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Improving photosynthetic efficiency toward food security: Strategies, advances, and perspectives

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ABSTRACT

Photosynthesis in crops and natural vegetation allows light energy to be converted into chemical energy and thus forms the foundation for almost all terrestrial trophic networks on Earth. The efficiency of photosynthetic energy conversion plays a crucial role in determining the portion of incident solar radiation that can be used to generate plant biomass throughout a growth season. Consequently, alongside the factors such as resource availability, crop management, crop selection, maintenance costs, and intrinsic yield potential, photosynthetic energy use efficiency significantly influences crop yield. Photosynthetic efficiency is relevant to sustainability and food security because it affects water use efficiency, nutrient use efficiency, and land use efficiency. This review focuses specifically on the potential for improvements in photosynthetic efficiency to drive a sustainable increase in crop yields. We discuss bypassing photorespiration, enhancing light use efficiency, harnessing natural variation in photosynthetic parameters for breeding purposes, and adopting new-to-nature approaches that show promise for achieving unprecedented gains in photosynthetic efficiency.

Key words: photosynthesis, photorespiration, photorespiratory bypass, natural variation, synthetic biology, plant metabolic engineering

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INTRODUCTION

Photosynthesis harnesses the energy of visible light quanta to extract electrons from water, utilizing them to convert atmospheric carbon dioxide (CO_2) into biomass. This crucial metabolic process originated over 2 billion years ago in an atmosphere abundant in CO_2 and low in oxygen (O_2). Throughout geological time, oxygenic photosynthesis caused a significant shift in the

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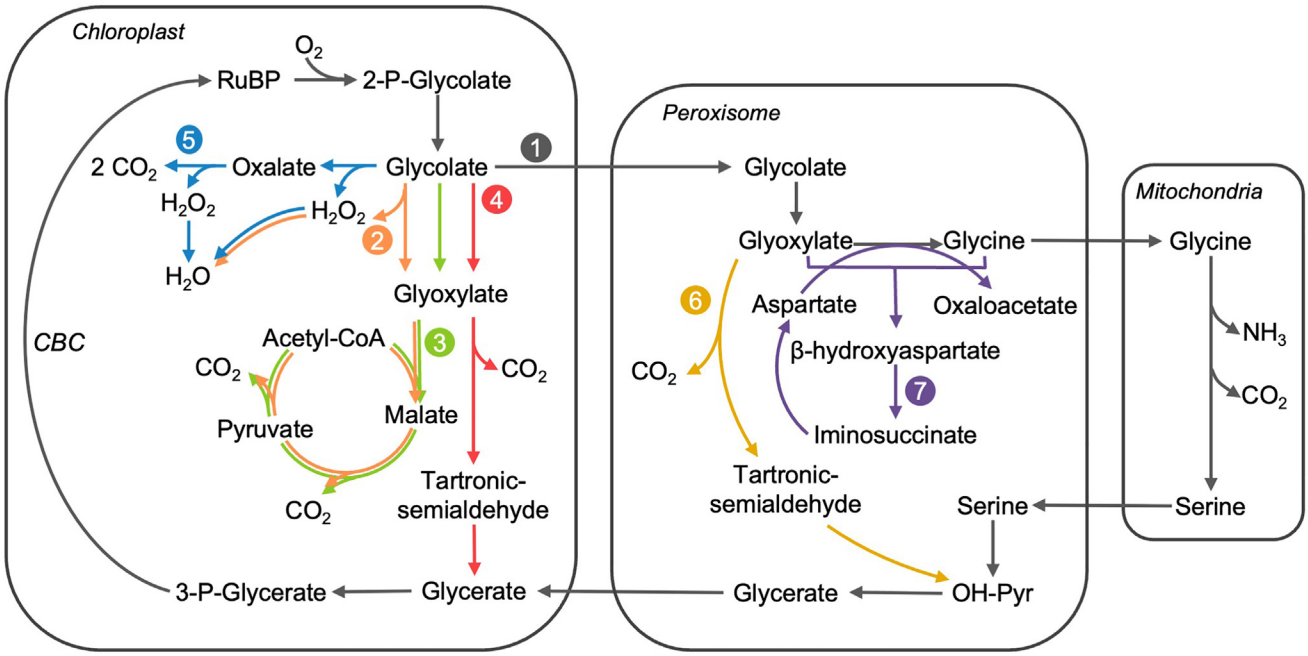

Figure 1. Bypasses to photorespiration discussed in this review.
Shown are (1) wild-type photorespiration, (2) Maier et al. (2012), (3) South et al. (2019), (4) Kebeish et al. (2007), (5) Shen et al. (2019), (6) Carvalho et al. (2011), and (7) Roell et al. (2021).

atmospheric O_2-to-CO_2 ratio, resulting in the present 500-fold excess of O_2 over CO_2. The enzyme responsible for CO_2 fixation in the Calvin–Benson cycle (CBC), known as ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), exhibits a higher affinity for CO_2 than for O_2. However, under current atmospheric conditions, Rubisco frequently reacts with O_2, leading to production of 3-phosphoglyceric acid (3PGA) and 2-phosphoglycolic acid (2PG). Notably, 2PG acts as an inhibitor of key enzymes in the CBC; namely, triose phosphate isomerase and sedoheptulose 1,7-bisphosphate phosphatase (Flügel et al., 2017).

The two carbon atoms present in 2PG cannot be further metabolized within the CBC. Instead, conversion of 2PG to 3PGA occurs via a metabolic pathway known as photorespiration (Bauwe, 2023). During photorespiration, one of four carbon atoms contained in two molecules of 2PG is released as CO_2; i.e., previously fixed carbon is lost. Additionally, the process results in release of ammonia and consumption of ATP and redox power. Overall, photorespiration significantly diminishes the efficiency of carbon assimilation in C_3 plants, leading to yield losses of approximately 30% or higher (Walker et al., 2016a). Despite its negative impact on photosynthetic efficiency, photorespiration is an essential process that enables photosynthesis in an O_2-containing atmosphere through breakdown of 2PG (Bauwe, 2023). Mutations affecting photorespiration are typically lethal, even in the presence of carbon-concentrating mechanisms such as carboxysomes in cyanobacteria, pyrenoids in algae, or C_4 photosynthesis in land plants (Eisenhut et al., 2008; Zelitch et al., 2009; Levey et al., 2019). The topic of photorespiration has been reviewed extensively (Bauwe et al., 2010; Hodges et al., 2016; Eisenhut et al., 2019; Fernie and Bauwe, 2020; Bauwe, 2023; Broncano et al., 2023), rendering a detailed account of the pathway unnecessary in this work. Instead, here we focus on recent approaches aiming to mitigate the impact of photorespiration on photosynthetic efficiency.

To mitigate the oxygenation reaction of Rubisco, land plants have evolved carbon-concentrating mechanisms, including C_4 and crassulacean acid metabolism photosynthesis. These pathways, however, are complex and require specific leaf anatomy. Despite significant efforts, introduction of these photosynthetic subtypes into C_3 plants remains an unsolved challenge. Instead of reducing oxygenation, several research groups have focused on improving the efficiency of 2PG recovery, designing and implementing alternative pathways that bypass photorespiration, converting 2PG into CO_2, intermediates of the CBC, or metabolites of C_4 photosynthesis. In this review, we will explore these photorespiration bypasses and other strategies to enhance photosynthetic efficiency, considering their potential contributions to increased crop yields. Additionally, we will examine naturally occurring variations in photosynthetic efficiency and explore how such variations could be leveraged to enhance photosynthetic efficiency through breeding. Finally, we will discuss the potential of new-to-nature pathways to increase photosynthetic efficiency.

INCREASING PHOTOSYNTHETIC EFFICIENCY THROUGH PHOTORESPIRATORY BYPASSES

Chloroplast-localized photorespiratory bypasses

We will first describe the design and implementation of bypasses to photorespiration, focusing on *in planta* experimentally validated approaches (Figure 1). We will then discuss how such bypasses improve yield and novel strategies that have not yet

been tested in plants. We will start the discussion with the designs proposed by Kebeish et al. (2007) and Maier et al. (2012) because most of the reported photorespiratory bypasses since 2012 are variations of these schemes.

Intraplastidic conversion of 2PG into 3PGA by glyoxylate carboligase (GCL) and tartronate semialdehyde reductase

Kebeish et al. (2007) successfully introduced a bacterial glycolate metabolic pathway into the chloroplasts of *Arabidopsis thaliana*, a process that involved incorporation of five pathway enzymes fused with chloroplast-targeting peptides. The ability of certain bacteria to utilize glycolate as the sole carbon source served as the basis for this implementation. Within the bacterial glycolate pathway, glycolate dehydrogenase (GDH), composed of three subunits (D, E, and F), converts glycolate to glyoxylate. Subsequently, GCL catalyzes ligation of two glyoxylate molecules, resulting in formation of tartronic semialdehyde and release of one molecule of CO_2. Tartronic semialdehyde is further transformed into glycerate through the action of tartronic semialdehyde reductase. Native glycerate kinase converts glycerate to 3PGA.

To establish the complete bacterial glycolate pathway, Kebeish et al. (2007) introduced the corresponding pathway components using three different plasmids. Through genetic crossings of lines expressing partial pathways, they eventually combined all of the components to create the full pathway. The transgenic *Arabidopsis* plants expressing the full pathway exhibited a two-fold increase in shoot biomass and a three-fold increase in root biomass. The transgenics also demonstrated a decrease in the glycine-to-serine ratio, which is indicative of reduced photorespiratory flux. Moreover, the post-illumination burst of CO_2 release, a measure of photorespiratory glycine in the light, was reduced.

Additionally, the lines expressing the complete bacterial pathway showed a minor reduction in O_2-inhibition of photosynthetic carbon assimilation and a slight decrease in the CO_2 compensation point. These findings suggest that implementation of the pathway resulted in a modest elevation of CO_2 levels at the site of Rubisco. Intriguingly, transgenic lines expressing only GDH D, E, and F also exhibited significant increases in biomass and rosette diameter, along with decreased Gly/Ser ratios. However, the reason behind the improved plant performance solely from expression of bacterial GDH remains unexplained.

Intraplastidic glycolate oxidation by glycolate oxidase

Maier et al. (2012) developed an alternative photorespiratory bypass strategy by redirecting peroxisomal enzymes to the chloroplasts. This pathway involves complete oxidation of glycolate to CO_2 within the chloroplast stroma. To achieve this, peroxisomal glycolate oxidase is targeted to the chloroplasts, where it catalyzes oxidation of glycolate to glyoxylate, releasing hydrogen peroxide as a byproduct. The chloroplast-targeted catalase then dissipates the hydrogen peroxide. Subsequently, glyoxylate and acetyl-coenzyme A (CoA) are condensed to malate by a plastid-targeted malate synthase, an enzyme from the peroxisomal glyoxylate cycle. Malate is decarboxylated by the native chloroplast NADP-malic enzyme, generating pyruvate and Nicotinamide adenine dinucleotide phosphate (NADPH). The resulting pyruvate is further decarboxylated by the native chloroplast pyruvate dehydrogenase, yielding acetyl-CoA and NADH. Acetyl-CoA, along with another glyoxylate molecule, re-enters the cycle through malate formation via malate synthase. Overall, this cycle completely oxidizes the carbon present in glycolate and releases CO_2 within the chloroplast stroma. Additionally, it generates NADPH and NADH as reducing equivalents. Unlike the pathway described by Kebeish et al. (2007), the Maier et al. (2012) pathway requires introduction of only three transgenes.

Transgenic *Arabidopsis* plants expressing the glycolate oxidizing pathway showed significantly increased rates of CO_2 assimilation and a decreased Gly/Ser ratio. However, in contrast to the findings of Kebeish et al. (2007), the CO_2 compensation point remained unchanged. This result is surprising considering that the local CO_2/O_2 ratio within the chloroplasts of these lines is expected to be higher than in the lines of Kebeish et al. (2007). One of the two lines analyzed by Maier et al. (2012) exhibited increased leaf fresh and dry weight, along with a reduction in leaf thickness.

Expression of GDH in chloroplasts

Kebeish et al. (2007) observed that transgenic *Arabidopsis* plants expressing all three subunits of *Escherichia coli* GDH exhibited enhanced biomass accumulation and reduced flux through the conventional photorespiration pathway. Building on this discovery, Nölke et al. (2014) created transgenic potato plants that were genetically modified to express a single GDH polyprotein, wherein the D, E, and F subunits were linked by a $(Gly_4Ser)_3$ linker sequence. The polyprotein was targeted to the chloroplasts using a Rubisco small subunit-targeting peptide (rbcS1).

The transgenic potato plants demonstrated elevated rates of CO_2 assimilation at a 400 ppm CO_2 concentration, decreased repression of CO_2 assimilation by O_2, and a lowered CO_2 compensation point. Moreover, these transgenic lines exhibited increased above-ground biomass accumulation compared with the control plants, with a more than 2-fold increase in tuber yield observed in lines showing the highest GDH activity. The precise mechanism underlying the augmented biomass production and yield in the transgenic plants was not extensively investigated in this study. However, the authors put forward the hypothesis that chloroplast-produced glycolate is decarboxylated by plastidial pyruvate dehydrogenase, resulting in a localized rise in CO_2 concentration at the Rubisco site.

Combination of plastid-localized photorespiration bypasses with reduced export of glycolate from chloroplasts

South et al. (2019) conducted a comparative analysis of three alternative designs for photorespiratory bypasses (AP1–AP3) in tobacco, a model crop, and evaluated the performance of transgenic plants in field trials. AP1 corresponds to the pathway described by Kebeish et al. (2007), AP2 is based on the pathway reported by Maier et al. (2012), and AP3 is a modified version of the Maier et al. (2012) pathway. In AP3, the combination of peroxisomal glycolate oxidase and catalase was replaced by mitochondrial GDH from *Chlamydomonas reinhardtii*, which was retargeted to the chloroplasts. Unlike the

bacterial GDH with its three subunits, the algal mitochondrial enzyme consists of a single subunit and does not produce H_2O_2 (the electron acceptor remains unknown), eliminating the need for co-expression of catalase. Consequently, the AP3 design only requires two transgenes: GDH and malate synthase.

Furthermore, all three alternative pathway designs were combined with a reduction in glycolate export from chloroplasts by employing antisense repression of the chloroplastic glycolate/glycerate transporter (PLGG1; Pick et al., 2013). T2 transformants were initially screened under high-light and low-CO_2 conditions to identify lines that exhibited enhanced protection against photorespiratory stress. This pre-selection step was crucial for identifying lines with optimal expression levels and stoichiometry of the pathway components. In greenhouse trials, all pathway designs were associated with increased biomass production, with AP2 and AP3 performing better when combined with repression of PLGG1. Subsequently, the AP3 design was evaluated in replicated field trials with and without repression of PLGG1. Surprisingly, contrary to the greenhouse experiments, the AP3 design performed best in the field without repression of PLGG1. The field-grown AP3 lines exhibited significantly higher biomass productivity, increased CO_2 assimilation rates, and decreased CO_2 compensation points. Additionally, AP3 lines displayed elevated levels of glyoxylate, while serine and glycerate levels were significantly reduced.

This study is significant because it validated previously reported bypass designs through a comparative analysis in the same model system, demonstrating that growth benefits observed in a greenhouse setting can be reproduced in the field. However, it is worth noting that lines expressing only a chloroplast-targeted GDH from *C. reinhardtii* were not included in this comparison. This omission is a limitation because it could have provided insights into whether the improved performance observed for AP3 is truly dependent on the coordinated activity of GDH and malate synthase or whether, similar to Nölke et al. (2014), GDH alone can confer growth benefits.

Complete oxidation of glycolate via oxalate oxidase

The study of Shen et al. (2019) describes a modified pathway, based on the work of Maier et al. (2012), that enables complete intraplastidic decarboxylation of glycolate. This pathway involves conversion of glycolate to oxalate through the action of rice glycolate oxidase isoform 3, previously demonstrated to oxidize glycolate and glyoxylate (Zhang et al., 2012). The resulting oxalate is then fully decarboxylated to CO_2 by rice oxalate oxidase 3. The accumulation of H_2O_2, a byproduct of glycolate and oxalate oxidation, is effectively eliminated by a chloroplast-targeted catalase. Consequently, implementation of this pathway necessitates incorporation of three transgenes encoding the chloroplast-targeted enzymes: glycolate oxidase, oxalate oxidase, and catalase.

Rice plants expressing these genetic constructs exhibited enhanced photosynthetic performance, characterized by a reduced CO_2 compensation point and a higher maximum photosynthetic rate under saturating light conditions. Furthermore, the Gly/Ser ratio and glycolate levels decreased, while glyoxylate and oxalate levels increased under ambient air conditions. Notably, the yield of single plant seeds varied depending on the seeding season, with a 27% increase observed in spring seeding but a yield penalty of 13%–16% in fall seeding.

In another variation of the pathways proposed by Kebeish et al. (2007) and Maier et al. (2012), Wang et al. (2020a) substituted the bacterial GDH component of the GCL route with the glycolate oxidase/catalase system in transgenic rice plants. This approach effectively combined aspects of the two previously reported bypasses. The transgenic plants exhibited improved photosynthetic parameters and yield in replicated field trials, further confirming the potential of photorespiratory bypasses to enhance crop performance under field conditions.

Peroxisome-localized photorespiratory bypasses

In the native photorespiration process, glycolate is transported from chloroplasts to peroxisomes. Within the peroxisomes, glycolate undergoes oxidation by glycolate oxidase, resulting in production of glyoxylate. Subsequently, glyoxylate is transaminated to form glycine. The glycine molecules are then transported to the mitochondria, where glycine decarboxylase and serine hydroxymethyltransferase catalyze the conversion of two glycine molecules into serine, CO_2, and ammonia. The generated serine is subsequently transported back to the peroxisomes, where it is converted into hydroxypyruvate by the action of serine:glyoxylate aminotransferase. Hydroxypyruvate is further reduced to glycerate, which is then transported back to the chloroplasts, thereby completing the photorespiratory cycle. Given the crucial role of peroxisomes in photorespiration, researchers have endeavored to develop strategies for bypassing the ammonia- and CO_2-releasing step that occurs in the mitochondria through alternate glycolate conversion within peroxisomes. Two such attempts have been documented in the literature.

Peroxisomal conversion of glyoxylate to hydroxypyruvate

This pathway design capitalizes on conversion of glycolate to glyoxylate by the inherent peroxisomal glycolate oxidase, aiming to redirect glyoxylate toward hydroxypyruvate through involvement of GCL and hydroxypyruvate isomerase. Similar to the pathway design proposed by Kebeish et al. (2007), bacterial GCL is employed to convert glyoxylate into tartronate semialdehyde, which is subsequently transformed into hydroxypyruvate by hydroxypyruvate isomerase. Essentially, this strategy enables retrieval of 75% of the carbon content present in two glyoxylate molecules while mitigating the release of ammonia mediated by mitochondrial glycine decarboxylase. Transgenic tobacco plants carrying constructs encoding peroxisome-targeted versions of these bacterial enzymes were generated.

Under non-photorespiratory conditions at high CO_2, the transgenic plants exhibited robust growth. However, when exposed to current ambient CO_2 conditions, the leaves displayed yellow lesions, and the plants exhibited a chlorotic phenotype. Metabolic labeling experiments employing [^{14}C]-glycolate revealed that glycolate was still predominantly converted into glycine and subsequently serine, suggesting that only a minor fraction of glycolate entered the engineered pathway. Surprisingly, amino acid analysis indicated that the leaves of the transgenic plants contained higher levels of glycine and serine compared with wild-type plants, contrary to initial expectations. Notably, the researchers were unable to detect the

presence of the hydroxypyruvate isomerase protein in the transgenic plants through immunoblotting despite detectable expression of the transgene confirmed by RNA gel blots. These findings suggest that the pathway may have been incomplete and that the observed phenotypes could arise from accumulation of undesired tartronate semialdehyde in peroxisomes, exerting adverse effects.

Peroxisomal glyoxylate-to-oxaloacetate conversion via the β-hydroxyaspartate shunt

In this study (Roell et al., 2021), a recently discovered microbial pathway involved in the metabolism of glyoxylate was introduced into plant peroxisomes. The pathway, known as the β-hydroxyaspartate cycle, encompasses four enzymatic steps that convert glyoxylate and glycine into oxaloacetate. The sequential actions of β-hydroxyaspartate aldolase, β-hydroxyaspartate dehydratase, and iminosuccinate reductase transform glyoxylate and glycine into β-hydroxyaspartate, iminosuccinate, and aspartate, respectively. Finally, aspartate: glyoxylate aminotransferase converts glyoxylate into glycine and releases oxaloacetate as the end product of the pathway. Transgenic *Arabidopsis* plants were engineered to express the pathway enzymes fused with peroxisomal targeting signals. Promoters that drive gene expression specifically in photosynthetic tissues were employed to prevent undesired pathway activity in non-photosynthetic plant organs.

Roell et al. (2021) hypothesized that reducing the conversion of glyoxylate to glycine would enhance metabolic flux through the β-hydroxyaspartate cycle. Therefore, apart from wild-type *Arabidopsis* plants, the pathway was introduced into the genetic background of the *ggt1-1* mutant, which lacks peroxisomal glutamate:glyoxylate aminotransferase 1 and exhibits a photorespiratory phenotype under ambient air conditions. This enabled investigation of the β-hydroxyaspartate cycle's function by assessing its ability to complement the visual phenotype of the *ggt1-1* mutant.

Similar to the findings of Carvalho et al. (2011), wild-type plants expressing the β-hydroxyaspartate cycle exhibited reduced growth and photosynthetic rates in ambient air. However, when exposed to elevated CO_2 concentrations that suppress photorespiration, their growth was comparable with that of wild-type controls. Importantly, expression of the β-hydroxyaspartate cycle in the *ggt1-1* mutant partially rescued the photorespiratory phenotype, indicating that the introduced pathway fulfilled its expected function to some extent.

Metabolic analysis was conducted to investigate the underlying reasons for the impaired growth observed in wild-type transgenic plants expressing the β-hydroxyaspartate cycle. These plants, as well as the transformed *ggt1-1* mutants, exhibited elevated levels of aspartate and malate, while glycine levels were reduced. Furthermore, intermediates of the CBC, such as 3-phosphoglycerate and sedoheptulose 7-phosphate, were depleted, suggesting a decrease in the availability of CBC intermediates and unproductive metabolic flux into C_4 acids.

Collectively, the studies by Carvalho et al. (2011) and Roell et al. (2021) indicate that perturbing the peroxisomal steps of the canonical photorespiration pathway does not yield the anticipated improvement in photosynthetic performance.

Although not extensively discussed in this review, we note that bypasses to photorespiration often led to a multitude of pleiotropic changes, including alterations in leaf shape and anatomy, metabolic changes, and developmental effects. For example, changes in leaf anatomy can have a multitude of effects on leaf photosynthetic activities. Changes in leaf thickness alone can result in increased photosynthetic activity because of more photosynthetic biomass per unit leaf area (Onoda et al., 2017). Changes in leaf anatomy, such as modifications of leaf thickness, intercellular airspace (IAS) volume, mesophyll cell wall thickness, and chloroplast size can also affect mesophyll conductance for CO_2 (g_m; see Figure 2 for a schematic explanation of g_m) and thereby alter the CO_2 concentration in chloroplasts (Flexas et al., 2012, 2013; Knauer et al., 2022). Given that observed changes in the CO_2 compensation points have often been small in the abovementioned studies, consideration of possible changes in g_m is important. We also note that the CO_2 compensation point does not only depend on Rubisco characteristics and CO_2 and O_2 concentrations but is also affected by the rate of mitochondrial respiration in light (R_d). R_d may change as a consequence of pathway engineering; for example, generation of extra respiratory substrate, such as malate transported out of chloroplasts.

The precise relationship between modified photorespiration and its impact on plant structure and function beyond photosynthesis remains incompletely understood. It is important to acknowledge that native photorespiration does not operate as a closed cycle in which 75% of the carbon derived from glycolate is reincorporated into the CBC as glycerate and 25% is released as CO_2. Instead, considerable amounts of carbon can be diverted toward synthesis of amino acids glycine and serine (Samuilov et al., 2018; Abadie and Tcherkez, 2019; Fu et al., 2023a), which serve as building blocks for protein biosynthesis and other plant metabolites. Moreover, photorespiration indirectly transfers redox equivalents from chloroplasts to mitochondria (Heber and Krause, 1980; Heber et al., 1996), where they are oxidized by the mitochondrial electron transport chain, contributing to ATP biosynthesis. The observed effects extending beyond photosynthetic metabolism may be linked to these additional, albeit less extensively studied and recognized, functions of the photorespiratory pathway. In the following paragraphs, we will provide a critical assessment of the assumptions as to how photorespiratory bypasses function, and we highlight unresolved questions regarding these pathways.

A critical qualitative assessment of mechanistic hypotheses

Despite the fact that some of the genetic implementations of photorespiratory bypasses increased photosynthetic efficiency and even yield under some conditions, many questions remain unresolved. In particular the precise molecular mechanisms that are responsible for higher performance are not yet understood. To develop informed hypotheses that are also backed up by theoretical considerations, we give some qualitative and quantitative arguments about attempts to explain increased photosynthetic rates.

First, it should be noted that an observation of biomass increases of a certain percentage after a growth period (usually several weeks long) cannot be directly translated into increased fluxes. Even marginal differences in flux will, over time, lead to an

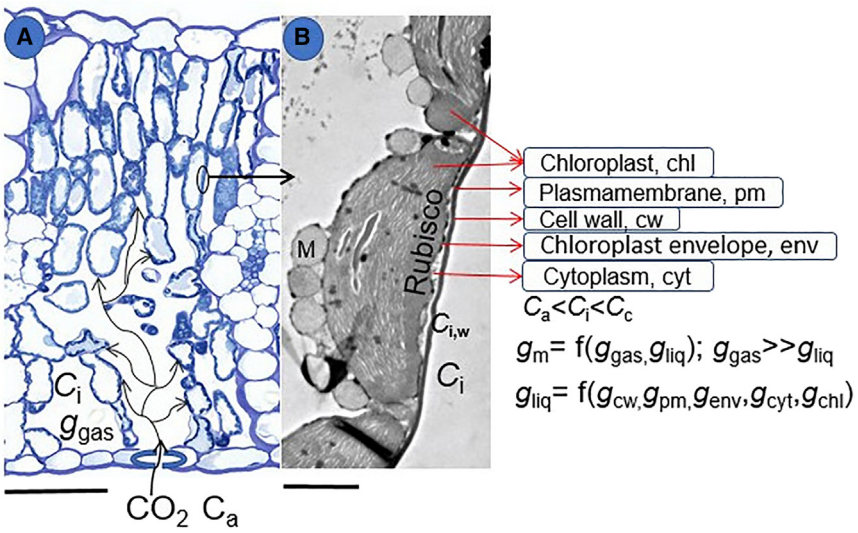

Figure 2. Semi- and ultrathin cross-sections of *Helianthus occidentalis* leaf mesophyll.

(A and B) Semi- and ultrathin cross-sections of *Helianthus occidentalis leaf mesophyll* ***(A)*** and palisade cells **(B)** to illustrate the CO_2 diffusion pathway from ambient air (C_a) to substomatal cavities (C_i) through IASs to the outer surface of the mesophyll cell wall ($C_{i,w}$) and farther into the chloroplast (C_c). The CO_2 concentration drawdown, C_a–C_i, is modulated by stomatal conductance. Mesophyll conductance (g_m) is determined by gas- and liquid-phase conductance. The C_i–$C_{i,w}$ drop is modulated by gas-phase diffusion conductance (g_{ias}), depending on mesophyll thickness and effective porosity of mesophyll airspace. The CO_2 drawdown from the outer surface of cell walls to chloroplasts, $C_{i,w}$–C_c, is determined by liquid-phase diffusion conductance (g_{liq}), which is determined by multiple liquid and lipid phase barriers: cell wall (cw), plasma membrane (pm), cytoplasm (cyt), chloroplast envelope (env), and chloroplast stroma (chl). Thus, the physical dimensions of each anatomical component of g_m determine its partial conductance, largely setting the maximum g_m in a given species (Tosens et al., 2012). In this context, the cell periphery facing the IAS is largely enveloped by chloroplasts ($S_c/S_{mes} \sim 1$, **B**). On a global scale, S_c/S_{mes} varies from 0.3–0.98. A high S_c/S_{mes} signifies the direct passage of CO_2 fluxes from the IAS into the chloroplasts; this configuration also facilitates efficient recycling of respiratory CO_2 fluxes as chloroplasts are covered by mitochondria (M) (Busch et al., 2013). Scale bars: **(A)** 0.03 mm, **(B)** 2 μm. Unpublished images by T.T.

exponential difference in overall biomass accumulation. For example, in South et al. (2019), it was shown that, under saturating CO_2, the assimilation rate is increased by approximately 10% (cf. South et al., 2019; Figure 5A). However, this increase is considerably lower than the reported 24% increase in biomass after a growth period of 6 weeks. This example illustrates that yield gains in percent stated for the diverse experimental approaches are not comparable on a quantitative level.

Other observations in South et al. (2019) are also challenging to explain. For example, it has been suggested that an increase in CO_2 locally in the chloroplast could explain a higher carbon fixation rate. However, increased CO_2 assimilation was observed even for saturating conditions, which entails that increasing the local concentration is not the primary cause for higher fixation rates. A similar result was observed when a complete glycolate decarboxylation pathway was expressed in rice, with several transgenic lines showing increased maximum rates of Rubisco carboxylation under saturating CO_2 (Shen et al., 2019). Another hypothesis was that, as a result of the glycolate oxidase activity, glycolate levels should be reduced, thus reducing its toxic effects. However, in various lines, the glycolate levels were actually increased, making this explanation unlikely. Similarly, although not explicitly measured, 2PG levels might be reduced as a result of the newly introduced pathways, but because dephosphorylation of 2PG to glycolate is highly irreversible, there is no convincing argument why 2PG levels should actually be reduced. We note, however, that it has been shown previously that more efficient removal of 2PG by overexpression of 2-phosphoglycolate phosphatase improved photosynthetic performance in *A. thaliana* under stress conditions (Timm et al., 2019).

Possibly, the growth promoting effect is of a more indirect nature. A possible explanation could be connected with the algal GDH, which transfers electrons not to H_2O_2 but to a so far unidentified electron acceptor. If this acceptor is one of the common electron carriers of the photosynthetic electron transport chain, such as plastoquinone or ferredoxin, then this new pathway would contribute to generation of redox equivalents and, thus, directly support the photosynthetic electron transport chain. The observation that the transformed plants exhibit a higher apparent quantum efficiency (φ; cf. South et al., 2019; Figure 6) is in line with this hypothesis. An experimental test would be to measure how the observed increase in growth depends on the light intensity under which plants are grown. If this speculation is correct, then the growth increase should be less pronounced the more saturating the light intensity.

In summary, these considerations illustrate the complexity of the system and the necessity to use mathematical models, which are based on clear mechanistic hypotheses and are designed to quantitatively reproduce experimental results and, thus, provide a platform to test different mechanistic hypotheses and, by making novel predictions, support the experimental design to confirm or falsify these.

Modeling at different scales can inform photorespiration engineering

The mechanisms of yield improvement caused by photorespiratory bypasses can be divided into four categories: improved stoichiometry and energy efficiency, improved kinetics (i.e., altered Rubisco carboxylation-to-oxygenation ratio), relief of inhibition by toxic intermediates, and indirect effects of altered physiology or development. Models at different scales, from simple cofactor accounting and stoichiometric models to kinetic models of photosynthesis, have provided some insight into how these mechanisms allow photorespiratory bypasses to be effective, but further work is still required if models are to explain all observed experimental results (Peterhansel et al., 2013; Xin et al., 2015; Basler et al., 2016; Trudeau et al., 2018; Khurshid et al., 2020; Osmanoglu et al., 2021).

Reference	Pathway name	CO_2 (α)	ATP (β)	Red. equiv. (γ)	Total ATP equiv.[a]	Total ATP equiv.[a] normalized to net 0 carbon lost/gained[b]
Trudeau et al., 2018	TaCo	1	7	4	17	9
Kebeish et al., 2007	chloroplast TSS	−0.5	3	1	5.5	9.5
Shih et al., 2014	3OHP[c]	1	8	4	18	10
Carvalho et al., 2011	peroxisome TSS	−0.5	3	2	8	12
Roell et al., 2021	BHAC[d]	−0.5	3	2	8	12
	photorespiration	−0.5	3.5	2	8.5	12.5
South et al., 2019	AP3	−2	2	−2	−3	13
Maier et al., 2012	GMK	−2	2	−1	−0.5	15.5
Shen et al., 2019	GOC	−2	2	1	4.5	20.5
	Calvin cycle	1	3	2	8	

Table 1. Photorespiratory bypass energy costs (adapted from Trudeau et al., 2018).

The table is based on the consumer model of photosynthesis, which describes the processes of photorespiration and the CBC as independent cycles that are able to regenerate ribulose 1,5-bisphosphate (RuBP) using ATP (β) and reducing equivalents (γ) and either consume or produce CO_2 and glyceraldehyde 3-phosphate (GAP) (α) (Trudeau et al., 2018). This description allows bypasses to be compared directly and separated from the CBC. Positive values represent consumption, and negative values represent production. See Supplemental Table 1 for detailed calculations.

3OHP, 3-hydroxypropionate; Red equiv, reducing equivalents; TaCo, tartonyl-CoA; TSS, tartronic-semialdehyde shunt; BHAC, β-hydroxyaspartate cycle; GMK, glycolate oxidase, malate synthase, catalase (KatE); AP3, alternative pathway 3; GOC, glycolate oxidase, oxalate oxidase, catalase.

[a]Assuming 2.5 ATP per reducing equivalent.

[b]Assuming CBC compensates for carbon lost by PR bypasses.

[c]3OHP bypass assuming that pyruvate is converted to GAP via pyruvate phosphate dikinase.

[d]BHAC bypass assuming that oxaloacetate is converted to GAP via phosphoenolpyruvate-carboxykinase to regenerate RuBP.

A common feature of photorespiratory bypasses is to avoid the energetic cost of ammonium refixation or to capture the reducing power from glycolate oxidation, thus decreasing the ATP and NADPH cost of photorespiration. Models for energy cofactor accounting can quantify these direct energetic benefits of bypassing photorespiration as well as indirect benefits, such as avoiding the cost of CO_2 refixation via the CBC in carbon fixing bypasses (Table 1; Peterhansel et al., 2013; Trudeau et al., 2018). Larger, genome-scale stoichiometric models can also calculate energy efficiency and have the advantage of predicting flux into biomass rather than just rates of carbon fixation, placing bypasses in the wider context of the plant metabolic network (Basler et al., 2016). For example, a curated stoichiometric model was able to predict a decrease in photorespiratory flux and a biomass output increase of ∼6.2%, qualitatively consistent with experimental data (Kebeish et al., 2007; Basler et al., 2016). However, stoichiometric models predict no benefit for bypasses that are energetically more costly than photorespiration, such as those that completely decarboxylate glycolate in the chloroplast. Experimentally, such bypasses still show increased yields when expressed in plants, suggesting either incorrect prediction of energy costs or benefits that are beyond just direct ATP and NADPH savings (Maier et al., 2012; Peterhansel et al., 2013; Xin et al., 2015; Shen et al., 2019; South et al., 2019).

Photorespiration involves transport of metabolites between three compartments as well as movement of reducing equivalents from the chloroplasts to the mitochondria. Photorespiratory bypasses can relocate reactions to different compartments, potentially avoiding the energetic costs of metabolite transport. Therefore, more complete modeling of transport reactions, including thermodynamic constraints, may improve the accuracy of energy accounting. However, even if all energetic costs were accurately modeled, stoichiometric models alone cannot account for changes in metabolite concentrations or reaction kinetics.

Several of the bypasses validated in plants aim to relocate the release of photorespiratory CO_2 from mitochondria to chloroplasts, which has two potential advantages: increasing the CO_2 concentration at the site of Rubisco and recapturing photorespiratory CO_2 that could otherwise be lost from the cell by diffusion out of mitochondria. Predicting such effects requires use of kinetic models or additional constraints on Rubisco carboxylation and oxygenation fluxes (Basler et al., 2016). A kinetic model predicted that, under high light conditions, the entire benefit of the Kebeish et al. (2007) bypass is relocation of CO_2 release, not the reduced ATP cost, which only contributes under low-light conditions (Xin et al., 2015). However, the same kinetic model failed to explain the benefit of complete glycolate decarboxylation in the chloroplast, predicting that the photosynthetic rate would be 31% lower than in the wild type, despite reported increases of more than 30% in carbon assimilation rate and biomass (Maier et al., 2012; Xin et al., 2015). Additionally, the kinetic model demonstrated that any benefit of relocating CO_2 release is dependent on the CO_2 permeability of the chloroplasts; predicting that, if more than 30% of photorespiratory CO_2 is already recaptured and refixed in wild-type plants, then there is no benefit to the bypass (Xin et al., 2015). It is therefore surprising that a glycolate decarboxylation bypass in rice, where wild-type plants already refix 38% of photorespiratory CO_2, still showed some biomass gains, suggesting additional benefits not captured by the kinetic model (Busch et al., 2013; Xin et al., 2015; Shen et al., 2019; Wang et al., 2020a; Zhang et al., 2022).

In contrast to Xin et al. (2015), a different kinetic model of a cyanobacterial complete glycolate decarboxylation bypass

expressed in the chloroplast predicted a 10% increase in photosynthetic rate, although this model did not explicitly account for the movement of CO_2 between compartments and has yet to be experimentally validated by expression of the full pathway in plants (Bilal et al., 2019; Khurshid et al., 2020; Abbasi et al., 2021). Kinetic models have been useful for estimating the potential effect of relocation of CO_2 release and distinguishing this from energy benefits, but they are still unable to explain all currently observed experimental results.

Reducing the concentration of inhibitory intermediates of photosynthetic metabolism could account for some of the benefit of photorespiratory bypasses, and this can be simulated using kinetic models. However, kinetic models of photorespiratory bypasses did not include parameters for the inhibitory effects of 2-phosphoglycolate on the CBC or the inhibitory effect of glyoxylate on Rubisco activation and Rubisco oxygenase activity (Oliver and Zelitch, 1977; Oliver, 1980; Campbell and Ogren, 1990; Xin et al., 2015; Flügel et al., 2017; Khurshid et al., 2020). Increasing the scale of kinetic models to include more inhibition terms may provide additional explanation for benefits, although this can be limited by the availability of accurate kinetic parameters.

As pointed out previously, a higher apparent φ could be responsible for yield improvements in the transformed plants. Modeling the effect of bypasses on φ would require a high-fidelity kinetic model of the photosynthetic electron transport chain and the CBC, such as in Saadat et al. (2021), to be combined with a model of photorespiration.

Additional effects beyond the cellular metabolic changes described by current models, such as altered gene expression, signaling, physiology, and pleiotropic effects, could also explain a large proportion of observed growth benefits (Maier et al., 2012; Shen et al., 2019). Using larger-scale integrated models could potentially provide more accurate prediction of the effect of engineering photorespiration in the field by accounting for interactions across scales (Wu, 2023). Additionally, attempting to model dynamic processes under non-steady-state conditions may also help identify advantages of photorespiratory bypasses not captured by current models (Fu et al., 2023b). Finally, extending current metabolic models to account for diurnal cycles could explain advantages of bypass reactions beyond altered photosynthetic metabolism, such as altered dark respiration and sucrose export during the night (Dalal et al., 2015).

No single modeling strategy can explain all of the observed phenotypes of plants expressing photorespiratory bypasses; instead, multiple models at different scales should be used as tools to help explain the underlying mechanisms of growth benefits. Questions still remain to be tested by exploring models: why does expression of GDH alone also increase photosynthetic performance and yield in *Arabidopsis*, potato, and *Camelina* (Kebeish et al., 2007; Nölke et al., 2014; Dalal et al., 2015; Abbasi et al., 2021), and how does the anatomy and physiology of different crop species affect CO_2 diffusion between cells and subcellular compartments? Future implementation of new bypass designs based on carbon-fixing rather than decarboxylating reactions will also provide valuable data for exploring the effect of a fundamentally different bypass mechanism (Trudeau et al., 2018; Scheffen et al., 2021) (see discussion of new-to-nature pathways below). With future modeling efforts and better understanding, it may be possible to further increase yields and improve the transferability of benefits between different crop species.

COMBINED OPTIMIZATION OF LIGHT USE AND CARBON ASSIMILATION EFFICIENCY TO ENHANCE PLANT PRODUCTIVITY

The intricate relationship between light conversion, carbon fixation, and their impact on plant productivity calls for a comprehensive exploration of their interdependencies. Through combining modifications of light utilization and carbon fixation, we have the potential to achieve synergistic enhancements. Here we discuss the interplay between these pathways and evaluate the possibility of maximizing overall productivity by improving the efficiency of converting light energy into ATP and NADPH while optimizing their utilization for carbon fixation.

In plants, pigments absorb light, and the excitation energy is utilized by photosystems to facilitate synthesis of ATP and NADPH, which are essential for all metabolic reactions, including carbon fixation. This initial phase of photosynthetic reactions effectively transforms light into chemical energy, and its efficiency significantly influences crop productivity (Zhu et al., 2010).

In highly dynamic environments, the amount of light absorbed by the photosynthetic apparatus can exceed the metabolic capacity of the cell, resulting in over-reduction of the photosynthetic electron transport chain and production of harmful reactive O_2 species. Photosynthetic organisms have evolved various mechanisms to regulate light utilization efficiency and photosynthetic electron transport, aiming to minimize the likelihood of over-reduction and cellular damage by safely dissipating excess excitation or electrons (Li et al., 2009). While protection of the photosynthetic apparatus plays a crucial biological role, it comes at the expense of reduced efficiency in converting sunlight into chemical energy (Alboresi et al., 2019).

Fluctuations in light intensity represent a particular challenge to regulation of photosynthesis and significantly impact primary productivity (Long et al., 2022). Abrupt increases in illumination can be detrimental because they do not allow sufficient time for activation of regulatory responses and modulation of metabolic reactions. Conversely, when light levels decrease from excessive to limiting, the photoprotective mode remains active for several minutes, leading to unnecessary energy dissipation and a subsequent reduction in carbon fixation efficiency (Wang et al., 2020b).

To address this limitation, modifications were made to the kinetics of non-photochemical quenching (NPQ), which is one of the mechanisms involved in light harvesting regulation. By overexpressing key proteins such as the Photosystem II subunit S, violaxanthine de-epoxidase, and zeaxanthin epoxidase the activation and relaxation kinetics of NPQ were accelerated. This overexpression allowed tobacco and soybean plants to effectively respond to changes in light and minimize potential damage during sudden increases in sunlight. Additionally, it

facilitated faster relaxation of NPQ when illumination intensity decreased. This approach has demonstrated positive effects on biomass productivity not only in the field, for crops like tobacco and soybean (Kromdijk et al., 2016; Souza et al., 2022), but also in high-density culture photobioreactors for the microalga *Nannochloropsis* (Perin et al., 2023). However, in *Arabidopsis*, the same approach was not as successful, suggesting that species-specific traits, including light distribution within the canopy, significantly impact plant productivity and the optimal balance between light harvesting and photoprotective responses (Garcia-Molina and Leister, 2020).

Another strategy to enhance the efficiency of sunlight utilization is modification of the light-harvesting apparatus. Leaves have evolved to efficiently capture light and outcompete other organisms for sunlight. However, in densely cultivated fields, this high light harvesting efficiency can have negative consequences. Light is primarily absorbed by the uppermost leaves in the canopy, leaving fewer photons available for the lower layers. While high light harvesting efficiency provides a competitive advantage in natural environments where individuals vie for a limited resource like light, it proves detrimental in cultivated fields. In this context, plants with paler leaves can enable a more uniform distribution of light within the canopy, which has been shown to enhance overall productivity (Rotasperti et al., 2022; Cutolo et al., 2023). Also, extra nitrogen that is no longer needed for construction of the pigment-binding machinery of photosynthesis could be invested in Rubisco and rate-limiting proteins of photosynthetic machinery, thereby increasing leaf photosynthetic capacity (Walker et al., 2017; Niinemets, 2023). It is interesting here to discuss whether these efforts in modulating regulation of light harvesting could be combined with improvements in carbon fixation efficiency. In principle, a higher-efficiency conversion of light energy into ATP and NADPH could be combined with more efficient utilization of these molecules for carbon fixation, with a potentially additive effect on productivity.

One possible implication to be considered is that photorespiration has been shown to be a major sink for photosynthetic electron transport and that this energy loss can have a beneficial effect under conditions where the photosynthetic apparatus is overexcited (Heber and Krause, 1980; Kozaki and Takeba, 1996; Hanawa et al., 2017). Light saturation occurs when electron transport is faster than the metabolic capacity of consuming ATP and NADPH produced, and, under these conditions, photorespiration can be beneficial in reducing oversaturation. If efficiency in carbon fixation is improved by introduction of photorespiratory bypasses, however, then this would also drive stronger ATP and NADPH consumption with a similar protective effect. This suggests that the critical point is that the new or modified pathways have a sufficient capacity to also compensate for ATP and NADPH consumption associated with photorespiration. If this is the case, then their introduction will increase CO_2 fixation while complementing the role of photorespiration in protection from light excess.

It is anticipated that altering crucial pathways in plant metabolism, including light conversion and carbon fixation, will inherently have interdependent effects that require additional investigation. Furthermore, there exists the possibility of enhancing the efficiency of converting light energy into ATP and NADPH while concurrently optimizing the utilization of these molecules for carbon fixation. Such combined improvements could potentially yield an additive outcome, maximizing the overall impact on productivity.

LEVERAGING NATURALLY OCCURRING VARIATION OF PHOTOSYNTHETIC PARAMETERS

Exploiting natural variation of Rubisco kinetic traits, Γ^*, and photorespiration in coordination with CO_2 diffusion

Leveraging the inherent diversity in essential photosynthetic traits presents two notable advantages: first, it provides engineering with essential knowledge about potential trade-offs, and second, it facilitates the breeding process, allowing accelerated improvements in crops. The crucial traits related to photorespiration in Rubisco include the Michaelis-Menten constant for CO_2 (K_c), the Michaelis-Menten constant for O_2 (K_o), Rubisco specificity to CO_2 over O_2 ($S_{c/o}$), and maximum turnover rates (specific activity per active center per mass or protein [s^{-1}]) for carboxylase (V_c) and oxygenase (V_o). Several authors have investigated the environmental and evolutionary trends of natural variation in Rubisco's kinetic properties and their temperature responses, including several recent meta-analyses (Galmés et al., 2019; Bouvier et al., 2021; Tcherkez and Farquhar, 2021). These studies have revealed substantial variability in major kinetic traits, which are highly sensitive to factors such as temperature, CO_2 availability, and photosynthetically active quantum flux density. Moreover, this variability is inherent among species adapted to different environments. There are several key trade-offs among Rubisco kinetic traits, most notably the reverse relationships between $S_{c/o}$ and V_c (Galmés et al., 2014, 2019), which are important to consider in Rubisco engineering.

Recent findings have demonstrated a strong co-regulation between the natural diversity of Rubisco kinetics and g_m as well as in underlying anatomical traits. For instance, plants adapted to drought exhibit reduced g_m and chloroplast CO_2 concentration (C_c) because of higher mesophyll cell wall thickness. Consequently, these plants possess Rubisco with higher $S_{c/o}$ but lower turnover rates (Galmés et al., 2019). This highlights the substantial influence of C_c associated with species adaptation on the diversity of Rubisco's kinetic properties and the trade-offs observed. Importantly, this regulation is significantly influenced by g_m, which exhibits considerable variation among species and is responsive to environmental stressors (Elferjani et al., 2021; Knauer et al., 2022).

It is noteworthy that g_m can limit photosynthesis, accounting for approximately 10%–70% of the limitation, thereby exerting an equally significant impact on photosynthetic assimilation (A) as stomatal conductance (g_s) does (Knauer et al., 2020).

The CO_2 compensation point (Γ) has traditionally served as a parameter reflecting Rubisco functionality and photosynthetic efficiency. It represents the equilibrium between the A and leaf respiration, making it a useful characteristic for categorizing crops and herbaceous species based on their inherent photosynthetic efficiency and stress resilience. In C_3 plants, Γ is typically

highest (40–100 μmol mol^{-1}), intermediate in C_3–C_4 intermediates (20–30 μmol mol^{-1}), and low in C_4 plants (3–10 μmol mol^{-1}) (Nobel, 1991; Schlüter et al., 2023).

However, Γ is a complex trait and can be estimated in a multitude of ways. To incorporate Rubisco kinetics, photorespiration, and more accurately, an alternative parameter called the photorespiratory CO_2 compensation point in absence of day respiration (Γ^*) has been proposed. Γ^* can be determined from A/C_c curves by using the common interception method measured at different light intensities and represents the C_c at which photosynthetic carbon uptake equals photorespiratory CO_2 release (Walker et al., 2016b). Γ^* and Γ differ significantly; the magnitude of difference depends on the variable g_m (Figure 2) and the fate of the mitochondrial CO_2 fluxes because both estimates affect the resulting C_c and Γ^*. An understanding of partitioning of respiratory fluxes between the proportion directed into the chloroplast and what diffuses into the cytoplasm and IAS is also crucial for correct g_m estimations. However, the fate of respiratory fluxes and g_m largely depend on similar anatomical traits, such as the proportion of mesophyll cell surface area lined with chloroplasts, and physical dimensions of the cell wall, chloroplasts, and cytoplasm (Figure 2B). All of these traits vary significantly across species, indicating substantial variability in partitioning of respiratory fluxes between chloroplasts and cytoplasm (Evans et al., 1994; Tosens et al., 2012; Ubierna et al., 2019). Thus, considering the physiological nature of CO_2 compensation points estimated through different methods, including Γ^*, offers valuable insights into how mitochondrial CO_2 effluxes curb C_c (Walker et al., 2016b; Busch, 2020; Sage, 2022).

Achieving optimal photosynthetic efficiency requires precise coordination between traits that regulate CO_2 diffusion efficiency and the functionality of Rubisco. Consequently, a major focus in improving photosynthesis, whether through breeding or engineering, is to explore and comprehend the natural diversity of key kinetic traits of Rubisco and their relationship with CO_2 availability in the chloroplast stroma (Walker et al., 2016b; Galmés et al., 2019; Flexas and Carriquí, 2020; Evans, 2021; Knauer et al., 2022; Iqbal et al., 2023). In angiosperms with elevated rates of turnover, the constraints of photosynthesis, including stomata, g_m, and photo/biochemical processes, typically coexist in a harmonious equilibrium unless stress-induced alterations disrupt this balance. Consequently, attaining optimal enhancements of photosynthesis and resource utilization efficiency requires the simultaneous manipulation of all three constraints or a shift in focus toward augmenting the g_m/g_s ratio. This approach enables a simultaneous increase in intrinsic water use efficiency (Gago et al., 2019; Flexas and Carriquí, 2020; Knauer et al., 2020; Clarke et al., 2022; Kromdijk and McCormick, 2022).

Previous studies have often addressed the kinetics components of A/C_i curves (Rubisco kinetics, RuBP turnover rate, electron transport limitations; R_d, respiration because of photorespiration [R_p], CO_2 compensation point) separately, combining *in vivo* and *in vitro* estimations, resulting in fragmented information (Bernacchi et al., 2013). However, employing state-of-the-art fast response gas-exchange and optical diagnostic systems, as described by Laisk et al. (2002), allows simultaneous measurement of all necessary parameters to comprehensively assess the photosynthetic apparatus in leaves. This approach enables a holistic understanding of how photosynthesis is optimized. A primer on measuring photorespiration is given in Supplemental File S1.

Natural genetic variation in photosynthetic parameters as a basis for breeding

One way to adapt plants to human requirements is by harnessing the natural genetic variation that has been generated through random mutations over historical time spans. This genetic variation can occur at the intraspecific and interspecific level.

The first evidence of natural genetic variation in the context of photorespiration was provided by Jordan and Ogren (1981). They observed differences in the specificity factors toward the substrates CO_2 and O_2, $S_{c/o}$, of Rubiscos purified from seven different species, ranging from 77–82. Subsequent studies, as reviewed by Hartman and Harpel (1994), revealed changes in the specificity factor because of replacement of the active-site metal, random and site-directed mutagenesis, chemical modification, and hybridization of heterologous subunits. The highest reported specificity factor for Rubisco is 238, found in the red alga *Galdieria partita* (Uemura et al., 1996), which is about three times higher than that reported for Rubisco from most crop plants (Parry et al., 1989).

Even before discovery of the oxygenase activity of Rubisco (Bowes et al., 1971), selection experiments aiming to manipulate the specificity factor of Rubisco through selection were conducted (cf. Cannell et al., 1969; Menz et al., 1969). In these experiments, plants were kept at or slightly above the compensation point, and plants with high rates of photorespiration were expected to perish.

As discussed in the previous section, over the recent years, the number of studies exploring the natural plasticity and inherent variability of Rubisco's key catalytic traits have increased significantly. Information about the interspecific variation in photorespiration has also been observed through carbon isotope fractionation (Lanigan et al., 2008). However, to the best of our knowledge, only the study by Cai et al. (2014) examined and observed natural genetic variation in CO_2 compensation points and evaluated these differences in the context of leaf anatomy variations among three *Rhododendron* species.

The genes or alleles responsible for the advantageous photorespiratory phenotype can be transferred through interspecific hybridization, protoplast fusion, transformation, or genome editing. To facilitate these processes, it is necessary to identify the underlying genes or alleles. One approach to achieve this is through large-scale comparative genomics, which incorporates phenotypic information and is referred to as phylogenetic association mapping (e.g., Collins and Didelot, 2018).

However, with the exception of Schlüter et al. (2023), no earlier study has considered natural variation in characters related to photorespiration in a densely sampled phylogenetic tree, making application of phylogenetic association mapping challenging for identifying the underlying genomic features.

An alternative approach for identifying the genomic features responsible for interspecific differences is utilization of segregating genetic material derived from interspecific hybrids.

Comparative transcriptomics is another approach that can aid in identifying the *cis* and *trans* factors responsible for interspecific differences. By comparing transcriptomes of accessions adapted to specific conditions or subjected to environmental perturbations, strategies for crop improvement can be guided. For example, in rice, RNA sequencing (RNA-seq) identified a transcription factor with the potential to enhance photosynthetic capacity and increase yield (Wei et al., 2022). However, when using bulk RNA-seq of an organ, it becomes challenging to detect differentially expressed genes specific to rare cell types because their expression is diluted. In the case of C_3–C_4 or C_4 photosynthesis, where the pathways operate in specific cell types of the leaf, obtaining cell-type-specific transcriptomes is crucial. Approaches such as mechanical separation of cell types (John et al., 2014) and laser capture microdissection followed by microarray/RNA seq have been used previously, but their scale and resolution are limited by the speed of sampling, and separating low-abundance cell types can still be challenging (Zhang et al., 2007; Aubry et al., 2014; Hua et al., 2021; Xiong et al., 2021).

The adoption of single-cell methods, particularly droplet-based technology, is gaining increasing acceptance in plant research. This approach allows capture of individual transcriptomic profiles of cells from a wide range of plant species, providing valuable insights at a remarkably low cost per cell. For example, comparative single-cell analysis of roots from three grass species provided significant insights into the evolution of cellular divergence in these crops (Guillotin et al., 2023). This approach therefore must hold potential for better understanding the compartmentation of gene expression in C_3–C_4 and C_4 species (Cuperus, 2021; Seyfferth et al., 2021). Currently the number of single-cell or single-nucleus datasets generated from leaf tissues is limited, and most studies have tended to focus on a single model species under one condition (Bezrutczyk et al., 2021; Kim et al., 2021; Lopez-Anido et al., 2021; Berrío et al., 2022; Procko et al., 2022; Sun et al., 2022). To enhance photosynthesis efficacy in crops, understanding the changes in gene expression associated with closely related C_3, C_4, or/and C_3–C_4 intermediate species is likely to provide insights into the molecular signatures of each of these traits and therefore how they might be rationally engineered. Comparative analyses of leaf anatomy, cellular ultrastructure, and photosynthetic traits between species within a genus (for example, in *Gynandropsis* [Marshall et al., 2007; Koteyeva et al., 2011], *Moricandia* [Schlüter et al., 2017], or *Flaveria* [McKown and Dengler, 2007; Kümpers et al., 2017]) have provided insights into traits that engender higher photosynthesis efficiency. Therefore, acquiring transcriptome data from individual bundle sheath and mesophyll cells of these closely related plants should provide new insights into how the patterns of transcript abundance alter in association with modifications to photosynthetic efficiency. We also anticipate that single-cell RNA-seq (scRNA-seq) will contribute to crop improvement by providing insights into underlying molecular mechanisms. Last, additional advantages can be obtained from scRNA-seq when the data are associated with a complex phenotype (single-cell transcriptome-wide association studies) and genotype (cell-type-specific expression quantitative trait loci) at the population level (Perez et al., 2022). It seems likely that such approaches will be adopted for high-resolution genotyping of bioengineered plants in synthetic biology projects. In summary, combination of scRNA-seq technology with comparative transcriptomics, population genetics, and synthetic biology is likely to serve as a powerful tool for crop improvement.

A technically simpler procedure to the above-described approach of exploiting interspecific natural genetic variation would be exploitation of the intraspecific variation within the species under consideration for photosynthetic properties because transfer or the enrichment of positive alleles is much easier to realize in comparison with interspecific variability. Within species, natural variation in leaf photosynthesis has been reported for model species (e.g., Tomeo and Rosenthal, 2018) but also for major crops (e.g., Gu et al., 2012; Driever et al., 2014). However, the improvements that can be realized in that way are up to now smaller compared with the approaches exploiting interspecific variability.

NEW-TO-NATURE APPROACHES TO IMPROVE PHOTOSYNTHETIC EFFICIENCY

Most efforts to improve carbon capture in plants have focused on engineering naturally existing enzymes and pathways (Kebeish et al., 2007; South et al., 2019). However, the emergence of synthetic biology has opened up the possibility to radically (re) draft plant metabolism to overcome the limitations of natural evolution, which is driven by co-linearity, tinkering, epistatic drift, and purifying selection, rather than by "design" (Wurtzel et al., 2019). Thus, such engineering approaches have the potential to expand the biological solution space and provide new-to-nature pathways that outcompete their natural counterparts in respect to thermodynamics and/or kinetics because they are drafted from first principles.

As an example, while nature has evolved seven different pathways for CO_2 fixation, more than 30 synthetic CO_2 fixation pathways have already been designed, which are all superior to the CBC (Bar-Even et al., 2010). Some of these new-to-nature solutions have even been realized successfully realized *in vitro* (Schwander et al., 2016; Luo et al., 2022; McLean et al., 2023) and are awaiting their transplantation *in vivo*.

The concept of designer metabolism has also been extended to photorespiration lately (Figure 3). Two studies have proposed alternative ways to use the photorespiratory metabolite glyoxylate to feed into synthetic carbon fixation cycles (Figure 3, pathways 1 and 2). The first cycle is the malyl-CoA-glycerate (MCG) pathway. In this cycle, the bacterial glyoxylate assimilation route condenses two molecules of glyoxylate to form a C3 compound, releasing CO_2 in the process. The resulting tartronate semialdehyde is then reduced and phosphorylated into 2PG. 2PG is converted to phosphoenolpyruvate and further carboxylated to oxaloacetate, subsequently reduced to malate and activated with CoA, followed by cleavage into glyoxylate and acetyl-CoA. Glyoxylate is then available to initiate the next cycle, while acetyl-CoA can be utilized for biosynthesis (Yu et al., 2018). The MGC pathway, therefore, does not result in a net loss of CO_2.

A second study proposed the 3-hydroxypropionate (3OHP) photorespiratory bypass, inspired by the naturally occurring 3OHP

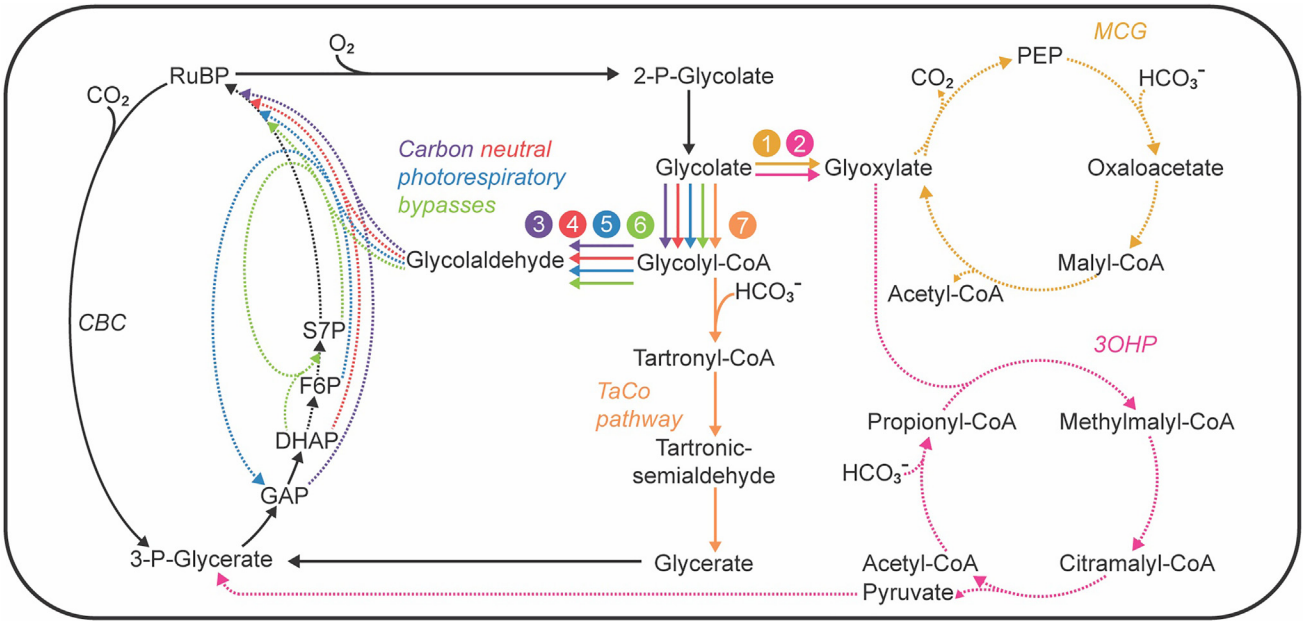

Figure 3. Designed (new-to-nature) photorespiratory bypasses.
Shown are (1) the malyl-CoA-glycerate pathway (MCG) (Yu et al., 2018), (2) 3-hydroxypropionate bypass (3OHP) (Shih et al., 2014), (3)–(6) carbon neutral bypasses (Trudeau et al., 2018), and (7) the tartronyl-CoA (TaCo) pathway (Scheffen et al., 2021).

bi-cycle (Zarzycki et al., 2009). The 3OHP bypass exploits malyl-CoA lyase to produce β-methylmalyl-CoA from glyoxylate and propionyl-CoA. Methylmalyl-CoA undergoes a series of interconversions that yield citramaly-CoA, which, in turn, serves as a substrate for malyl-CoA lyase and produces pyruvate and acetyl-CoA. Acetyl-CoA is carboxylated to malonyl-CoA, which is then reduced and further activated into the starter compound of the cycle: propionyl-CoA. Pyruvate, on the other hand, can be further converted to phosphoglycerate and re-enter the CBC (Shih et al., 2014), resulting in a net gain in carbon.

Both studies implemented their proposed cycles in cyanobacteria. Regardless of the CO_2 fixing ability of 3OHP bypass, strains carrying the cycle did not present a conclusive phenotype. However, the authors set an example for future *in vivo* implementation of carbon-capturing photorespiratory bypasses. On the other hand, strains containing the MCG pathway showed increased bicarbonate assimilation and acetyl-CoA accumulation and achieved higher optical densities than strains lacking MCG.

The aforementioned cycles represent what has been described in literature as "mix and match" synthetic pathways because they described novel pathways relying on known reactions and enzymes (Erb et al., 2017). Nevertheless, a recent study has identified several new-to-nature pathways by systematically developing reaction sequences from the pool of feasible biochemical transformations that could convert (phospho)glycolate back into a central intermediate of the CBC cycle (Trudeau et al., 2018). The design of these solutions was guided by two additional principles. First, the new reaction sequences should require as little energy as possible, and second, these pathways should be CO_2 neutral (i.e., not release CO_2) or even capture CO_2.

Through these efforts, the authors proposed four "carbon-neutral pathways" to reincorporate the C2 compound product of the oxygenation reaction of Rubisco into the CBC (Figure 3, pathways 3–6). These pathways rely on a novel reduction of glycolate to glycolaldehyde via engineered enzymes. Taking advantage of the high reactivity of glycolaldehyde, it is further combined with sugar phosphates present on the CBC, such as glyceraldehyde 3-phosphate (GAP), dihydroxyacetone phosphate, fructose 6-phosphate, or sedoheptulose 7-phosphate, via aldolase, transketolase, or transaldolase reactions. In all cases, a C5 compound is produced and further converted into the substrate of Rubisco. In the case of transketolase and transaldolase reactions, side products are formed that are also part of the CBC and, therefore, can be reused directly. Trudeau et al. (2018) proved the *in vitro* feasibility of one of their proposed carbon neutral bypasses; however, to date, no further improvement or *in vivo* implementation has been reported.

Furthermore, in the same study, another photorespiration bypass comprising novel reactions was proposed, the tartonyl-CoA (TaCo) pathway (Figure 3, pathway 7). The TaCo pathway is a five-reaction sequence that first converts photorespiratory glycolate into glycolyl-CoA, which is subsequently carboxylated into tartronyl-CoA, the namesake compound of the pathway. In two subsequent steps, tartronyl-CoA is then reduced to glycerate, which can re-enter the CBC at the level of phosphoglycerate. Compared with natural photorespiration, the TaCo pathway sequence is about 50% shorter, requires about 20% less ATP and 30% less reducing power, and does not release ammonia or, notably, CO_2 but instead captures additional CO_2 during photorespiration. In other words, through the TaCo pathway, photorespiration can be turned into a carbon-capturing process in which the oxygenation reaction of Rubisco will still lead to subsequent fixation of carbon.

Although this pathway outcompetes natural photorespiration, a challenge has been that the central TaCo pathway sequence relies on enzyme reactions that have not been described so far. This

apparent challenge was addressed through re-engineering the active sites of enzyme candidates that catalyze similar reactions to establish and/or improve the desired reactions (Scheffen et al., 2021). The full pathway was reconstituted and prototyped *in vitro*.

However, this proof of principle is only the starting point for further developments to successfully realize the TaCo pathway in the context of the living plant. This could be achieved through a combination of complementary approaches. The *in vitro* prototyping efforts could be expanded by combining high-throughput combinatorics with machine-learning-guided experimentation to optimize the enzyme stoichiometry and robustness of the pathway (Pandi et al., 2022; Vögeli et al., 2022). The optimized network could then be (partially) transplanted in a suitable (micro)organism to test its feasibility and use adaptive laboratory evolution to further improve the network and its cellular integration. The latter strategy has been recently used successfully to establish the CBC, the reverse glycine cleavage pathway, or a modified serine cycle in *E. coli* (Antonovsky et al., 2016; Yishai et al., 2017; Gleizer et al., 2019; Luo et al., 2022), in which selection strains were designed, which need to form (part) of their biomass through the new pathways. Having established a functional cycle inside of a microorganisms will guide further efforts to integrate this new reaction sequence in plants.

CONCLUDING REMARKS AND PERSPECTIVES

Sustainably increasing crop yields is crucial to meet the growing demands of a rising global population for food, feed, and other plant-derived products. Maximizing photosynthetic efficiency is hence an important factor for food security in the context of anthropogenic climate change and resource limitations. In the quest to increase crop productivity through improvement of photosynthetic efficiency, bypasses to canonical photorespiration and modifications of excess light protection have been designed, implemented in several model species and in crops, and tested in the field. Jointly, these data provide a proof of concept for yield increases through engineering of photorespiration and light harvesting.

While progress has been made, there are still unanswered questions that need to be addressed, essential to fully gather the potential improvements achievable from these approaches. For example, how do photorespiratory bypasses actually work? What are the mechanisms that underpin the observed yield increases? Resolving questions such as these will require additional experiments and comprehensive analysis by mathematical models of plant metabolism.

Available data suggest that combining photorespiratory bypasses with optimized energy dissipation holds promise to maximize the energy available for CO_2 assimilation. New-to-nature pathways show potential to exceed the yield improvements that have been achieved by current photorespiratory bypasses. To date, these pathways have been prototyped *in vitro* and in bacteria, and they are awaiting testing in plants.

Furthermore, natural variation in photosynthetic efficiency exists in domesticated crops and their wild relatives and so provides a valuable resource for breeding strategies. Techniques such as intra- and interspecific hybridization and genome editing could leverage promising alleles identified through pan-genomic association mapping and systems biology approaches to introduce beneficial genetic variants into crops. Knowledge of the natural variation in photosynthetic parameters should be combined with synthetic photorespiratory bypasses for synergistic effects. For example, alternative forms of Rubisco with higher V_c at the cost of lower V_c/V_o could be combined with more energy-efficient or CO_2-concentrating photorespiratory bypasses, resulting in additive benefits. Also, it should be possible to combine more performant Rubisco variants with better sourcing of CO_2 via optimization of g_m. The CO_2 compensation point serves as a key parameter when screening for photosynthetic efficiency; multiple methods have been employed for its determination, but its precision relies on accurate estimation of cellular CO_2 efflux and influx.

The efficacy of increasing photosynthetic efficiency in crops in the field has been clearly demonstrated, but further improvements, or reliable transfer of traits from model organisms to crop species or between crop species, will likely require additional modifications of sink tissues or harvestable biomass to reap the full benefits of increased photosynthetic efficiency. Only when sink strength can keep up with source capacity will it be possible to capitalize on gains in efficiency.

The practicality of implementing different strategies to improve photosynthetic efficiency that range from selective breeding to new-to-nature pathways depends on government regulation of genetic modification, which must be also considered when applying scientific discoveries to solve real-world problems.

SUPPLEMENTAL INFORMATION

Supplemental information is available at *Molecular Plant Online*.

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REFERENCES

Abadie, C., and Tcherkez, G. (2019). Plant sulphur metabolism is stimulated by photorespiration. Commun. Biol. **2**:379.

Abbasi, A.Z., Bilal, M., Khurshid, G., Yiotis, C., Zeb, I., Hussain, J., Baig, A., Shah, M.M., Chaudhary, S.U., Osborne, B., and Ahmad, R. (2021). Expression of cyanobacterial genes enhanced CO2 assimilation and biomass production in transgenic *Arabidopsis thaliana*. PeerJ **9**, e11860.

Alboresi, A., Storti, M., and Morosinotto, T. (2019). Balancing protection and efficiency in the regulation of photosynthetic electron transport across plant evolution. New Phytol. **221**:105–109.

Antonovsky, N., Gleizer, S., Noor, E., Zohar, Y., Herz, E., Barenholz, U., Zelcbuch, L., Amram, S., Wides, A., Tepper, N., et al. (2016). Sugar Synthesis from CO_2 in *Escherichia coli*. Cell **166**:115–125.

Aubry, S., Kelly, S., Kümpers, B.M.C., Smith-Unna, R.D., and Hibberd, J.M. (2014). Deep Evolutionary Comparison of Gene Expression Identifies Parallel Recruitment of Trans-Factors in Two Independent Origins of C_4 Photosynthesis. PLoS Genet. **10**, e1004365.

Bar-Even, A., Noor, E., Lewis, N.E., and Milo, R. (2010). Design and analysis of synthetic carbon fixation pathways. Proc. Natl. Acad. Sci. USA **107**:8889–8894.

Basler, G., Küken, A., Fernie, A.R., and Nikoloski, Z. (2016). Photorespiratory Bypasses Lead to Increased Growth in Arabidopsis thaliana: Are Predictions Consistent with Experimental Evidence? Front. Bioeng. Biotechnol. **4**:31.

Bauwe, H. (2023). Photorespiration – Rubisco's repair crew. J. Plant Physiol. **280**, 153899.

Bauwe, H., Hagemann, M., and Fernie, A.R. (2010). Photorespiration: players, partners and origin. Trends Plant Sci. **15**:330–336.

Bernacchi, C.J., Bagley, J.E., Serbin, S.P., Ruiz-Vera, U.M., Rosenthal, D.M., and Vanloocke, A. (2013). Modelling C_3 photosynthesis from the chloroplast to the ecosystem. Plant Cell Environ. **36**:1641–1657.

Berrío, R.T., Verstaen, K., Vandamme, N., Pevernagie, J., Achon, I., Van Duyse, J., Van Isterdael, G., Saeys, Y., De Veylder, L., Inzé, D., and Dubois, M. (2022). Single-cell transcriptomics sheds light on the identity and metabolism of developing leaf cells. Plant Physiol. **188**:898–918.

Bezrutczyk, M., Zöllner, N.R., Kruse, C.P.S., Hartwig, T., Lautwein, T., Köhrer, K., Frommer, W.B., and Kim, J.-Y. (2021). Evidence for phloem loading via the abaxial bundle sheath cells in maize leaves. Plant Cell **33**:531–547.

Bilal, M., Abbasi, A.Z., Khurshid, G., Yiotis, C., Hussain, J., Shah, M.M., Naqvi, T., Kwon, S.-Y., Park, Y.-I., Osborne, B., and Ahmad, R. (2019). The expression of cyanobacterial glycolate–decarboxylation pathway genes improves biomass accumulation in *Arabidopsis thaliana*. Plant Biotechnol. Rep. **13**:361–373.

Bouvier, J.W., Emms, D.M., Rhodes, T., Bolton, J.S., Brasnett, A., Eddershaw, A., Nielsen, J.R., Unitt, A., Whitney, S.M., and Kelly, S. (2021). Rubisco Adaptation Is More Limited by Phylogenetic Constraint Than by Catalytic Trade-off. Mol. Biol. Evol. **38**:2880–2896.

Bowes, G., Ogren, W.L., and Hageman, R.H. (1971). Phosphoglycolate production catalyzed by ribulose diphosphate carboxylase. Biochem. Biophys. Res. Commun. **45**:716–722.

Broncano, L.S., Pukacz, K.R., Reichel-Deland, V., Schlüter, U., Triesch, S., and Weber, A.P. (2023). Photorespiration is the solution, not the problem. J. Plant Physiol. **282**, 153928.

Busch, F.A. (2020). Photorespiration in the context of Rubisco biochemistry, CO_2 diffusion and metabolism. Plant J. **101**:919–939.

Busch, F.A., Sage, T.L., Cousins, A.B., and Sage, R.F. (2013). C_3 plants enhance rates of photosynthesis by reassimilating photorespired and respired CO_2. Plant Cell Environ. **36**:200–212.

Cai, Y.-F., Li, S.-F., Li, S.-F., Xie, W.-J., and Song, J. (2014). How do leaf anatomies and photosynthesis of three Rhododendron species relate to their natural environments? Bot. Stud. **55**:36.

Campbell, W.J., and Ogren, W.L. (1990). A novel role for light in the activation of ribulosebisphosphate carboxylase/oxygenase. Plant Physiol. **92**:110–115.

Cannell, R.Q., Brun, W.A., and Moss, D.N. (1969). A Search for High Net Photosynthetic Rate among Soybean Genotypes1. Crop Sci. **9**:840–841.

Carvalho, J.d.F.C., Madgwick, P.J., Powers, S.J., Keys, A.J., Lea, P.J., and Parry, M.A.J. (2011). An engineered pathway for glyoxylate metabolism in tobacco plants aimed to avoid the release of ammonia in photorespiration. BMC Biotechnol. **11**:111.

Clarke, V.C., De Rosa, A., Massey, B., George, A.M., Evans, J.R., von Caemmerer, S., and Groszmann, M. (2022). Mesophyll conductance is unaffected by expression of Arabidopsis PIP1 aquaporins in the plasmalemma of Nicotiana. J. Exp. Bot. **73**:3625–3636.

Collins, C., and Didelot, X. (2018). A phylogenetic method to perform genome-wide association studies in microbes that accounts for population structure and recombination. PLoS Comput. Biol. **14**, e1005958.

Cuperus, J.T. (2021). Single-cell genomics in plants: current state, future directions, and hurdles to overcome. Plant Physiol. **188**:749–755.

Cutolo, E.A., Guardini, Z., Dall'Osto, L., and Bassi, R. (2023). A paler shade of green: engineering cellular chlorophyll content to enhance photosynthesis in crowded environments. New Phytol. **239**:1567–1583. https://doi.org/10.1111/nph.19064.

Dalal, J., Lopez, H., Vasani, N.B., Hu, Z., Swift, J.E., Yalamanchili, R., Dvora, M., Lin, X., Xie, D., Qu, R., and Sederoff, H.W. (2015). A photorespiratory bypass increases plant growth and seed yield in biofuel crop *Camelina sativa*. Biotechnol. Biofuels **8**:175.

Driever, S.M., Lawson, T., Andralojc, P.J., Raines, C.A., and Parry, M.A.J. (2014). Natural variation in photosynthetic capacity, growth, and yield in 64 field-grown wheat genotypes. J. Exp. Bot. **65**:4959–4973.

Eisenhut, M., Ruth, W., Haimovich, M., Bauwe, H., Kaplan, A., and Hagemann, M. (2008). The photorespiratory glycolate metabolism is essential for cyanobacteria and might have been conveyed endosymbiontically to plants. Proc. Natl. Acad. Sci. USA **105**:17199–17204.

Eisenhut, M., Roell, M.S., and Weber, A.P.M. (2019). Mechanistic understanding of photorespiration paves the way to a new green revolution. New Phytol. **223**:1762–1769.

Elferjani, R., Benomar, L., Momayyezi, M., Tognetti, R., Niinemets, Ü., Soolanayakanahally, R.Y., Théroux-Rancourt, G., Tosens, T., Ripullone, F., Bilodeau-Gauthier, S., et al. (2021). A meta-analysis of mesophyll conductance to CO_2 in relation to major abiotic stresses in poplar species. J. Exp. Bot **72**:erab127.

Erb, T.J., Jones, P.R., and Bar-Even, A. (2017). Synthetic metabolism: metabolic engineering meets enzyme design. Curr. Opin. Chem. Biol. **37**:56–62.

Evans, J.R. (2021). Mesophyll conductance: walls, membranes and spatial complexity. New Phytol. **229**:1864–1876.

Evans, J., Caemmerer, S., Setchell, B., and Hudson, G. (1994). The relationship between CO_2 transfer conductance and leaf anatomy in transgenic tobacco with a reduced content of rubisco. Funct. Plant Biol. **21**:475–495.

Fernie, A.R., and Bauwe, H. (2020). Wasteful, essential, evolutionary stepping stone? The multiple personalities of the photorespiratory pathway. Plant J. **102**:666–677.

Flexas, J., Barbour, M.M., Brendel, O., Cabrera, H.M., Carriquí, M., Díaz-Espejo, A., Douthe, C., Dreyer, E., Ferrio, J.P., Gago, J., et al. (2012). Mesophyll diffusion conductance to CO_2: An unappreciated central player in photosynthesis. Plant Sci. **193–194**:70–84.

Flexas, J., Niinemets, U., Gallé, A., Barbour, M.M., Centritto, M., Diaz-Espejo, A., Douthe, C., Galmés, J., Ribas-Carbo, M., Rodriguez, P.L., et al. (2013). Diffusional conductances to CO_2 as a target for increasing photosynthesis and photosynthetic water-use efficiency. Photosynth. Res. **117**:45–59.

Flexas, J., and Carriquí, M. (2020). Photosynthesis and photosynthetic efficiencies along the terrestrial plant's phylogeny: lessons for improving crop photosynthesis. Plant J. **101**:964–978.

Flügel, F., Timm, S., Arrivault, S., Florian, A., Stitt, M., Fernie, A.R., and Bauwe, H. (2017). The Photorespiratory Metabolite 2-Phosphoglycolate Regulates Photosynthesis and Starch Accumulation in Arabidopsis. Plant Cell **29**:2537–2551.

Fu, X., Gregory, L.M., Weise, S.E., and Walker, B.J. (2023a). Integrated flux and pool size analysis in plant central metabolism reveals unique roles of glycine and serine during photorespiration. Nat. Plants **9**:169–178.

Fu, X., Smith, K., Gregory, L., Roze, L., and Walker, B. (2023b). Understanding and improving crop photosynthesis. Burleigh Dodds Ser. Agric. Sci. 203–222. https://doi.org/10.19103/as.2022.0119.12.

Gago, J., Carriquí, M., Nadal, M., Clemente-Moreno, M.J., Coopman, R.E., Fernie, A.R., and Flexas, J. (2019). Photosynthesis Optimized across Land Plant Phylogeny. Trends Plant Sci. **24**:947–958.

Galmés, J., Capó-Bauçà, S., Niinemets, Ü., and Iñiguez, C. (2019). Potential improvement of photosynthetic CO_2 assimilation in crops by exploiting the natural variation in the temperature response of Rubisco catalytic traits. Curr. Opin. Plant Biol. **49**:60–67.

Galmés, J., Kapralov, M.V., Andralojc, P.J., Andralojc, P.J., Conesa, M.À., Keys, A.J., Parry, M.A., and Flexas, J. (2014). Expanding knowledge of the Rubisco kinetics variability in plant species: environmental and evolutionary trends. Plant Cell Environ **37**:1989–2001.

Garcia-Molina, A., and Leister, D. (2020). Accelerated relaxation of photoprotection impairs biomass accumulation in Arabidopsis. Nat. Plants **6**:9–12.

Gleizer, S., Ben-Nissan, R., Bar-On, Y.M., Antonovsky, N., Noor, E., Zohar, Y., Jona, G., Krieger, E., Shamshoum, M., Bar-Even, A., and Milo, R. (2019). Conversion of *Escherichia coli* to Generate All Biomass Carbon from CO_2. Cell **179**:1255–1263.e12.

Gu, J., Yin, X., Stomph, T.-J., Wang, H., and Struik, P.C. (2012). Physiological basis of genetic variation in leaf photosynthesis among rice (*Oryza sativa* L.) introgression lines under drought and well-watered conditions. J. Exp. Bot. **63**:5137–5153.

Guillotin, B., Rahni, R., Passalacqua, M., Mohammed, M.A., Xu, X., Raju, S.K., Ramírez, C.O., Jackson, D., Groen, S.C., Gillis, J., and Birnbaum, K.D. (2023). A pan-grass transcriptome reveals patterns of cellular divergence in crops. Nature **617**:785–791.

Hanawa, H., Ishizaki, K., Nohira, K., Takagi, D., Shimakawa, G., Sejima, T., Shaku, K., Makino, A., and Miyake, C. (2017). Land plants drive photorespiration as higher electron-sink: Comparative study of post-illumination transient O_2-uptake rates from liverworts to angiosperms through ferns and gymnosperms. Physiol. Plantarum **161**:138–149.

Hartman, F.C., and Harpel, M.R. (1994). Structure, Function, Regulation, and Assembly of D-Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase. Annu. Rev. Biochem. **63**:197–234.

Heber, U., and Krause, G.H. (1980). What is the physiological role of photorespiration? Trends Biochem. Sci. **5**:32–34.

Heber, U., Bligny, R., Streb, P., and Douce, R. (1996). Photorespiration is Essential for the Protection of the Photosynthetic Apparatus of C_3 Plants Against Photoinactivation Under Sunlight. Bot. Acta **109**:307–315.

Hodges, M., Dellero, Y., Keech, O., Betti, M., Raghavendra, A.S., Sage, R., Zhu, X.-G., Allen, D.K., and Weber, A.P.M. (2016). Perspectives for a better understanding of the metabolic integration of photorespiration within a complex plant primary metabolism network. J. Exp. Bot. **67**:3015–3026.

Hua, L., Stevenson, S.R., Reyna-Llorens, I., Xiong, H., Kopriva, S., and Hibberd, J.M. (2021). The bundle sheath of rice is conditioned to play an active role in water transport as well as sulfur assimilation and jasmonic acid synthesis. Plant J. **107**:268–286.

Iqbal, W.A., Lisitsa, A., and Kapralov, M.V. (2023). Predicting plant Rubisco kinetics from RbcL sequence data using machine learning. J. Exp. Bot. **74**:638–650.

John, C.R., Smith-Unna, R.D., Woodfield, H., Covshoff, S., and Hibberd, J.M. (2014). Evolutionary Convergence of Cell-Specific Gene Expression in Independent Lineages of C_4 Grasses. Plant Physiol. **165**:62–75.

Jordan, D.B., and Ogren, W.L. (1981). Species variation in the specificity of ribulose biphosphate carboxylase/oxygenase. Nature **291**:513–515.

Kebeish, R., Niessen, M., Thiruveedhi, K., Bari, R., Hirsch, H.-J., Rosenkranz, R., Stäbler, N., Schönfeld, B., Kreuzaler, F., and Peterhänsel, C. (2007). Chloroplastic photorespiratory bypass increases photosynthesis and biomass production in *Arabidopsis thaliana*. Nat. Biotechnol. **25**:593–599.

Khurshid, G., Abbassi, A.Z., Khalid, M.F., Gondal, M.N., Naqvi, T.A., Shah, M.M., Chaudhary, S.U., and Ahmad, R. (2020). A cyanobacterial photorespiratory bypass model to enhance photosynthesis by rerouting photorespiratory pathway in C_3 plants. Sci. Rep. **10**, 20879.

Kim, J.-Y., Symeonidi, E., Pang, T.Y., Denyer, T., Weidauer, D., Bezrutczyk, M., Miras, M., Zöllner, N., Hartwig, T., Wudick, M.M., et al. (2021). Distinct identities of leaf phloem cells revealed by single cell transcriptomics. Plant Cell **33**:511–530.

Knauer, J., Zaehle, S., De Kauwe, M.G., Haverd, V., Reichstein, M., and Sun, Y. (2020). Mesophyll conductance in land surface models: effects on photosynthesis and transpiration. Plant J. **101**:858–873.

Knauer, J., Cuntz, M., Evans, J.R., Niinemets, Ü., Tosens, T., Veromann-Jürgenson, L.L., Werner, C., and Zaehle, S. (2022). Contrasting anatomical and biochemical controls on mesophyll conductance across plant functional types. New Phytol. **236**:357–368.

Koteyeva, N.K., Voznesenskaya, E.V., Roalson, E.H., and Edwards, G.E. (2011). Diversity in forms of *C4* in the genus Cleome (Cleomaceae). Ann. Bot. **107**:269–283.

Kozaki, A., and Takeba, G. (1996). Photorespiration protects C_3 plants from photooxidation. Nature **384**:557–560.

Kromdijk, J., and McCormick, A.J. (2022). Genetic variation in photosynthesis: many variants make light work. J. Exp. Bot. **73**:3053–3056.

Kromdijk, J., Głowacka, K., Leonelli, L., Gabilly, S.T., Iwai, M., Niyogi, K.K., and Long, S.P. (2016). Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. Science **354**:857–861.

Kümpers, B.M.C., Burgess, S.J., Reyna-Llorens, I., Smith-Unna, R., Boursnell, C., and Hibberd, J.M. (2017). Shared characteristics underpinning C_4 leaf maturation derived from analysis of multiple C_3 and C_4 species of Flaveria. J. Exp. Bot. **68**:177–189.

Laisk, A., Oja, V., Rasulov, B., Rämma, H., Eichelmann, H., Kasparova, I., Pettai, H., Padu, E., and Vapaavuori, E. (2002). A computer-operated routine of gas exchange and optical measurements to diagnose photosynthetic apparatus in leaves: Diagnosing photosynthesis. Plant Cell Environ. **25**:923–943.

Lanigan, G.J., Betson, N., Griffiths, H., and Seibt, U. (2008). Carbon Isotope Fractionation during Photorespiration and Carboxylation in Senecio. Plant Physiol. **148**:2013–2020.

Levey, M., Timm, S., Mettler-Altmann, T., Luca Borghi, G., Koczor, M., Arrivault, S., Pm Weber, A., Bauwe, H., Gowik, U., and Westhoff, P. (2019). Efficient 2-phosphoglycolate degradation is required to maintain carbon assimilation and allocation in the C_4 plant. J. Exp. Bot. **70**:575–587.

Li, Z., Wakao, S., Fischer, B.B., and Niyogi, K.K. (2009). Sensing and Responding to Excess Light. Annu. Rev. Plant Biol. **60**:239–260.

Long, S.P., Taylor, S.H., Burgess, S.J., Carmo-Silva, E., Lawson, T., De Souza, A.P., Leonelli, L., and Wang, Y. (2022). Into the Shadows and Back into Sunlight: Photosynthesis in Fluctuating Light. Annu. Rev. Plant Biol. **73**:617–648.

Lopez-Anido, C.B., Vatén, A., Smoot, N.K., Sharma, N., Guo, V., Gong, Y., Anleu Gil, M.X., Weimer, A.K., and Bergmann, D.C. (2021). Single-cell resolution of lineage trajectories in the Arabidopsis stomatal lineage and developing leaf. Dev. Cell **56**:1043–1055.e4.

Luo, S., Lin, P.P., Nieh, L.-Y., Liao, G.-B., Tang, P.-W., Chen, C., and Liao, J.C. (2022). A cell-free self-replenishing CO_2-fixing system. Nat. Catal. **5**:154–162.

Maier, A., Fahnenstich, H., von Caemmerer, S., Engqvist, M.K.M., Weber, A.P.M., Flügge, U.I., and Maurino, V.G. (2012). Transgenic Introduction of a Glycolate Oxidative Cycle into *A. thaliana* Chloroplasts Leads to Growth Improvement. Front. Plant Sci. **3**:38.

Marshall, D.M., Muhaidat, R., Brown, N.J., Liu, Z., Stanley, S., Griffiths, H., Sage, R.F., and Hibberd, J.M. (2007). Cleome, a genus closely related to Arabidopsis, contains species spanning a developmental progression from C_3 to C_4 photosynthesis. Plant J. **51**:886–896.

McKown, A.D., and Dengler, N.G. (2007). Key innovations in the evolution of Kranz anatomy and C_4 vein pattern in Flaveria (Asteraceae). Am. J. Bot. **94**:382–399.

McLean, R., Schwander, T., Diehl, C., Cortina, N.S., Paczia, N., Zarzycki, J., and Erb, T.J. (2023). Exploring alternative pathways for the in vitro establishment of the HOPAC cycle for synthetic CO_2 fixation. Sci. Adv. **9**, eadh4299.

Menz, K.M., Moss, D.N., Cannell, R.Q., and Brun, W.A. (1969). Screening for Photosynthetic Efficiency1. Crop Sci. **9**:692–694.

Niinemets, Ü. (2023). Variation in leaf photosynthetic capacity within plant canopies: optimization, structural and physiological constraints and inefficiencies. Photosynth. Res. 1–19.

Nobel, P.S. (1991). Achievable productivities of certain CAM plants: basis for high values compared with C_3 and C_4 plants. New Phytol. **119**:183–205.

Nölke, G., Houdelet, M., Kreuzaler, F., Peterhänsel, C., and Schillberg, S. (2014). The expression of a recombinant glycolate dehydrogenase polyprotein in potato (Solanum tuberosum) plastids strongly enhances photosynthesis and tuber yield. Plant Biotechnol. J. **12**:734–742.

Oliver, D.J. (1980). The effect of glyoxylate on photosynthesis and photorespiration by isolated soybean mesophyll cells. Plant Physiol. **65**:888–892.

Oliver, D.J., and Zelitch, I. (1977). Increasing photosynthesis by inhibiting photorespiration with glyoxylate. Science **196**:1450–1451.

Onoda, Y., Wright, I.J., Evans, J.R., Hikosaka, K., Kitajima, K., Niinemets, Ü., Poorter, H., Tosens, T., and Westoby, M. (2017). Physiological and structural tradeoffs underlying the leaf economics spectrum. New Phytol. **214**:1447–1463.

Osmanoglu, Ö., Khaled AlSeiari, M., AlKhoori, H.A., Shams, S., Bencurova, E., Dandekar, T., and Naseem, M. (2021). Topological Analysis of the Carbon-Concentrating CETCH Cycle and a Photorespiratory Bypass Reveals Boosted CO_2-Sequestration by Plants. Front. Bioeng. Biotechnol. **9**, 708417.

Pandi, A., Diehl, C., Yazdizadeh Kharrazi, A., Scholz, S.A., Bobkova, E., Faure, L., Nattermann, M., Adam, D., Chapin, N., Foroughijabbari, Y., et al. (2022). A versatile active learning workflow for optimization of genetic and metabolic networks. Nat. Commun. **13**:3876.

Parry, M.A.J., Keys, A.J., and Gutteridge, S. (1989). Variation in the Specificity Factor of C_3 Higher Plant Rubiscos Determined by the Total Consumption of Ribulose-P 2. J. Exp. Bot. **40**:317–320.

Perez, R.K., Gordon, M.G., Subramaniam, M., Kim, M.C., Hartoularos, G.C., Targ, S., Sun, Y., Ogorodnikov, A., Bueno, R., Lu, A., et al. (2022). Single-cell RNA-seq reveals cell type–specific molecular and genetic associations to lupus. Science **376**, eabf1970.

Perin, G., Bellan, A., Michelberger, T., Lyska, D., Wakao, S., Niyogi, K.K., and Morosinotto, T. (2023). Modulation of xanthophyll cycle impacts biomass productivity in the marine microalga Nannochloropsis. Proc. Natl. Acad. Sci. USA **120**, e2214119120.

Peterhansel, C., Blume, C., and Offermann, S. (2013). Photorespiratory bypasses: how can they work? J. Exp. Bot. **64**:709–715.

Pick, T.R., Bräutigam, A., Schulz, M.A., Obata, T., Fernie, A.R., and Weber, A.P.M. (2013). PLGG1, a plastidic glycolate glycerate transporter, is required for photorespiration and defines a unique class of metabolite transporters. Proc. Natl. Acad. Sci. USA **110**:3185–3190.

Procko, C., Lee, T., Borsuk, A., Bargmann, B.O.R., Dabi, T., Nery, J.R., Estelle, M., Baird, L., O'Connor, C., Brodersen, C., et al. (2022). Leaf cell-specific and single-cell transcriptional profiling reveals a role for the palisade layer in UV light protection. Plant Cell **34**:3261–3279.

Roell, M.-S., Schada von Borzykowski, L., Westhoff, P., Plett, A., Paczia, N., Claus, P., Urte, S., Erb, T.J., and Weber, A.P.M. (2021). A synthetic C_4 shuttle via the β-hydroxyaspartate cycle in C_3 plants. Proc. Natl. Acad. Sci. USA **118**, e2022307118.

Rotasperti, L., Tadini, L., Chiara, M., Crosatti, C., Guerra, D., Tagliani, A., Forlani, S., Ezquer, I., Horner, D.S., Jahns, P., et al. (2022). The barley mutant *happy under the sun 1* (*hus1*): An additional contribution to pale green crops. Environ. Exp. Bot. **196**, 104795.

Saadat, N.P., Nies, T., van Aalst, M., Hank, B., Demirtas, B., Ebenhöh, O., and Matuszyńska, A. (2021). Computational Analysis of Alternative Photosynthetic Electron Flows Linked With Oxidative Stress. Front. Plant Sci. **12**, 750580.

Sage, R.F. (2002). Variation in the k_{cat} of Rubisco in C_3 and C_4 plants and some implications for photosynthetic performance at high and low temperature. J. Exp. Bot. **53**:609–620.

Samuilov, S., Brilhaus, D., Rademacher, N., Flachbart, S., Arab, L., Alfarraj, S., Kuhnert, F., Kopriva, S., Weber, A.P.M., Mettler-Altmann, T., and Rennenberg, H. (2018). The Photorespiratory BOU Gene Mutation Alters Sulfur Assimilation and Its Crosstalk With Carbon and Nitrogen Metabolism in *Arabidopsis thaliana*. Front. Plant Sci. **9**:1709.

Scheffen, M., Marchal, D.G., Beneyton, T., Schuller, S.K., Klose, M., Diehl, C., Lehmann, J., Pfister, P., Carrillo, M., He, H., et al. (2021). A new-to-nature carboxylation module to improve natural and synthetic CO_2 fixation. Nat. Catal. **4**:105–115.

Schlüter, U., Bräutigam, A., Gowik, U., Melzer, M., Christin, P.-A., Kurz, S., Mettler-Altmann, T., and Weber, A.P. (2017). Photosynthesis in C_3–C_4 intermediate Moricandia species. J. Exp. Bot. **68**:191–206.

Schlüter, U., Bouvier, J.W., Guerreiro, R., Malisic, M., Kontny, C., Westhoff, P., Stich, B., and Weber, A.P.M. (2023). Brassicaceae display diverse photorespiratory carbon recapturing mechanisms. J. Exp. Bot. erad250. https://doi.org/10.1093/jxb/erad250.

Schwander, T., Schada von Borzyskowski, L., Burgener, S., Cortina, N.S., and Erb, T.J. (2016). A synthetic pathway for the fixation of carbon dioxide in vitro. Science **354**:900–904.

Seyfferth, C., Renema, J., Wendrich, J.R., Eekhout, T., Seurinck, R., Vandamme, N., Blob, B., Saeys, Y., Helariutta, Y., Birnbaum, K.D., et al. (2021). Advances and Opportunities of Single-Cell Transcriptomics for Plant Research. Annu. Rev. Plant Biol. **72**:1–20.

Shen, B.-R., Wang, L.-M., Lin, X.-L., Yao, Z., Xu, H.-W., Zhu, C.-H., Teng, H.-Y., Cui, L.-L., Liu, E.E., Zhang, J.-J., et al. (2019). Engineering a New Chloroplastic Photorespiratory Bypass to Increase Photosynthetic Efficiency and Productivity in Rice. Mol. Plant **12**:199–214.

Shih, P.M., Zarzycki, J., Niyogi, K.K., and Kerfeld, C.A. (2014). Introduction of a synthetic CO_2-fixing photorespiratory bypass into a cyanobacterium. J. Biol. Chem. **289**:9493–9500.

South, P.F., Cavanagh, A.P., Liu, H.W., and Ort, D.R. (2019). Synthetic glycolate metabolism pathways stimulate crop growth and productivity in the field. Science **363**, eaat9077.

De Souza, A.P., Burgess, S.J., Doran, L., Hansen, J., Manukyan, L., Maryn, N., Gotarkar, D., Leonelli, L., Niyogi, K.K., and Long, S.P. (2022). Soybean photosynthesis and crop yield are improved by accelerating recovery from photoprotection. Science **377**:851–854.

Sun, G., Xia, M., Li, J., Ma, W., Li, Q., Xie, J., Bai, S., Fang, S., Sun, T., Feng, X., et al. (2022). The maize single-nucleus transcriptome comprehensively describes signaling networks governing movement and development of grass stomata. Plant Cell **34**:1890–1911.

Tcherkez, G., and Farquhar, G.D. (2021). Rubisco catalytic adaptation is mostly driven by photosynthetic conditions – Not by phylogenetic constraints. J. Plant Physiol. **267**, 153554.

Timm, S., Woitschach, F., Heise, C., Hagemann, M., and Bauwe, H. (2019). Faster Removal of 2-Phosphoglycolate through Photorespiration Improves Abiotic Stress Tolerance of Arabidopsis. Plants **8**:563.

Tomeo, N.J., and Rosenthal, D.M. (2018). Photorespiration differs among *Arabidopsis thaliana* ecotypes and is correlated with photosynthesis. J. Exp. Bot. **69**:5191–5204.

Tosens, T., Niinemets, Ü., Westoby, M., and Wright, I.J. (2012). Anatomical basis of variation in mesophyll resistance in eastern Australian sclerophylls: news of a long and winding path. J. Exp. Bot. **63**:5105–5119.

Trudeau, D.L., Edlich-Muth, C., Zarzycki, J., Scheffen, M., Goldsmith, M., Khersonsky, O., Avizemer, Z., Fleishman, S.J., Cotton, C.A.R., Erb, T.J., et al. (2018). Design and in vitro realization of carbon-conserving photorespiration. Proc. Natl. Acad. Sci. USA **115**:E11455–E11464.

Ubierna, N., Cernusak, L.A., Holloway-Phillips, M., Busch, F.A., Cousins, A.B., and Farquhar, G.D. (2019). Critical review: incorporating the arrangement of mitochondria and chloroplasts into models of photosynthesis and carbon isotope discrimination. Photosynth. Res. **141**:5–31.

Uemura, K., Suzuki, Y., Shikanai, T., Wadano, A., Jensen, R.G., Chmara, W., and Yokota, A. (1996). A Rapid and Sensitive Method for Determination of Relative Specificity of RuBisCO from Various Species by Anion-Exchange Chromatography. Plant Cell Physiol. **37**:325–331.

Vögeli, B., Schulz, L., Garg, S., Tarasava, K., Clomburg, J.M., Lee, S.H., Gonnot, A., Moully, E.H., Kimmel, B.R., Tran, L., et al. (2022). Cell-free prototyping enables implementation of optimized reverse β-oxidation pathways in heterotrophic and autotrophic bacteria. Nat. Commun. **13**:3058.

Walker, B.J., Vanloocke, A., Bernacchi, C.J., and Ort, D.R. (2016a). The Costs of Photorespiration to Food Production Now and in the Future. Annu. Rev. Plant Biol. **67**:107–129.

Walker, B.J., Skabelund, D.C., Busch, F.A., and Ort, D.R. (2016b). An improved approach for measuring the impact of multiple CO_2 conductances on the apparent photorespiratory CO_2 compensation point through slope–intercept regression. Plant Cell Environ. **39**:1198–1203.

Wang, L.-M., Shen, B.-R., Li, B.-D., Zhang, C.-L., Lin, M., Tong, P.-P., Cui, L.-L., Zhang, Z.-S., and Peng, X.-X. (2020a). A Synthetic Photorespiratory Shortcut Enhances Photosynthesis to Boost Biomass and Grain Yield in Rice. Mol. Plant **13**:1802–1815.

Walker, B.J., Drewry, D.T., Slattery, R.A., VanLoocke, A., Cho, Y.B., and Ort, D.R. (2017). Chlorophyll Can Be Reduced in Crop Canopies with Little Penalty to Photosynthesis. Plant Physiol. **176**:1215–1232.

Wang, X., Wang, Y., Ling, A., Guo, Z., Asim, M., Song, F., Wang, Q., Sun, Y., Khan, R., Yan, H., and Shi, Y. (2020b). Rationale: Photosynthesis of Vascular Plants in Dim Light. Front. Plant Sci. **11**, 573881.

Wei, S., Li, X., Lu, Z., Zhang, H., Ye, X., Zhou, Y., Li, J., Yan, Y., Pei, H., Duan, F., et al. (2022). A transcriptional regulator that boosts grain yields and shortens the growth duration of rice. Science **377**, eabi8455.

Wu, A. (2023). Modelling plants across scales of biological organisation for guiding crop improvement. Funct. Plant Biol. **50**:435–454.

Wurtzel, E.T., Vickers, C.E., Hanson, A.D., Millar, A.H., Cooper, M., Voss-Fels, K.P., Nikel, P.I., and Erb, T.J. (2019). Revolutionizing agriculture with synthetic biology. Nat. Plants **5**:1207–1210.

Xin, C.-P., Tholen, D., Devloo, V., and Zhu, X.-G. (2015). The benefits of photorespiratory bypasses: how can they work? Plant Physiol. **167**:574–585.

Xiong, H., Hua, L., Reyna-Llorens, I., Shi, Y., Chen, K.-M., Smirnoff, N., Kromdijk, J., and Hibberd, J.M. (2021). Photosynthesis-independent production of reactive oxygen species in the rice bundle sheath during high light is mediated by NADPH oxidase. Proc. Natl. Acad. Sci. USA **118**, e2022702118.

Yishai, O., Goldbach, L., Tenenboim, H., Lindner, S.N., and Bar-Even, A. (2017). Engineered Assimilation of Exogenous and Endogenous Formate in *Escherichia coli*. ACS Synth. Biol. **6**:1722–1731.

Yu, H., Li, X., Duchoud, F., Chuang, D.S., and Liao, J.C. (2018). Augmenting the Calvin-Benson-Bassham cycle by a synthetic malyl-CoA-glycerate carbon fixation pathway. Nat. Commun. **9**:2008.

Zarzycki, J., Brecht, V., Müller, M., and Fuchs, G. (2009). Identifying the missing steps of the autotrophic 3-hydroxypropionate CO2 fixation cycle in *Chloroflexus aurantiacus*. Proc. Natl. Acad. Sci. USA **106**:21317–21322.

Zelitch, I., Schultes, N.P., Peterson, R.B., Brown, P., and Brutnell, T.P. (2009). High glycolate oxidase activity is required for survival of maize in normal air. Plant Physiol. **149**:195–204.

Zhang, X., Madi, S., Borsuk, L., Nettleton, D., Elshire, R.J., Buckner, B., Janick-Buckner, D., Beck, J., Timmermans, M., Schnable, P.S., and Scanlon, M.J. (2007). Laser Microdissection of Narrow Sheath Mutant Maize Uncovers Novel Gene Expression in the Shoot Apical Meristem. PLoS Genet. **3**, e101.

Zhang, Z., Lu, Y., Zhai, L., Deng, R., Jiang, J., Li, Y., He, Z., and Peng, X. (2012). Glycolate Oxidase Isozymes Are Coordinately Controlled by GLO1 and GLO4 in Rice. PLoS One **7**, e39658.

Zhang, C., Zhong, X., Lin, D., Wu, K., Wu, Z., Zhang, Z., and Peng, X. (2022). Grain Quality Affected by Introducing Photorespiratory Bypasses into Rice. Agronomy **12**:566.

Zhu, X.-G., Long, S.P., and Ort, D.R. (2010). Improving photosynthetic efficiency for greater yield. Annu. Rev. Plant Biol. **61**:235–261.