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Optimizing photorespiration for improved crop productivity[FA]

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Abstract In C3 plants, photorespiration is an energy-expensive process, including the oxygenation of ribulose-1,5-bisphosphate (RuBP) by ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) and the ensuing multi-organellar photorespiratory pathway required to recycle the toxic byproducts and recapture a portion of the fixed carbon. Photorespiration significantly impacts crop productivity through reducing yields in C3 crops by as much as 50% under severe conditions. Thus, reducing the flux through, or improving the efficiency of photorespiration has the potential of large improvements in C3 crop productivity. Here, we review an array of approaches intended to engineer photorespiration in a range of plant systems with the goal of increasing crop productivity. Approaches include optimizing flux through the native photorespiratory pathway, installing non-native alternative photorespiratory pathways, and lowering or even eliminating Rubisco-catalyzed oxygenation of RuBP to reduce substrate entrance into the photorespiratory cycle. Some proposed designs have been successful at the proof of concept level. A plant systems-engineering approach, based on new opportunities available from synthetic biology to implement *in silico* designs, holds promise for further progress toward delivering more productive crops to farmer's fields.

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INTRODUCTION

Global agricultural demand is rapidly increasing as the global human population climbs towards 9 billion by mid-century with increasing affluence (UN Population Division 2017). It is projected that agricultural output will need to increase 70% to 100% to meet this demand (Tilman et al. 2011; Ray et al. 2013), even while available arable land is stagnant or even decreasing. The Green Revolution resulted in a more-than doubling of global crop production, through selective breeding and increased fertilizer inputs, while improving both yield potential and resilience to environm ental and biotic stresses. Improved photosynthetic efficiency played little role in yield potential improvement, during the Green Revolution, whereas those traits that did are now near their maximum efficiency (Zhu et al. 2010; Ray et al. 2013; Ort et al. 2015). While there is a suite of improvements to crop plants and cropping systems that will be needed to meet the challenge of doubling production, improving yield potential must play a central role for which improving photosynthetic efficiency must be the central focus.

The primary carboxylase of the C3 photosynthetic cycle is ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), which generates two molecules of 3-phosphoglycerate (3-PGA) by catalyzing the addition of CO_2 to the five-carbon acceptor, ribulose-1,5-bisphosphate (RuBP). A major inefficiency of the C3 cycle occurs when Rubisco catalyzes oxygenation of RuBP, which results in the generation of one molecule of 3-PGA and one molecule of 2-phosphoglycolate (2-PG) (Bowes et al. 1971; Ogren and Bowes 1971; Somerville and Ogren 1979a; Lorimer 1981). The 2-PG is toxic to plants, as accumulation can lead to reduction in RuBP regeneration by limiting the function of phosphofructokinase and triose phosphate isomerase (Kelly and Latzko 1976; Artus et al. 1986; Gonzalez Moro et al. 1997). The photorespiratory carbon oxidative pathway both prevents the accumulation of 2-PG as well as recovers a portion of previously fixed carbon in 2-PG (Somerville and Ogren 1981; Ogren 1984; Artus et al. 1986; Peterhansel et al. 2010).

The first step in the photorespiratory pathway is the dephosphorylation of 2-PG by 2-phosphoglycolate phosphatase to produce glycolate (Somerville and Ogren 1979b). Glycolate is transported out of the chloroplast by a plastidic glycolate glycerate transporter (PLGG1) and a bile acid sodium symporter (BASS6) (Figure 1) (Pick et al. 2013; Walker et al. 2016a; South et al. 2017). Glycolate then undergoes a multi-step conversion to glycine in the peroxisome, after which it moves to the mitochondria. In the mitochondria, glycine decarboxylation and conversion to serine produces ammonia (NH_3) and releases CO_2. Serine generated in the mitochondria moves to the peroxisome where it is converted to glycerate. Finally, PLGG1 transports glycerate into the chloroplast (Pick et al. 2013; South et al. 2017) where it is phosphorylated and reenters the C3 cycle (Figure 1).

The photorespiratory pathway recovers 75% of the fixed carbon lost due to oxygenation, with the remaining 25% released as CO_2 in the mitochondria (Bauwe and Kolukisaoglu 2003; Peterhansel et al. 2010). Whereas C3 plants can function well without this recovery pathway, so long as Rubisco oxygenase activity is fully repressed (e.g., very high [CO_2] or very low [O_2]), it is required for survival under all normal air conditions and is energetically quite costly to the plant. Each turn of the photorespiratory cycle requires the equivalent of 12.25 ATP (calculated in [Peterhansel et al. 2010]), which is largely due to the energy demands of re-fixing the released CO_2 and re-assimilating NH_3.

The oxygenation of RuBP increases with increasing temperature due to decreases in Rubisco specificity and, under drought conditions, when internal CO_2

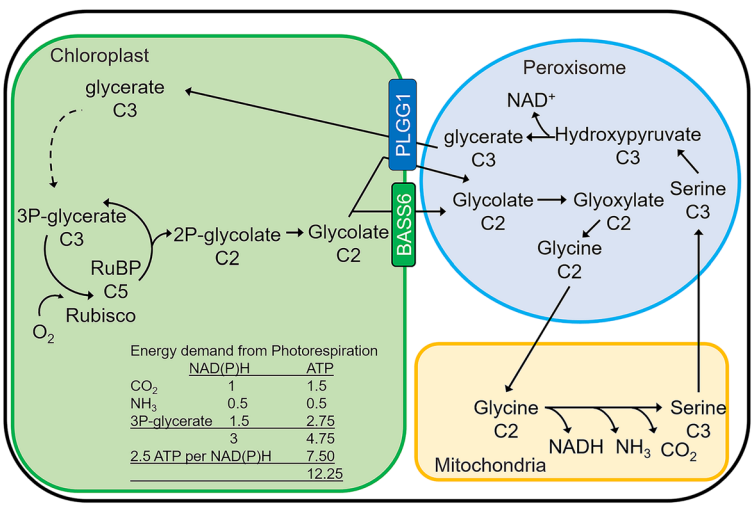

Figure 1. Photorespiration in plants
Photorespiration is a multi-organellar process in photosynthetic cells involving the chloroplast (green), peroxisome (blue), mitochondria (yellow), and cytosol (white). Known transporters between organelles are depicted. For every two oxygenation reactions catalyzed by Rubisco in the chloroplast, one molecule of glycerate is generated in the peroxisome and transported to the chloroplast for reintroduction into the C3 cycle and one carbon is released as CO_2 in the mitochondria. Number of carbons per molecule are indicated. Energy demand of photorespiration depicted in reducing equivalents (NAD(P)H) and ATP reviewed in Peterhansel et al. (2010).

concentration is lowered due to declining stomatal conductance (Ku et al. 1977; Jordan and Ogren 1984; Brooks and Farquhar 1985; Sharkey 1988; Zhu et al. 2008). During such periods of high temperatures or severe drought as much as 50% of the ATP produced through photosynthesis may be used for photorespiration (Peterhansel et al. 2013; Walker et al. 2016b). The high energetic cost of photorespiration represents a significant reduction in yield potential of C3 crops (Walker et al. 2016b) explaining the decades-long effort into reducing it.

CURRENT APPROACHES TO OPTIMIZING PHOTORESPIRATION

Three main approaches have been taken to lower the cost of photorespiration with the goal of increasing plant productivity. The first is to reduce oxygenation of RuBP by increasing the efficiency of Rubisco through either genetic manipulation of the enzyme, or by concentrating CO_2 around Rubisco (Raines 2006). The second is to manipulate the native photorespiratory pathway through gene mutation or overexpression to increase the rate of toxic byproduct recycling and carbon recovery (Peterhansel et al. 2013b). Lastly, the third approach is to install non-native alternative metabolic pathways to reduce the energetic cost of photorespiration (Figure 2) (Peterhansel and Maurino 2011; Maurino and Weber 2013).

Alternate Rubiscos

Rubisco has long been a target of genetic manipulation, with the goal of improving its selectivity and kinetic performance (Somerville and Ogren 1982; Zhu et al. 2004; Mueller-Cajar and Whitney 2008; Whitney and Sharwood 2008). However, attempts to engineer a better enzyme have so far been unsuccessful. Most Form I Rubiscos (those found in land plants, green algae, and cyanobacteria) appear to exhibit a trade-off between catalytic turnover rate (speed) and substrate specificity. Thus, attempts to reduce the Rubisco oxygenase activity, through enhanced specificity for CO_2, have impaired CO_2 reactivity at the catalytic site (Tcherkez et al. 2006; Savir et al. 2010; Camille et al. 2018).

This trend is not observed in Form I Rubisco from diatoms, which contain a carbon concentrating mechanism and sustain near-C3 levels of enzyme specificity and carboxylation turnover rates, in addition to much slower rates of oxygenation (Young et al. 2016). This highlights the need to eliminate sampling bias towards crop plants and model species and survey Rubisco kinetic data from diverse sources to identify alternative evolutionary pathways to lower oxygenase activity (Orr et al. 2016; Prins et al. 2016). Implementing a non-native Rubisco, such as a high-specificity red algal or cyanobacterial version, into crop plants could offer a greater benefit than enhancing native Rubisco kinetics alone, particularly when coupled with a carbon-concentrating mechanism (Zhu et al. 2004; Lin et al. 2014b). However, recent unsuccessful attempts to replace tobacco Rubisco with large and small red algal Rubisco highlight the importance of co-expression of compatible chaperones in the successful assembly of foreign Rubisco in plants (Lin and Hanson 2018).

Recently, a reconstituted Rubisco holoenzyme was assembled in a bacterial host (Aigner et al. 2017). Coupled with recent insights in Rubisco species-specific structure-function relationships (Valegård et al. 2018a, 2018b), and assembly requirements (Saschenbrecker et al. 2007; Feiz et al. 2012; Whitney et al. 2015), this provides a much-needed technological breakthrough in our ability to screen Rubisco variants (Saschenbrecker et al. 2007; Feiz et al. 2012; Whitney et al. 2015; Valegård et al. 2018a, 2018b).

Concentrating carbon near Rubisco

In addition to modifying Rubisco directly, other approaches aim to decrease oxygenation reactions by concentrating CO_2 within the chloroplast (Rae et al. 2017). One strategy to increase the concentration of CO_2 around Rubisco uses non-plant carbon concentrating mechanisms (CCMs) (Figure 2). CCMs have evolved in cyanobacteria and algae, and the components needed for a functional CCM include carboxysome or pyrenoid structures around Rubisco, carbonic anhydrase, along with inorganic carbon transporters (Morita et al. 1998; Kinney et al. 2011; Sinetova et al. 2012; Niederhuber et al. 2017; Sharwood 2017; Sommer et al. 2017).

Carboxysomes are microcompartments within the chloroplast of oxygenic photosynthetic bacteria that are made of a protein shell, which contains carbonic anhydrase and Rubisco proteins (Rae et al. 2013a, 2013b; Sommer et al. 2017). Similar in function, pyrenoids present in many algae and the hornwort group of land plants act as a subcellular microcompartment CCM. Unlike carboxysomes, pyrenoids are surrounded by a starch sheath and protein layer (Sharwood 2017). To

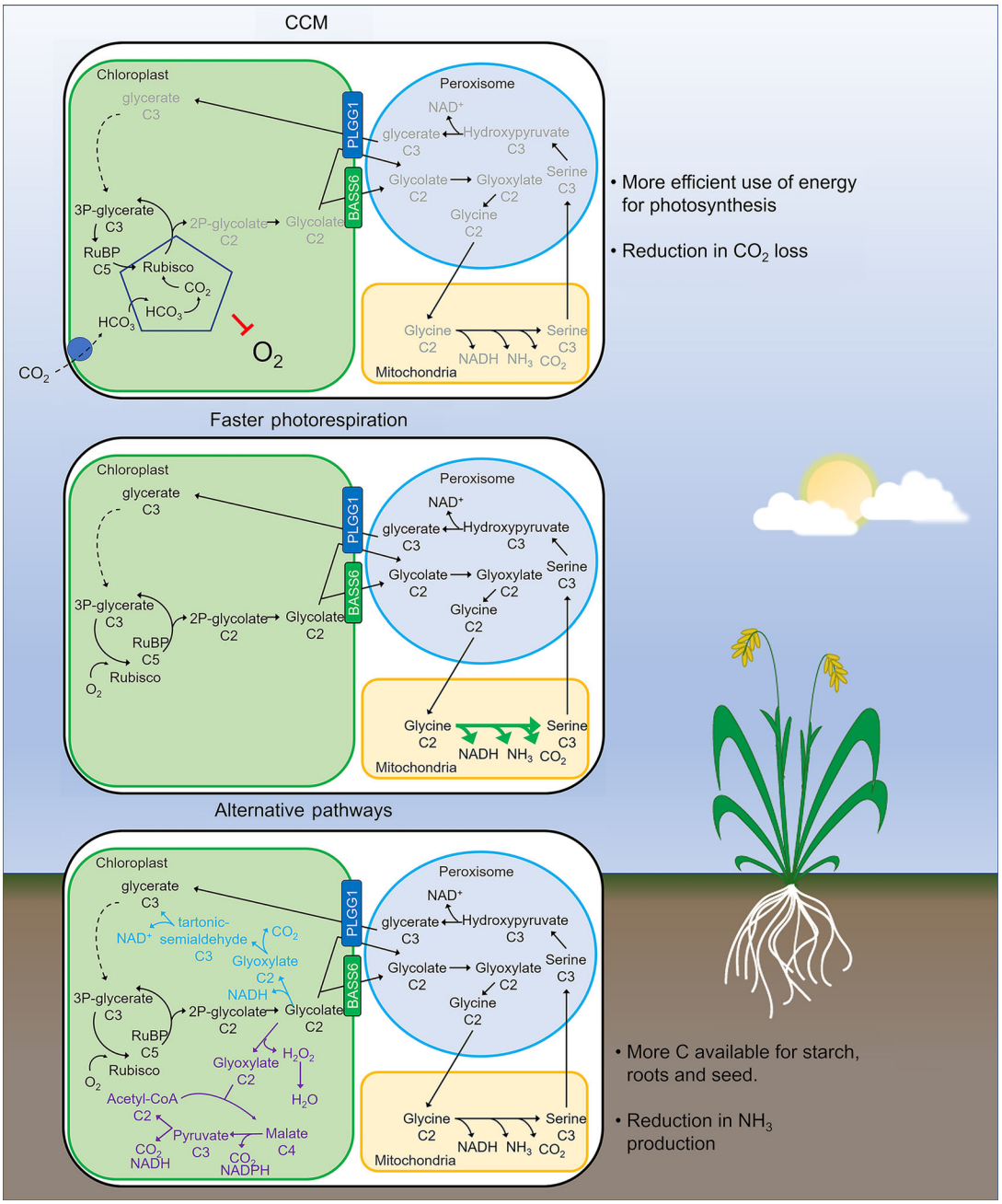

Figure 2. Current approaches to optimizing photorespiration

Three models depicting current efforts to optimize photorespiration in C3 crops. Alternative pathways. Non-native genes are used to more efficiently process glycolate either back to glycerate similar to native photorespiration or, by fully decarboxylating glycolate to CO_2 to be re-fixed by Rubisco. Carbon concentrating mechanisms (CCM). The installation of pyrenoid or carboxysome structures and the expression of bicarbonate transporters to prevent Rubisco oxygenation and enrich CO_2 at the Rubisco active site. Faster photorespiration. Increased expression of native genes in the photorespiration pathway facilitate the faster rate of conversion of glycolate to glycerate, preventing the accumulation of toxic intermediates. Approaches to optimizing photorespiration could lead to more efficient energy use, reduction in CO_2 loss and more ATP and carbon available for plant growth.

date, some of these structures have been introduced into plants representing promising initial steps toward transplanting a functional CCM (Hanson et al. 2016; Occhialini et al. 2016).

The β-carboxysome proteins have been introduced into the chloroplasts of tobacco plants where higher-order structures have been shown to self-assemble (Lin et al. 2014a). In addition to the structure of the CCM microcompartment, Rubisco must be incorporated into the microcompartment to realize a fully functional structure, and this is complicated by the structural requirements needed for Rubisco recruitment. In pyrenoid formation, Rubisco recruitment is mediated through α-helices contained on the small subunit of the Form 1 Rubisco, that are predicted to interact with the linking protein present in the pyrenoid (Meyer et al. 2012; Mackinder et al. 2016). Engineering native small-subunits through direct replacement of the two surface α-helices from *Chlamydomonas reinhardtii* results in Rubiscos that are catalytically competent and represent an ideal background to test candidates for new recruitment and linker proteins as they emerge (Atkinson et al. 2017).

To date, fully assembled carboxysomes and pyrenoid assembly in plant chloroplasts has remained elusive. Further work needs to be completed to identify the minimum number of genes responsible for assembly and proper targeting of Rubisco into the CCM microcompartments. Further understanding the CCM structure, assembly, and function could have significant implications in increasing crop productivity (Sharwood 2017). Alternatively, previous work suggests that the introduction of bicarbonate transporters from CCMs would, alone, have a net benefit to photosynthesis. Modelling suggests that installation of the cyanobacterial bicarbonate transporter, BicA could increase light-saturated photosynthesis by 9% and using all known bicarbonate transporters could increase rates of photosynthesis by 16% (McGrath and Long 2014). Recent attempts at integrating individual components of algal CCM include the expression of the carbonic anhydrase, CAH3 in the thylakoid lumen and the bicarbonate transporter, LCIA in the chloroplast inner membrane of tobacco generating individual lines with enhanced CO_2 uptake, increased photosynthetic efficiency and higher biomass levels (Nolke et al. 2018).

An alternative method to increase CO_2 concentration at Rubisco is to introduce C4 photosynthesis into C3 crops. C4 photosynthesis has independently evolved from C3 photosynthesis over 60 times (Sage 2004; Sage et al. 2011; Sage et al. 2012; Furbank 2017) and is thought to be an adaption to higher photorespiratory pressures (Sage et al. 2012). Most C4 plant species are located in the grasslands of tropical and subtropical regions around the world (Schluter and Weber 2016; Furbank 2017).

The CO_2 concentration near Rubisco occurs in C4 plants by dividing photosynthesis activities between the mesophyll and bundle sheath cells. In mesophyll cells, CO_2 is first converted into four-carbon malate by the non-oxygen sensitive PEP carboxylase. This four-carbon dicarboxylic acid is then actively transported into the bundle sheath (Furbank 2017) where it is decarboxylated, increasing the CO_2 concentration near Rubisco. This C4 "CO_2 pump" requires two additional ATPs for every mole of CO_2 fixed. In addition, introduction of C4 photosynthesis into C3 plants requires directed changes in both the biochemistry of photosynthesis and leaf structure with increased photosynthetically active bundle sheath cells, although single cell C4 photosynthesis and C3-C4 intermediates could also be sources of engineering strategies (Matsuoka et al. 2001; Schuler et al. 2016). Currently, there has been some success in engineering C4 photosynthesis into rice through the C4Rice project, but further investigation into how C4 photosynthesis evolves and the regulatory elements needed to significantly convert C3 photosynthesis to C4 is needed to fully realize the benefits in crops (https://c4rice.com).

ACCELERATING FLUX THROUGH NATIVE PHOTORESPIRATORY PATHWAY

Discovery of the enzymatic steps involved in the photorespiratory pathway was largely driven by mutational studies in *Arabidopsis*. T-DNA lines were identified primarily by their requirement of a high CO_2 environment for growth. Most of the lines with insertions in genes encoding key enzymes involved in the photorespiratory pathway demonstrated lethality, or poor growth phenotypes under ambient air conditions, but could be rescued with elevated CO_2 concentrations (Somerville and Ogren 1979b; Hall et al. 1987; Murray et al. 1989; Boldt et al. 2005; Schwarte and Bauwe 2007; Timm et al. 2008; Timm et al. 2012b; Pick et al. 2013; South et al. 2017).

Although this mutational approach efficiently deciphered the photorespiratory pathway, it did not reveal

strategies to optimize the pathway for improved growth. Furthermore, a comprehensive study analyzing data from 40 years of field trials, in soybean and wheat, showed that cultivars with increased photosynthetic rates also had higher rates of photorespiration, suggesting that using natural variation in photorespiration to identify plants with lower levels of photorespiration and higher productivity would likely not be successful (Aliyev 2012). Yet, studies of natural variation in photorespiration, in tobacco, described the selection of plants with low photorespiration, which also exhibited higher rates of photosynthesis and growth. However, the effect appeared to be more related to higher levels of peroxisomal catalase than to reduced levels of photorespiration, and did not appear to stabilize in successive generations (Zelitch and Day 1973; Zelitch 1989, 1992).

Some C3 plants, including rice and wheat, appear able to trap and re-assimilate photorespired CO_2 (Sage and Sage 2009; Busch et al. 2013), suggesting that plants can use anatomical adaptation strategies to mitigate the loss of CO_2 to the atmosphere without concurrent reductions in rates of photorespiration. Hence, these might be relevant traits which could be harnessed for development of higher yielding crops.

An alternative approach to reduce photorespiratory yield drag on crop productivity has focused on increasing the rate of photorespiratory pathway enzymes, and is showing promising results (Figure 2). The notion here being that increasing the flux through the photorespiratory pathway would minimize the accumulation and toxic effects of 2-PG and glycolate in the chloroplast, while also accelerating the rate of carbon recapture and return of PGA to the C3 cycle, thereby boosting the rate of RuBP regeneration.

Increased expression of two of the components of the mitochondrial glycine decarboxylase complex, the L-protein and the H-protein, separately, result in increased photosynthesis and plant growth, potentially due to increased flux through the photorespiratory pathway (Timm et al. 2012a; Timm et al. 2015; Simkin et al. 2017; Lopez-Calcagno et al. 2018). In addition, overexpressing the H-protein in tobacco reduced damage to photosystem II when plants were exposed to high photorespiratory stress conditions (Lopez-Calcagno et al. 2018). That these plants may be able to cope better with the high photorespiratory stress experienced in agricultural settings due to enhanced photorespiratory pathway flux would explain the 26%–47% increase in biomass observed in these overexpressors in the field (Lopez-Calcagno et al. 2018).

Therefore, genetic engineering of the native photorespiratory pathway, in combination with anatomical modifications to increase recovery of photorespired CO_2 and manipulation of other areas of metabolism closely associated with photorespiration, could be important strategies when developing crops able to sustain increased yields to meet the predicted future food demands (Betti et al. 2016; Timm et al. 2016; Lopez-Calcagno et al. 2018).

ALTERNATIVE PHOTORESPIRATORY PATHWAYS

As an alternative to decreasing Rubisco oxygenation, or increasing efficiency of the native photorespiratory pathway, there have been several efforts to re-engineer the photorespiratory pathway using non-native genes and alternative metabolic pathways. One strategy uses the *E. coli* glyoxylate oxidation pathway, which is intended to convert photorespiratory glycolate to glycerate entirely within the chloroplast, thereby reducing energy demand by using less ATP, avoiding the production of NH_3 and releasing photorespired CO_2 within the chloroplast in close proximity to Rubisco (Figure 2) (Kebeish et al. 2007; Nolke et al. 2014; Dalal et al. 2015).

The *E. coli* pathway converts glycolate to glyoxylate using the three-subunit glycolate dehydrogenase. Glyoxylate is then converted to tartonic semi-aldehyde, by glyoxylate carboligase (GCL), which is then converted to glycerate by tartonic semi-aldehyde reductase (Figure 2). Of the published alternative photorespiratory pathways, this *E. coli* pathway has been tested most extensively and has reported increases in photosynthesis and biomass in several species, including *Arabidopsis*, potato and camelina (Kebeish et al. 2007; Nolke et al. 2014; Dalal et al. 2015). However, expression of the entire pathway appears not to be required to observe improvements in plant performance. Expression of glycolate dehydrogenase, alone, has been observed to increase growth, revealing that more work needs to be done to fully understand the biochemical changes occurring in the leaf (Kebeish et al. 2007; Nolke et al. 2014; Dalal et al. 2015).

A related pathway has been attempted in tobacco, where expression of GCL and hydroxypyruvate isomerase, in the peroxisome, was predicted to convert glyoxylate to glycerate thus bypassing the mitochondria (Figure 2). However, hydroxypyruvate isomerase was not successfully installed in the peroxisome and these plants did not show a growth benefit (Carvalho et al. 2011). In addition, an alternative pathway not yet tested in plants involves recycling glycolate without releasing CO_2, such as through the 3-hydroxypropionate pathway, which converts glycolate to pyruvate in some bacteria (Shih et al. 2014). More work will need to be done to determine if either of these alternative pathways to photorespiration could result in increases in photosynthetic efficiency.

Another non-native photorespiratory pathway tested in plants uses the glycolate oxidase pathway intended to fully decarboxylate glycolate within the chloroplast (Figure 2). This glycolate oxidase pathway requires expression of the glycolate oxidase normally expressed in the peroxisome, malate synthase to convert glyoxylate to malate, and a catalase enzyme because the conversion of glycolate to glyoxylate, by glycolate oxidase, generates hydrogen peroxide as a byproduct. In addition to confining all steps of glycolate metabolism to the chloroplast, this alternative pathway would theoretically increase the CO_2 concentration around Rubisco, thereby decreasing oxygenation reactions, which could result in increased biomass (Maier et al. 2012).

Indeed, expression of the glycolate oxidase pathway in *Arabidopsis* (Maier et al. 2012) led to increased growth. However, this alternative pathway is expected to expend more energy compared to the native photorespiratory pathway (Xin et al. 2015) and fails to return any P-glycerate to the photosynthetic carbon reduction cycle, suggesting some alternative metabolism not yet understood is at play (Maier et al. 2012; Peterhansel et al. 2013).

To better assess how these alternative photorespiratory pathways could lead to an increase in crop production, an engineering approach may be necessary. With current rapid cloning techniques, such as Gibson assembly and Golden Gate cloning (Engler et al. 2009; Gibson et al. 2009), it is now possible to clone entire biochemical pathways into a single construct for single plant transformation. This could lead to multiple up-front designs to test variations of promoter gene combinations to optimize gene expression. In addition, multiple different enzymes could be tested in the same pathway. For example, the *E. coli* and the glycolate oxidase pathway both require the conversion of glycolate to glyoxylate by different means. The glycolate oxidase pathway may benefit from an enzyme that does not produce hydrogen peroxide as a byproduct, using an enzyme such as the algal glycolate dehydrogenase or a recently described glycolate dehydrogenase present in the mitochondria of diatoms that can use electron acceptors other than oxygen (Aboelmy and Peterhansel 2014; Schmitz et al. 2017). In addition, testing a variety of alternative pathway designs maximizing flux through the alternative pathways can be accomplished by shutting down the native photorespiratory pathway.

Modelling has suggested that an optimized *E. coli* pathway could increase photosynthetic efficiency by 16%, as long as all the glycolate produced enters the alternate path (Xin et al. 2015). Turning off native photorespiration could be accomplished by either targeted RNA interference or a gene editing approach. It is also imperative to begin testing how alternative pathways to photorespiration perform under agricultural conditions. With the goal of increasing crop productivity, field trials will need to be completed to provide a proof of concept that this should also work in crop species, similar to work on accelerating relaxation of photoprotection and speeding up photorespiration (Kromdijk et al. 2016; Lopez-Calcagno et al. 2018).

Altogether, the alternative pathways currently tested are only a small fraction of the possible metabolic pathways that could lead to improvements in photorespiration. Further, it has become clear that fine-tuning of gene expression, more active enzymes, and inducible systems could be used to fully optimize photorespiration under agricultural and environmental stress conditions.

FUTURE PROSPECTS IN ENGINEERING PHOTORESPIRATION

Traditional genetic engineering (*i.e.,* gene mutation and single gene transformations) has been used in most of the above-described approaches in manipulating photorespiration. Recent advances in genome engineering and synthetic biology (Liu and Stewart 2015; Patron

et al. 2015; Fuentes et al. 2016; Patron 2016) are now opening up new opportunities in altering photorespiration. With the power of synthetic biology, it is now possible to imagine a systems engineering approach to conceptualize, design, build, and test a multitude of ways to re-engineer photorespiratory metabolism, with the goal of crop improvement.

Initial algorithms for *in silico* design of the alternative photorespiratory pathways described above have been tested (Xin et al. 2015). Modelling of projected improvements suggested that the *E. coli* pathway could result in increased photosynthetic efficiency and biomass, by as much as 16%, especially if flux through the native pathway is reduced or eliminated (Xin et al. 2015). In addition, completely non-tested novel pathways can be evaluated based on stoichiometric and kinetic models of enzyme activity. Modelling manipulations to photorespiration also can provide unexpected results, such as how changes in photorespiration could affect nitrogen use, which is integral to the role of photorespiration in maintaining photosynthetic efficiency during NH_3 re-assimilation. Indeed, large scale computational modelling projects have been created to better design next generation crops (Zhu et al. 2016; Marshall-Colon et al. 2017; Busch et al. 2018).

For computer modelling to be usefully predictive *in planta*, more detailed characterizations of photorespiratory pathways are needed. Much of the work describing the function of photorespiratory enzymes comes from genetic mutation and *in vitro* enzymatic assays (Somerville and Ogren 1982; Ogren 1984; Bauwe et al. 2010; Peterhansel et al. 2010). Metabolic flux analysis, *in vivo*, has long been an aspirational goal toward a better understanding of photorespiratory metabolism, especially in agriculturally important crops and under field conditions (Rachmilevitch et al. 2004; Zhu et al. 2007; Timm et al. 2012a; Xin et al. 2015; Timm et al. 2016; Flugel et al. 2017).

With a deeper understanding of photorespiratory flux, it will be possible to better determine how manipulating photorespiration impacts other branches of central carbon metabolism and secondary pathways important for plant function. For example, photorespiration is a large contributor to serine production in C3 plants and the photorespiratory pathway has been implicated in abiotic and biotic stress responses, via a role in reactive oxygen species (ROS) signaling (Fernie et al. 2013; Timm and Bauwe 2013). In addition, decreased rates of photorespiration, facilitated by growth at elevated CO_2, have been reported to exhibit a negative feedback on nitrogen assimilation. Indeed, plants can increase their rates of photosynthetic CO_2 uptake when assimilating nitrogen, *de novo*, via the photorespiratory pathway by fixing carbon as amino acids in addition to carbohydrates (Bloom et al. 2018; Busch et al. 2018).

Deeper understanding of the role of photorespiratory intermediates in other metabolic pathways and determining how altered photorespiration will affect plant growth and yield under different growth environments is needed. Although photorespiration is involved in these aspects of plant metabolism, it is not clear if this is essential for plant function, or a result of the evolutionary pathway that led to the photorespiratory cycle in land plants (Hagemann et al. 2016). It is well known, for example, that under many conditions, C3 plants benefit from reduced, including full suppression, of Rubisco oxygenation and subsequent photorespiratory metabolism (Wheeler et al. 1996; Long et al. 2006).

Synthetic biology combines the principles of engineering with molecular biology to provide the ability to design and build biological parts. This ability to design and build is beginning to make it possible to standardize parts, similar to manufacturing principles, to quickly assemble a wide variety of designs to be tested in biological systems. The first set of standardized biological parts were BioBricks designed primarily for the engineering of prokaryotic organisms. Golden Gate and GoldenBraid, as well as other similar cloning techniques now provide standardized parts available for plant synthetic biology (Norville et al. 2010; Engler et al. 2014; Liu and Stewart 2015; Marillonnet and Werner 2015; Patron et al. 2015; Fuentes et al. 2016; Shih et al. 2016).

Limitations to engineering plants arises from the complexity of specialized metabolites, complex genomes, and high degrees of regulation that result in several unknowns in terms of predictability in manipulation as well as from low transformation efficiency in most crops of interest. Candidate gene discovery, promoter analysis, and regulatory functions of promoter elements are needed to optimize the design portion of plant synthetic biology. One benefit to the ease of design is the ability to generate a whole metabolic pathway on a single construct with individual promoter gene combinations.

The use of many different promoters could prevent homology-dependent gene silencing that can result from repeated use of a constitutive promoter, after multiple generations (Matzke and Matzke 1995; Meyer and Saedler 1996; Matzke et al. 2002). In addition, this upfront design could test a range of promoter strengths without *a priori* knowledge of expression level predictions using phenotype as the selectable pressure in optimized design. Once a desired phenotype is identified (*e.g.*, decreased photorespiration stress and increased plant growth), gene expression can be correlated with phenotype. Using engineering cycle principles and machine learning, the information acquired from multiple rounds of optimization could lead to phenotypes not necessarily achievable with only a single round of the design, build, test, and learn cycle.

With the ability to generate large data sets, model photorespiration *in silico*, and generate large libraries of standardized parts, a systems approach may be the best tool for realizing increased crop productivity through changes in photorespiration. The number of genome annotated crops is increasing at a rapid pace (Matasci et al. 2014). The increasing amount of genomic information becoming available will provide insight into the genetic diversity and potential plasticity of the native photorespiratory pathway. This information can then be used in engineering approaches. For example, the peroxisomal glyoxylate cycle could convert glyoxylate to malate, as opposed to glycine, as a photorespiratory intermediate, which is proposed from *in silico* analysis and select *in vivo* results (Davis et al. 2017). Different environmental pressures may have also induced evolutionary changes to photorespiration that may be elucidated by expanding genomic data and could drive changes in engineering strategies.

To fully understand how changes in photorespiration affect plant growth, source/sink relationships in engineered plants will also need to be examined. Potato plants expressing part of the *E. coli* glyoxylate pathway show increased tuber production (Nolke et al. 2014; Ahmad et al. 2016) but, would this design translate into increased production in other root crops, such as cassava, or seed crops, such as soybean or cowpea? It may be that optimization of alternatives to photorespiration will be accomplished only on a species-specific basis.

In conclusion, current efforts to optimize photorespiration have shown promising results. These concepts, and potentially better options that can be achieved through synthetic biology, will eventually need to be moved from model organisms to target crops and assessed under a range of relevant agricultural settings.

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AUTHOR CONTRIBUTIONS

P.F.S., A.P.C., P.E.L-C., C.A.R., and D.R.O. wrote and edited the manuscript.

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