improve the photosynthetic CO₂ uptake rate. The default flux through the Kebeish bypass in our model was about equal to the flux through the normal photorespiratory pathway. We found that the GDH enzyme in this bypass is rate limiting for the flux, and increasing the maximal activity of GDH leads

to an increased flux through the Kebeish bypass and an enhanced rate of photosynthesis (Fig. 8; Supplemental Fig. S3). By contrast, increasing the flux through the Maier bypass by increasing the maximal activity of GDH leads to a decreased photosynthetic rate as a result of the large amount of CO₂ that would be lost (Supplemental Fig. S4). The benefit of the bypass also was increased when the Rubisco carboxylation capacity decreased or the Rubisco oxygenation capacity increased (Fig. 7). However, such changes of the Rubisco kinetics would result in lower photosynthetic rates for both wild-type and bypass plants (Supplemental Table S7). It is worth emphasizing here that the actual benefit of the proposed targets for manipulation to gain increased photosynthesis in the bypass plants will be dependent on the existing activities of enzymes in the bypass plants. Once enzyme activities of all those involved enzymes can be measured, the modeling framework presented here can be used to determine the precise targets for engineering for increased efficiency.

CONCLUSION

Using a systems model, this study demonstrated that photorespiratory bypasses can increase carbon assimilation under specific conditions. Based on this theoretical analysis, not all previously described bypasses are expected to be functional or beneficial to photosynthesis. Relocation of the photorespiratory CO₂ release from mitochondria into chloroplast and reducing energy costs by avoiding ammonium release were shown to be the main factors that contribute to an improved photosynthetic efficiency. The g_{chl} greatly influences the potential benefit of a photorespiratory bypass. Specifically, the benefit of a bypass is expected to decrease with an increase in g_{chl} . Given the scarcity and uncertainty of the available estimates for membrane permeability, it remains difficult to predict whether introducing a bypass is a viable approach to optimize photosynthetic rates in crop species. The photorespiratory pathway may interact closely with many other pathways, such as nitrogen metabolism, respiration, and mitochondrial one-carbon metabolism (for review, see Ogren 1984; Foyer et al., 2009; Bauwe et al., 2010). More research on unraveling the regulatory networks controlling the association between photorespiration and other biochemical pathways is needed.

MATERIALS AND METHODS

Model Development

We extended the photosynthetic carbon metabolism model developed by Zhu et al. (2007) by incorporation of a more detailed description of the light reaction, CO₂ diffusion, ammonia refixation during photorespiration, and dark respiration (Fig. 1). In addition, some of the parameters (e.g. enzyme activities) were updated based on the literature. The default values for all parameters used in the current model are listed in Supplemental Tables S1 and S2. Here, we briefly describe the reactions that were added to the model.

Light Reactions

We assumed that the electron transport rate is much faster than the biochemical reactions of carbon fixation such as the CBB cycle. Therefore, we based the light reaction in the kinetic model on the steady-state biochemical description by von Caemmerer (2000). The relationship between the electron transport and the absorbed irradiance was described using an empirical equation (Ogren and Evans, 1993; von Caemmerer, 2000):

$$J = \frac{I_2 + J_{\max} - \sqrt{(I_2 + J_{\max})^2 - 4\theta I_2 J_{\max}}}{2\theta} \quad (1)$$

where I_2 is the light ($\mu\text{mol m}^{-2} \text{s}^{-1}$) absorbed by PSII, J_{\max} is the maximal electron transport rate, and θ is an empirical curvature factor (default value is 0.7; von Caemmerer, 2000). J is the electron transport rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$) that is directly related to the rate of ATP synthesis in the model as described below. I_2 is related to incident irradiance I by:

$$I_2 = \frac{I\alpha(1-f)}{2} \quad (2)$$

where α is the leaf absorptance (default value is 0.85; von Caemmerer, 2000), f corrects for the spectral quality of the light (default value is 0.15; Evans, 1987), and the 2 in the denominator indicates that 50% of the light is absorbed by each photosystem.

The maximum rate of ATP synthesis is described as:

$$V_{\text{ATPsynth}} = \min(V_{\text{maxE}}, V_{\text{maxJ}}) \quad (3)$$

where V_{maxE} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) is the maximum rate of ATP synthesis reactions determined by the enzyme kinetics and V_{maxJ} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) is the maximum rate of ATP synthesis reactions limited by the electron transport rate:

$$V_{\text{maxJ}} = \beta J \quad (4)$$

where J is the electron transport rate (in $\mu\text{mol m}^{-2} \text{s}^{-1}$) calculated by the steady-state model (Eq. 1). β is the ATP:e⁻ ratio. By assuming that the H⁺:ATP ratio is 4, and assuming that protons are generated through the whole electron transport chain with the Q cycle through cytochrome b_6f , the default value of β used in our model is 0.75 (von Caemmerer, 2000). The NADPH concentration is assumed to be a constant in the current model.

CO₂ Diffusion

The diffusion of CO₂ from intercellular airspaces to the site of fixation in the chloroplasts forms a significant limitation to photosynthesis in C₃ plants (Evans et al., 2009). Since some photorespiratory bypasses release CO₂ in other cellular compartments than in wild-type plants, the effects of the diffusion within the cell have to be taken into account by our model. Tholen and Zhu (2011) described this diffusion on a subcellular level using a reaction-diffusion model, but such a detailed approach is beyond the scope of this work. However, the diffusion of CO₂ between the different compartments of the biochemical model were explicitly considered using conductances. To calculate the contribution of CO₂ mass transfer between different organelles on the biochemical fluxes, we added to following rate equation for each compartment:

$$V = \frac{S}{\text{Vol}} \times g(\Delta\text{CO}_2) \quad (5)$$

where S is the surface area of a compartment (i.e. chloroplast, cytosol, or mitochondrion), Vol is the volume of the compartment, g is the conductance for CO₂ (through the cell wall and plasmalemma, chloroplast envelopes, or mitochondrial envelopes), and ΔCO_2 is the CO₂ concentration difference between two compartments.

Calculation of the CO₂ Refixation Ratio

CO₂ released by photorespiration can escape to the atmosphere or be refixed by Rubisco in the chloroplasts and phosphoenolpyruvate carboxylase (PEPC) in the cytosol. To quantify the amount of refixation, Tholen et al. (2012b) defined the refixation ratio as the flux of refixed (photo)respired CO₂ relative to the total flux of CO₂ that is released from respiration and photorespiration. We used individual identifiers for (photo)respiratory and atmospheric CO₂ in the model;

this allowed us to distinguish between the flux of CO₂ that was released by (photo)respiration and subsequently refixed by Rubisco or PEPc (FR) and the flux of CO₂ released from respiration and photorespiration (F_{release}):

$$R_{\text{refix}} = \frac{FR}{F_{\text{release}}} \quad (6)$$

The net flux of CO₂ that is lost to the atmosphere as a result of photorespiration (FL) relative to the CO₂ fixation rate by Rubisco (F_{Rubisco}) and PEPc (F_{PEPc}) is:

$$\phi = \frac{FL}{F_{\text{Rubisco}} + F_{\text{PEPc}}} \quad (7)$$

$$FL = F_{\text{release}} - FR \quad (8)$$

Implementation of Three Different Photorespiratory Bypass Pathways

Three different models representing different photorespiratory bypass pathways were implemented. We refer to the pathways described by Kebeish et al. (2007), Maier et al. (2012), and Carvalho et al. (2011) as the Kebeish bypass, the Maier bypass, and the Carvalho bypass, respectively. The different pathways are described in Figure 1. The Kebeish bypass converts glycolate into glycerate in the chloroplast, and CO₂ is released in the chloroplast stroma instead of in the mitochondria. The Maier bypass consists of glycolate dehydrogenase, malate synthase, malic enzyme, and pyruvate dehydrogenase. This pathway allows for the complete decarboxylation of glycolate, and the resulting CO₂ is released in the chloroplast stroma. In the Carvalho bypass, CO₂ is released in the peroxisome instead of in mitochondria or chloroplasts.

The metabolites and reactions used by the different photorespiratory bypass pathways were added to the Zhu et al. (2007) model (Fig. 1). The initial concentrations of metabolites and the kinetic parameters used in our model are listed in Supplemental Tables S1 and S3. The reaction rates of the additional enzymes are described using Michaelis-Menten kinetics (Supplemental Equations S1).

Analysis of Three Scenarios to Study the Mechanisms Underlying Increased Photosynthesis in the Kebeish Bypass Pathway

Peterhansel et al. (2013) suggested several characteristic features of photorespiratory bypasses that can explain why such bypasses can achieve a higher rate of photosynthesis and growth. To quantitatively analyze such features, we designed three theoretical scenarios that captured features of photorespiratory bypasses (Fig. 2). These theoretical scenarios enable us to test whether photosynthesis enhancement in the bypass plants was due to reduced ATP cost for photorespiration or was a consequence of releasing CO₂ inside the chloroplast stroma instead of in mitochondria. In the first scenario, we assumed that CO₂ was released in chloroplasts and that there was also no ATP cost for NH₃ refixation in the chloroplast (Fig. 4B). In the second scenario, we assumed that CO₂ was released in mitochondria but there was no ATP cost for NH₃ refixation in the chloroplast (Fig. 4C). For the third scenario, we assumed that the CO₂ was released inside chloroplasts but NH₃ was released and its refixation in the chloroplast required ATP (Fig. 4D). The first scenario is similar to the situation in which all photorespiratory flux would enter the Kebeish bypass pathway, the second scenario examines the effect of reduced ATP costs, and the third scenario tests whether releasing photorespiratory CO₂ in the chloroplast instead of in mitochondria has an effect on photosynthesis.

The Benefit of a Photorespiratory Bypass to Photosynthesis

The benefit of a photorespiratory bypass to photosynthesis was defined as the percentage of increase in photosynthetic rates in plants with a photorespiratory bypass pathway compared with the wild type:

$$\text{Bypass benefit} = \frac{A_{\text{bypass}} - A_{\text{wt}}}{A_{\text{wt}}} \times 100\% \quad (9)$$

where A_{bypass} is the photosynthetic rate of a plant with a photorespiratory bypass pathway and A_{wt} is the photosynthetic rate of the wild type.

Our model is built with the Simbiology Toolbox provided by MATLAB (version 2008a; MathWorks). The sundials solver of Simbiology was chosen to solve the system. The solution of the ordinary differential equations provided

by sundials provides the time series changes of each metabolite in every compartment, which in turn were used to calculate reaction fluxes.

Supplemental Data

The following supplemental materials are available.

Supplemental Figure S1. Simulated photosynthetic rate of wild type and different bypass pathways under multiple combinations of light intensity and CO₂ concentrations.

Supplemental Figure S2. Simulated CO₂ response curves (PAR = 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and light response curves ($C_i = 27 \text{ Pa}$) of wild type and the bypass described by Kebeish et al. (2007).

Supplemental Figure S3. The predicted effect of GDH activity (V_{maxGDH}) on the proportion of photorespiratory flux through the Kebeish bypass pathway and the photosynthetic rate of Kebeish bypass.

Supplemental Figure S4. The predicted effect of GDH activity (V_{maxGDH}) on the proportion of photorespiratory flux through the Maier bypass pathway and the photosynthetic rate of the Maier bypass.

Supplemental Table S1. Enzyme kinetic parameters used in the model.

Supplemental Table S2. Initial values of metabolite concentrations used in the model.

Supplemental Table S3. Additional parameters used in the model.

Supplemental Table S4. Predicted photosynthesis of wild type plants under ambient conditions.

Supplemental Table S5. The effects of glyoxylate carboligase and hydroxypyruvate isomerase enzyme parameters on the photosynthetic rate and on the proportion of the photorespiratory fluxes through the Carvalho bypass under low light conditions.

Supplemental Table S6. The photosynthetic rate predicted for several hypothetical scenarios based on the Kebeish pathway.

Supplemental Table S7. Effect of variation in the maximal enzyme activity (V_{max}) on the photosynthetic rates of wild type and Kebeish bypass plants under ambient conditions.

Supplemental Equations S1. Equations used in the systems models.

Supplemental Model S1: The model used in the study.

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