The Company of **Biologists**

A Year at the Forefront of Engineering Photosynthesis

Sophie L. Johnson*

ABSTRACT

Multiple proof-of-principle experiments and successful field trials have demonstrated that engineering photosynthesis is a viable strategy for improving crop yields. Advances to engineering technologies have accelerated efforts to improve photosynthesis, generating a large volume of published literature: this Review therefore aims to highlight the most promising results from the period February 2021 to January 2022. Recent research has demonstrated the importance of understanding the impact of changing climates on photosynthesis to ensure that proposed engineering strategies are resilient to climate change. Encouragingly, there have been several reports of strategies that have benefits at temperatures higher than current ambient conditions. There has also been success in engineering synthetic bypass pathways, providing support for the feasibility of a synthetic biology approach. Continued developments in all areas of engineering photosynthesis will be necessary for sustainably securing sufficient crop yields for the future.

This article has an associated First Person interview with the first author of the paper.

KEY WORDS: Engineering photosynthesis, Synthetic biology

Introduction

The growing global population and increasing demand for bioenergy are predicted to result in future crop yields falling substantially behind demand without significant improvements to productivity (Ray et al., 2013). Future yield shortages will be further exacerbated by increasing temperatures resulting from climate change, with large losses for major crops such as maize, rice and soybean predicted (Jägermeyr et al., 2021). International efforts are therefore underway to improve crop yields, notably by targeting photosynthetic efficiency (Ort et al., 2015; Kubis and Bar-Even, 2019; Batista-Silva et al., 2020). Indeed, theoretical yield potential calculations have shown that improving photosynthesis is the only viable option to achieve the necessary yield improvements given the plateauing benefits of the Green Revolution (Murchie et al., 2009; Zhu et al., 2010; Long et al., 2015). Multiple strategies have been explored to this end, including targeting light capture efficiency, optimising photosynthetic enzymes, introducing a carbon-concentrating mechanism (CCM) or alternative form of photosynthesis to C3 species, optimising photorespiration, and engineering a smart canopy, with the aim of attaining sustainable crop improvements.

Targeting light capture is a major strategy for engineering photosynthesis. The light reactions of photosynthesis convert light to chemical energy through the fixation of atmospheric carbon dioxide: subsequent carboxylation of ribulose 1,5-bisphosphate (RuBP) by the enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) results in the generation of 3-phosphoglycerate (3PGA), which can be integrated into the Calvin–Benson–Bassham cycle and converted to organic sugars. Light capture depends on light-harvesting complexes arranged around reaction centres that form photosystems for the absorption of specific wavelengths. Light harvesting therefore makes partial use of the available solar spectrum. Moreover, only a fraction of the theoretical maximum of 12% solar energy conversion efficiency is achieved since plants are subjected to fluctuating light conditions and tend to absorb more light than can be productively used (Blankenship et al., 2011). In addition, although photoprotective mechanisms prevent damage to the photosystems at saturating light levels, slow downregulation of these processes can limit photosynthetic efficiency (Kromdijk et al., 2016). These limitations to photosynthesis could be overcome by (i) engineering pigments/light-harvesting complexes to access a wider range of the solar spectrum, for example as proposed by the PhotoRedesign consortium (Hitchcock et al., 2021), and (ii) improving the efficiency of protective mechanisms.

In addition, an alternative strategy is to target Rubisco itself. Rubisco is an inefficient enzyme with a low catalytic rate and low CO_2 affinity (Flamholz et al., 2019) that is poor at distinguishing CO_2 from O_2, particularly at elevated temperatures (Sage and Kubien, 2007). This promiscuity results in a competing oxygenation reaction at the Rubisco active site, generating toxic 2-phosphoglycolate (2PG) that must be recycled via the energetically costly photorespiratory pathway (Zhu et al., 2010; Walker et al., 2016; Busch, 2020). Further strategies to improve photosynthesis therefore include engineering of Rubisco to increase its CO_2 specificity, introducing a CCM, or engineering a novel synthetic photorespiratory bypass. For example, transgenic tobacco plants expressing the *E. coli* glycolate oxidation pathway had increased photosynthesis and biomass compared with wild type (South et al., 2019). Novel synthetic biology approaches have also been proposed (Bar-Even et al., 2010; Schwander et al., 2016; Miller et al., 2020), and their implementation is rapidly becoming feasible with improving technologies (e.g. Scheffen et al., 2021). This Review aims to highlight the most promising advances in engineering photosynthesis in the period February 2021 to January 2022 (Table 1).

A year at the forefront of engineering photosynthesis Discoveries

Engineering of photosynthesis relies on the development of molecular and synthetic biology approaches that are guided by detailed molecular knowledge. Advances in understanding the fundamental processes in different organisms therefore represent a significant contribution towards engineering photosynthesis. Progress has been made in both fundamental mechanistic understandings and technologies to implement engineering strategies in the period February 2021 to January 2022.

Department of Plant Sciences, University of Oxford, Oxford, OX1 3RB, UK.

*Author for correspondence (sophie.johnson@plants.ox.ac.uk)

S.L.J., 0000-0002-6219-6331

Table 1. Summary of key developments in engineering photosynthesis in the period February 2021 to January 2022

Experiment	Strategy	Major contribution	Limitations	Reference
Incorporation of CbbQ and CbbO into synthetic α-carboxysomes	CCM engineering	CbbQO improved carbon fixation	Unlikely to be a universal strategy; needs to be tested *in planta*	Chen et al. (2022)
$\Delta\beta$-*ca1ca5* tobacco lines	CCM engineering	Reduced stromal CA had no impact on photosynthetic efficiency	Transgenic plants had developmental defects	Hines et al. (2021)
AP3 lines at elevated temperatures	Photorespiratory bypass	AP3 shown to improve thermostability due to enhanced photorespiration	Needs to be tested in crop plants	Cavanagh et al. (2022)
BHAC expression in *Arabidopsis*	Photorespiratory bypass	BHAC lines generated OAA and have potential for improving carbon and nitrogen use efficiency	Transgenic plants had reduced growth and higher CO_2 compensation point	Roell et al. (2021)
ECGC pathway expression in rice	Photorespiratory bypass	Both full and partial ECGC pathway improved yield	Needs to be tested in field trials	Nayak et al. (2022)
In vitro construction of TaCo	Photorespiratory bypass	Engineering of a fully synthetic bypass pathway	Needs to be tested *in planta*	Scheffen et al. (2021)
ictB expression in maize	Single candidate expression	Improved yield in field trials	Yield variations seen in different growing seasons and location	Koester et al. (2021)
RBCS and *RCA* overexpression in rice	Rubisco engineering	Enhanced photosynthesis at elevated temperature	No improvements seen at ambient temperature	Suganami et al. (2021)
OsRBCS and *ZmRCA* overexpression in rice	Rubisco engineering	Enhanced photosynthesis at elevated temperature	No improvements seen at ambient temperature	Qu et al. (2021)
Measurement of Rubisco deactivation kinetics in cowpea	Rubisco engineering	Rubisco deactivation is faster than predicted, providing an engineering opportunity	Needs to be tested *in planta*	Taylor et al. (2022)

AP3, alternative pathway from South et al. (2019); BHAC, β-hydroxyaspartate cycle; CA, carbonic anhydrase; CbbO, Rubisco adaptor protein; CbbQ, AAA$^+$-type ATPase; CCM, carbon-concentrating mechanism; ECGC, *E. coli* glycolate catabolic; ictB, (previously annotated as) a possible bicarbonate transporter; OAA, oxaloacetate; *RBCS*, RIBULOSE BISPHOSPHATE CARBOXYLASE/OXYGENASE SMALL SUBUNIT; *RCA*, RUBISCO ACTIVASE; TACO, tartronic-CoA cycle.

Cyanobacterial CCM engineering

C_3 yields are predicted to be enhanced by up to 60% by introduction of a cyanobacterial CCM into chloroplasts (McGrath and Long, 2014; Yin and Struik, 2017). All cyanobacterial CO_2 fixation is enhanced by protein complexes called carboxysomes that consist of Rubisco, carbonic anhydrase (CA), a nucleating protein, and often Rubisco activase (Rca) (reviewed in Turmo et al., 2017; Hennacy and Jonikas, 2020). Inorganic HCO_3^- transporters facilitate CO_2 concentration within the cell, resulting in elevated CO_2 concentrations in the carboxysomes and improved Rubisco carbon
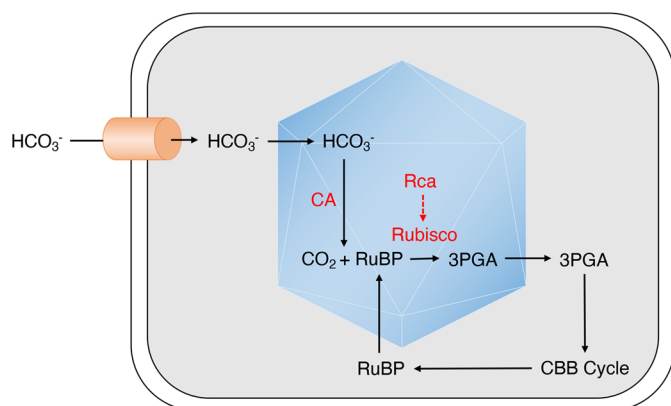

Fig. 1. Simplified cyanobacterial CMM. Hydrogen carbonate (HCO_3^-) transporters (orange) in the membrane of cyanobacteria import HCO_3^- that is concentrated in proteinaceous carboxysomes (blue). Rubisco catalyses the carboxylation of ribulose 1,5-bisphosphate, generating 3-phosphoglycerate, a Calvin–Benson–Bassham (CBB) cycle substrate. Some species require Rubisco activase to activate Rubisco (red dashed arrow). 3PGA, 3-phosphoglycerate; CA, carbonic anhydrase; Rca, Rubisco activase; Rubisco, ribulose 1,5-bisphosphate carboxylase/oxygenase; RuBP, ribulose 1,5-bisphosphate.

fixation (Fig. 1) (Price, 2011). Despite the recent reconstitution of a minimal carboxysome in flowering plants (Long et al., 2018), a functional plant CCM is yet to be demonstrated, suggesting that additional molecular components are required. It is possible that Rubisco requires chaperones for folding and activation in this context (Long et al., 2018). Indeed, a new discovery is that Rca components CbbQ and CbbO can increase overall carbon fixation when engineered into the α-carboxysomes of *Halothiobacillus neapolitanus* (Chen et al., 2022), although the dependence of carboxysome-localised Rubisco on activase activity is hypothesised to vary between organisms (Tsai et al., 2022). In addition to optimising Rubisco activity, functionalisation of an engineered chloroplast CCM will also require the elimination of native stromal CA to allow for the accumulation of HCO_3^- within carboxysomes (Dean Price et al., 2011). Hines et al. recently demonstrated the feasibility of this by knocking out the major stromal isoforms β-CA1 and β-CA5 in tobacco. Mutant plants exhibited no photosynthetic defects, instead showing developmental perturbations that included low germination rates, accumulation of necrotic lesions and early cessation of flowering (Hines et al., 2021). The authors hypothesised that this was the result of disrupted bicarbonate biosynthesis, the effects of which would be mitigated by the exogenous expression of bicarbonate transporter(s) in engineered CCMs (Hines et al., 2021). Generation of a fully functional C_3 CCM could therefore be a realistic prospect.

Synthetic biology approaches to optimising photorespiration

In the absence of a CCM, photosynthetic efficiency is limited by photorespiration (Fig. 2A), which has the potential to be improved for energy and carbon use efficiencies (Shen et al., 2019; South et al., 2019). Building on the success of the introduction of a synthetic alternative pathway (AP3) in tobacco to increase biomass (Fig. 2B) (South et al., 2019), Cavanagh et al. tested whether AP3
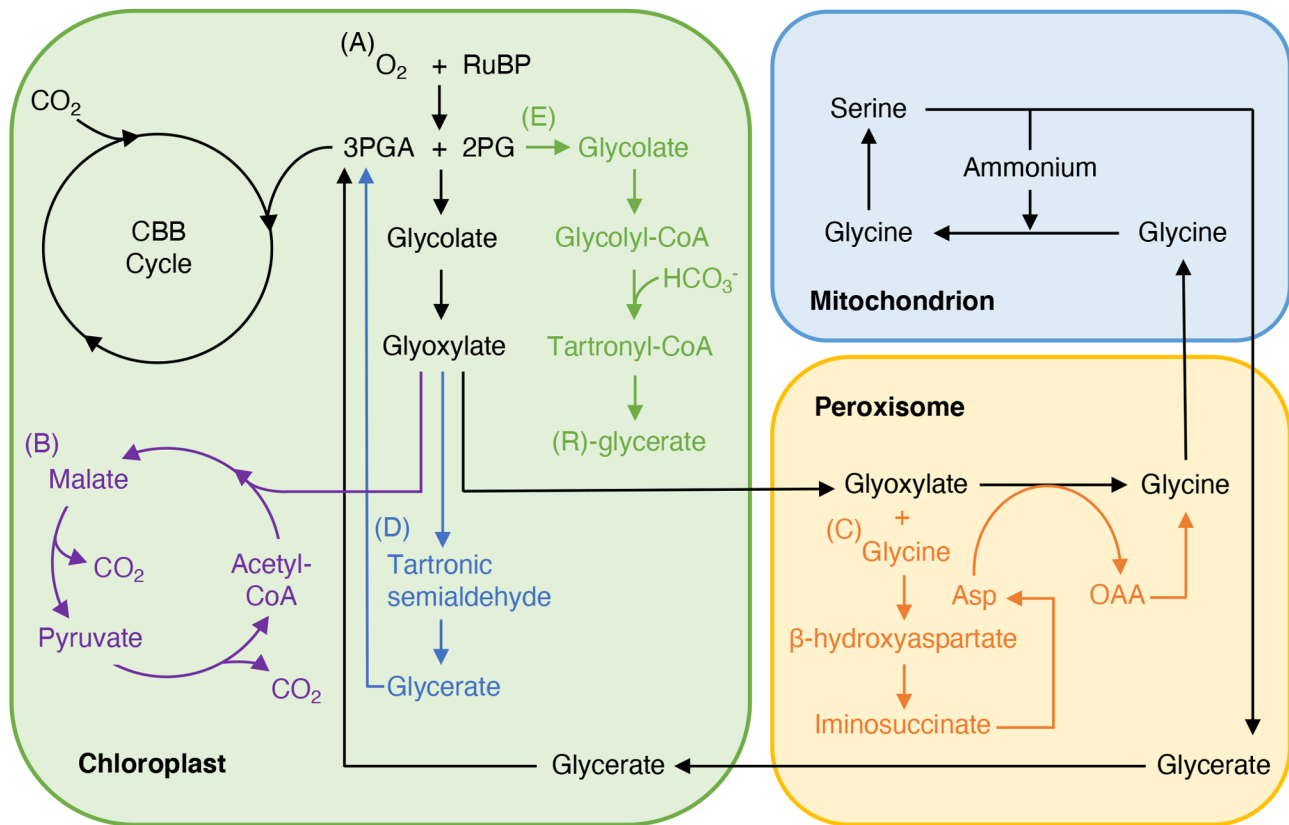
Fig. 2. Integration of the Calvin–Benson–Bassham cycle with native photorespiration and different photorespiratory bypasses. (A) Rubisco oxygenation generates 2-phosphoglycolate that is recycled via the native photorespiratory pathway (black). (B) Alternative pathway 3 (purple) (South et al., 2019; Cavanagh et al., 2021) bypasses photorespiration by metabolising glycolate in the chloroplast. (C) The β-hydroxyaspartate cycle (orange) (Roell et al., 2021) generates oxaloacetate from glycolate while conserving more carbon and nitrogen than native photorespiration. (D) *E. coli* glycolate catabolic pathway (blue) (Nayak et al., 2022) generates 3-phosphoglycerate for Calvin–Benson–Bassham cycle metabolism without requiring catalytic steps in additional compartments to the chloroplast. (E) Fully synthetic tartronyl-CoA pathway (green) (Scheffen et al., 2021) provides a more direct route for glycolate assimilation than native photorespiration. Enzymes, stoichiometries and co-substrates have been omitted for clarity. 2PG, 2-phosphoglycolate; 3PGA, 3-phosphoglycerate; Asp, aspartate; CBB, Calvin–Benson–Bassham; OAA, oxaloacetate; RuBP, ribulose 1,5-bisphosphate.

could confer improved thermal tolerance. Notably, higher photosynthetic rates were found to be maintained in AP3 plants under short-term temperature stress, resulting in transgenic plants retaining 19% more biomass than wild-type plants under the same conditions (Cavanagh et al., 2022). No increase in maximum carboxylation or electron transport rate was observed in the transgenic lines, consistent with the observed thermostability being conferred by improved photorespiration (Cavanagh et al., 2022). Alternative photorespiratory bypass strategies based on the introduction of the β-hydroxyaspartate cycle (BHAC) into *Arabidopsis thaliana* or the *Escherichia coli* glycolate catabolic (ECGC) pathway into rice were met with more variable success (Roell et al., 2021; Nayak et al., 2022) (Fig. 2C,D). The BHAC from marine proteobacteria oxidises glycolate to glyoxylate, which is then converted to oxaloacetate (OAA) via four enzymatic steps without the loss of carbon or nitrogen (in contrast to native photorespiration). Four BHAC enzymes were introduced into *Arabidopsis*, targeted to the peroxisome, and shown to function *in planta* to the extent that relevant metabolic intermediates could be detected. However, transgenic plants had reduced growth compared to wild type and BHAC was not found to improve the CO_2 compensation point (Roell et al., 2021). Despite this, BHAC lines have a potential use in engineering C_4 photosynthesis (an alternative CCM) as they accumulate key C_4 metabolite OAA without requiring the establishment of phosphoenolpyruvate-dependent CO_2 fixation

(Roell et al., 2021). Introduction of the ECGC pathway into rice involved constitutive expression of five genes in rice chloroplasts (Nayak et al., 2022). Transgenic plants expressing both the full (FB) and partial (PB) ECGC pathway were found to maintain higher CO_2 assimilation rates and growth than wild type, which translated into up to 46.7% and 67.0% yield increases in FB and PB plants, respectively (Nayak et al., 2022). Introduction of orthogonal metabolic pathways to bypass photorespiration therefore has real potential for improving crop yields.

A significant advance in the period under review has been the use of state-of-the-art enzyme engineering and directed evolution methods to make the previously hypothetical tartronyl-CoA (TaCo) photorespiratory bypass a reality (Fig. 2E). Theoretical calculations suggested that interfacing TaCo with photorespiration would increase the carbon efficiency of the Calvin–Benson–Bassham cycle from 75 to 100%, while using 21% less ATP and 29% less reducing equivalents than native photorespiration (Scheffen et al., 2021). Tartronyl-CoA, formed by the carboxylation of glycolyl-CoA, and the reactions of the TaCo pathway are not known to occur in nature, so Scheffen et al. identified *Methylorubrum extorquens* propionyl-CoA carboxylase *Me*PCC as a potential candidate for engineering TaCo. Structure-guided rational design was used to engineer the substrate preference of *Me*PCC, resulting in a glycolyl-CoA carboxylase (GCC) that had a 15-fold increase in catalytic efficiency towards glycolyl-CoA.

Directed evolution using a high-throughput approach based on a novel microfluidics screen identified a GCC variant with catalytic efficiency of 3.6×10^4 M^{-1} s^{-1} for glycolyl-CoA carboxylation and reduced futile ATP hydrolysis. Tests *in vitro* found that reconstituted TaCo could generate the physiologically relevant glycerate stereoisomer, suggesting that TaCo has the potential to be successfully implemented *in planta* (Scheffen et al., 2021). Realisation of TaCo in plants remains to be achieved, but the results so far have provided a clear proof-of-principle for the engineering of fully synthetic pathways to improve photosynthesis.

Technological innovations and new resources

Efforts to engineer photosynthesis are limited by available technologies, particularly for stable nuclear transformation of large, multigene constructs (>10 genes) into nuclear genomes (Ort et al., 2015) and for the transformation of chloroplast genomes (Bock, 2015; Jackson et al., 2021). However, introduction of point mutations to plastome-localised *RbcL* via a restriction enzyme-based system has been re-demonstrated with a 40% editing efficiency (Lin et al., 2021). Improvements have also been made to an already established proteoliposome system for testing of photoprotective strategies (Nicol and Croce, 2021).

Development of open access, online resources is crucial for photosynthesis research and related engineering strategies (Zhu et al., 2020). This is particularly important for comparing results between labs. For example, Rubisco kinetics were found to vary significantly depending on the lab and method, with k_{cat} and K_c found to vary >200% in vascular plants (Iñiguez et al., 2021). A normalised Rubisco kinetic database and methods for correcting empirical values have therefore been proposed and will be invaluable for future Rubisco engineering (Iñiguez et al., 2021). Recently published genome and transcriptome data for species with high photosynthetic efficiencies will also help to inform future research (Xi et al., 2021; Garassino et al., 2022 preprint), as will an analysis of photosynthetic and morphological traits in widely used *indica* rice (Acevedo-Siaca et al., 2021).

Implementation of future design strategies is also likely to require novel promoters with well-defined characteristics (strength, tissue specificity, etc.). A comprehensive analysis of all core promoters in *Arabidopsis*, maize and sorghum revealed determinants of promoter strength (Jores et al., 2021). This allowed for the design of synthetic promoters with chosen characteristics and informed the generation of a computational model to predict promoter strength (Jores et al., 2021). This is likely to make an impact in the future of photosynthetic engineering projects, as well as in other biological engineering contexts.

New hypotheses

The results produced in the period February 2021 to January 2022 have informed or challenged previous hypotheses. For example, Hines et al. demonstrated that loss of stromal CA activity does not result in photosynthetic defects, contrary to the hypothesis that removal of stromal CA would significantly reduce mesophyll conductance and consequently photosynthesis (Tholen and Zhu, 2011). There has also historically been confusion as to the role of IctB in cyanobacterial CCMs. IctB was originally identified as a bicarbonate transporter (Bonfil et al., 1998) but was subsequently shown not to contribute to bicarbonate transporter loss-of-function lines (Shibata et al., 2002; Xu et al., 2008). If IctB is not a bicarbonate transporter, previous suggestions that *ictB* expression in C_3 species enhances photosynthesis by facilitating a CCM (Lieman-Hurwitz et al., 2003; Simkin et al., 2015; Gong et al., 2015; Hay et al., 2017)

must be overlooking an alternative function. *ictB* expression in a C_4 species should therefore recapitulate the enhanced photosynthesis phenotype of *ictB* expression in C_3 plants (Koester et al., 2021). In agreement with this, transgenic *RbcS::ictB* maize lines had a 3.49% increase in yield compared to controls in field trials, with the increased performance attributed to enhanced carbohydrate production (Koester et al., 2021). Yield increase was associated with an increase in photosystem II operating efficiency, suggesting that enhanced photosynthesis was responsible for the improvements (Koester et al., 2021). These results support the hypothesis that IctB is not a CCM bicarbonate transporter: a complete understanding of how CCMs operate therefore remains lacking.

It has long been hypothesised that overexpression of Rubisco could enhance photosynthesis; however, Rubisco overexpression has been associated with a decrease in Rubisco activation and lower Rca:Rubisco ratio (Suzuki et al., 2009; Suganami et al., 2018). Two groups therefore recently tested the effect of Rubisco and Rca co-overexpression. Although the rate of CO_2 assimilation in Rubisco/Rca-overexpressing rice lines (*RBCS-RCA-ox*) was similar to wild type under ambient conditions, CO_2 assimilation was enhanced in *RBCS-RCA-ox* lines at 32–36°C (Suganami et al., 2021). Similar results were found with the co-overexpression of *OsRBCS* and *ZmRCA* in rice, with a 26% increase in dry biomass found in transgenic plants compared with wild type when grown at 40°C (Qu et al., 2021). In addition, Rubisco reactivation on shade–sun transitions is known to be slow (Sassenrath-Cole et al., 1994; Tanaka et al., 2019), providing an opportunity for engineering. Notably, a recent paper that determined *in vitro* and *in vivo* Rubisco deactivation half-times in cowpea found faster Rubisco deactivation than previously predicted from wheat values (Taylor et al., 2022). Furthermore, the speed of Rubisco response to sun–shade transitions differed more than Rubisco induction on shade–sun transitions, leading the authors to hypothesise that Rubisco deactivation could be a new target for engineering (Taylor et al., 2022). The significance of this finding will likely be tested in transgenic plants in the future.

Future prospects

Despite the bleak outlook on future crop security, engineering photosynthesis is a promising approach for improvement, particularly in the light of the recent success of synthetic biology approaches. Given that photosynthesis is a complex trait, it is challenging to predict which strategy will yield the best result: it therefore seems astute to continue developing all avenues of photosynthetic research to ensure a robust strategy for sustainably safeguarding yields in a changing climate. Indeed, engineering strategies are not mutually exclusive, and a combined approach may prove to be the most effective.

References
Acevedo-Siaca, L. G., Coe, R., Quick, W. P. and Long, S. P. (2021). Evaluating natural variation, heritability, and genetic advance of photosynthetic traits in rice (*Oryza sativa*). *Plant Breed.* **140**, 745-757. doi:10.1111/pbr.12965

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. doi:10.1073/pnas.0907176107

Batista-Silva, W., da Fonseca-Pereira, P., Martins, A. O., Zsögön, A., Nunes-Nesi, A. and Araújo, W. L. (2020). Engineering improved photosynthesis in the era of synthetic biology. *Plant Commun.* **1**, 100032. doi:10.1016/j.xplc.2020.100032

Blankenship, R. E., Tiede, D. M., Barber, J., Brudvig, G. W., Fleming, G., Ghirardi, M., Gunner, M. R., Junge, W., Kramer, D. M., Melis, A. et al. (2011). Comparing photosynthetic and photovoltaic efficiencies and recognizing the potential for improvement. *Science* **332**, 805-809. doi:10.1126/science.1200165

Bock, R. (2015). Engineering plastid genomes: methods, tools, and applications in basic research and biotechnology. *Ann. Rev. Plant Biol.* **66**, 211-241. doi:10.1146/annurev-arplant-050213-040212

Bonfil, D. J., Ronen-Tarazi, M., Sültemeyer, D., Lieman-Hurwitz, J., Schatz, D. and Kaplan, A. (1998). A putative HCO_3^- transporter in the cyanobacterium *Synechococcus* sp. strain PCC 7942. *FEBS Lett.* **430**, 236-240. doi:10.1016/S0014-5793(98)00662-0

Busch, F. A. (2020). Photorespiration in the context of Rubisco biochemistry, CO_2 diffusion and metabolism. *Plant J.* **101**, 919-939. doi:10.1111/tpj.14674

Cavanagh, A. P., South, P. F., Bernacchi, C. J. and Ort, D. R. (2022). Alternative pathway to photorespiration protects growth and productivity at elevated temperatures in a model crop. *Plant Biotechnol. J.* **20**, 711-721. doi:10.1111/pbi.13750

Chen, T., Fang, Y., Jiang, Q., Dykes, G. F., Lin, Y., Price, G. D., Long, B. M. and Liu, L. N. (2022). Incorporation of functional Rubisco activases into engineered carboxysomes to enhance carbon fixation. *ACS Synth. Biol.* **11**, 154-161. doi:10.1021/acssynbio.1c00311

Dean Price, G., Badger, M. R. and von Caemmerer, S. (2011). The prospect of using cyanobacterial bicarbonate transporters to improve leaf photosynthesis in C3 crop plants. *Plant Physiol.* **155**, 20-26. doi:10.1104/pp.110.164681

Flamholz, A. I., Prywes, N., Moran, U., Davidi, D., Bar-On, Y. M., Oltrogge, L. M., Alves, R., Savage, D. and Milo, R. (2019). Revisiting trade-offs between Rubisco kinetic parameters. *Biochem* **58**, 3365-3376. doi:10.1021/acs.biochem.9b00237

Garassino, F., Wijfjes, R. Y., Boesten, R., Becker, F. F. M., Clapero, V., van den Hatert, I., Holmer, R., Schranz, , M. E., Harbinson, J., de Ridder, D. et al. (2022). The genome sequence of *Hirschfeldia incana*, a species with high photosynthetic light-use efficiency. *bioRxiv*. doi: 10.1101/2022.01.29.478283

Gong, H. Y., Li, Y., Fang, G., Hu, D. H., Jin, W. b., Wang, Z. H. and Li, Y. S. (2015). Transgenic rice expressing Ictb and FBP/Sbpase derived from cyanobacteria exhibits enhanced photosynthesis and mesophyll conductance to CO_2. *PLoS One* **10**, e0140928. doi:10.1371/journal.pone.0140928

Hay, W. T., Bihmidine, S., Mutlu, N., Le Hoang, K., Awada, T., Weeks, D. P., Clemente, T. E. and Long, S. P. (2017). Enhancing soybean photosynthetic CO_2 assimilation using a cyanobacterial membrane protein, *ictB*. *J. Plant Physiol.* **212**, 58-68. doi:10.1016/j.jplph.2017.02.003

Hennacy, J. H. and Jonikas, M. C. (2020). Prospects for engineering biophysical CO_2 concentrating mechanisms into land plants to enhance yields. *Ann. Rev. Plant Biol.* **71**, 461-485. doi:10.1146/annurev-arplant-081519-040100

Hines, K. M., Chaudhari, V., Edgeworth, K. N., Owens, T. G. and Hanson, M. R. (2021). Absence of carbonic anhydrase in chloroplasts affects C_3 plant development but not photosynthesis. *Proc. Natl. Acad. Sci. USA* **118**, e2107425118. doi:10.1073/pnas.2107425118

Hitchcock, A., Hunter, C. N., Sobotka, R., Komenda, J., Dann, M. and Leister, D. (2021). Redesigning the photosynthetic light reactions to enhance photosynthesis - the PhotoRedesign consortium. *Plant J.* **109**, 23-34. doi:10.1111/tpj.15552

Iñiguez, C., Niinemets, Ü., Mark, K. and Galmés, J. (2021). Analyzing the causes of method-to-method variability among Rubisco kinetic traits: from the first to the current measurements. *J. Exp. Bot.* **72**, 7846-7862. doi:10.1093/jxb/erab356

Jackson, H. O., Taunt, H. N., Mordaka, P. M., Smith, A. G. and Purton, S. (2021). The algal chloroplast as a testbed for synthetic biology designs aimed at radically rewiring plant metabolism. *Front. Plant Sci.* **12**, 708370. doi:10.3389/fpls.2021.708370

Jägermeyr, J., Müller, C., Ruane, A. C., Elliott, J., Balkovic, J., Castillo, O., Faye, B., Foster, I., Folberth, C., Franke, J. A. et al. (2021). Climate impacts on global agriculture emerge earlier in new generation of climate and crop models. *Nat. Food.* **2**, 873-885. doi:10.1038/s43016-021-00400-y

Jores, T., Tonnies, J., Wrightsman, T., Buckler, E. S., Cuperus, J. T., Fields, S. and Queitsch, C. (2021). Synthetic promoter designs enabled by a comprehensive analysis of plant core promoters. *Nat. Plants* **7**, 842-855. doi:10.1038/s41477-021-00932-y

Koester, R. P., Pignon, C. P., Kesler, D. C., Willison, R. S., Kang, M., Shen, Y., Priest, H. D., Begemann, M. B., Cook, K. A., Bannon, G. A. et al. (2021). Transgenic insertion of the cyanobacterial membrane protein ictB increases grain yield in *Zea mays* through increased photosynthesis and carbohydrate production. *PLoS ONE* **16**, e0246359. doi:10.1371/journal.pone.0246359

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. doi:10.1126/science.aai8878

Kubis, A. and Bar-Even, A. (2019). Synthetic biology approaches for improving photosynthesis. *J. Exp. Bot.* **70**, 1425-1433. doi:10.1093/jxb/erz029

Lieman-Hurwitz, J., Rachmilevitch, S., Mittler, R., Marcus, Y. and Kaplan, A. (2003). Enhanced photosynthesis and growth of transgenic plants that express *ictB*, a gene involved in HCO_3^- accumulation in cyanobacteria. *Plant Biotechnol. J.* **1**, 43-50. doi:10.1046/j.1467-7652.2003.00003.x

Lin, M. T., Orr, D. J., Worrall, D., Parry, M. A. J., Carmo-Silva, E. and Hanson, M. R. (2021). A procedure to introduce point mutations into the Rubisco large subunit gene in wild-type plants. *Plant J.* **106**, 876-887. doi:10.1111/tpj.15196

Long, B. M., Hee, W. Y., Sharwood, R. E., Rae, B. D., Kaines, S., Lim, Y. L., Nguyen, N. D., Massey, B., Bala, S., von Caemmerer, S. et al. (2018). Carboxysome encapsulation of the CO_2-fixing enzyme Rubisco in tobacco chloroplasts. *Nat. Commun.* **9**, 3570. doi:10.1038/s41467-018-06044-0

Long S. P. and Marshall-Colon A. & Zhu. X. (2015) Meeting the global food demand of the future by engineering crop photosynthesis and yield potential. *Cell* **161**: 56–66

McGrath, J. M. and Long, S. P. (2014). Can the cyanobacterial carbon-concentrating mechanism increase photosynthesis in crop species? A theoretical analysis. *Plant Physiol.* **164**, 2247-2261. doi:10.1104/pp.113.232611

Miller, T. E., Beneyton, T., Schwander, T., Diehl, C., Girault, M., McLean, R., Chotel, T., Claus, P., Cortina, N. S., Baret, J. C. et al. (2020). Light-powered CO_2 fixation in a chloroplast mimic with natural and synthetic parts. *Science* **368**, 649-654. doi:10.1126/science.aaz6802

Murchie, E. H., Pinto. M. and Horton, P. (2009) Agriculture and the new challenges for photosynthesis research. *New Phytol* **181**, 532-552

Nayak, L., Panda, D., Dash, G. K., Lal, M. K., Swain, P., Baig, M. J. and Kumar, A. (2022). A chloroplast glycolate catabolic pathway bypassing the endogenous photorespiratory cycle enhances photosynthesis, biomass and yield in rice (*Oryza sativa L.*). *Plant Sci.* **314**, 111103. doi:10.1016/j.plantsci.2021.111103

Nicol, L. and Croce, R. (2021). The PsbS protein and low pH are necessary and sufficient to induce quenching in the light-harvesting complex of plants LHCII. *Sci. Rep.* **11**, 7415. doi:10.1038/s41598-021-86975-9

Ort, D. R., Merchant, S. S., Alric, J., Barkan, A., Blankenship, R. E., Bock, R., Croce, R., Hanson, M. R., Hibberd, J. M., Long, S. P. et al. (2015). Redesigning photosynthesis to sustainably meet global food and bioenergy demand. *Proc. Natl. Acad. Sci. USA* **112**, 8529-8536. doi:10.1073/pnas.1424031112

Price, G. D. (2011). Inorganic carbon transporters of the cyanobacterial CO_2 concentrating mechanism. *Photosynth. Res.* **109**, 47-57. doi:10.1007/s11120-010-9608-y

Qu, Y., Sakoda, K., Fukayama, H., Kondo, E., Suzuki, Y., Makino, A., Terashima, I. and Yamori, W. (2021). Overexpression of both Rubisco and Rubisco activase rescues rice photosynthesis and biomass under heat stress. *Plant Cell Environ.* **44**, 2308-2320. doi:10.1111/pce.14051

Ray, D. K., Mueller, N. D., West, P. C. and Foley, J. A. (2013). Yield trends are insufficient to double global crop production by 2050. *PLoS ONE* **8**, e66428. doi:10.1371/journal.pone.0066428

Roell, M. S., von Borzykowski, L. S., 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. doi:10.1073/pnas.2022307118

Sage, R. F. and Kubien, D. S. (2007). The temperature response of C_3 and C_4 photosynthesis. *Plant Cell Environ.* **30**, 1086-1106. doi:10.1111/j.1365-3040.2007.01682.x

Sassenrath-Cole, G. F., Pearcy, R. W. and Steinmaus, S. (1994). The role of enzyme activation state in limiting carbon assimilation under variable light conditions. *Photosynth. Res.* **41**, 295-302. doi:10.1007/BF00019407

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. doi:10.1038/s41929-020-00557-y

Schwander, T., Schada von Borzyskowski, L. S., 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. doi:10.1126/science.aah5237

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. doi:10.1016/j.molp.2018.11.013

Shibata, M., Katoh, H., Sonoda, M., Ohkawa, H., Shimoyama, M., Fukuzawa, H., Kaplan, A. and Ogawa, T. (2002). Genes essential to sodium-dependent bicarbonate transport in cyanobacteria: function and phylogenetic analysis. *J. Biol. Chem.* **277**, 18658-18664. doi:10.1074/jbc.M112468200

Simkin, A. J., McAusland, L., Headland, L. R., Lawson, T. and Raines, C. A. (2015). Multigene manipulation of photosynthetic carbon assimilation increases CO_2 fixation and biomass yield in tobacco. *J. Exp. Bot.* **66**, 4075-4090. doi:10.1093/jxb/erv204

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. doi:10.1126/science.aat9077

Suganami, M., Suzuki, Y., Sato, T. and Makino, A. (2018). Relationship between Rubisco activase and Rubisco contents in transgenic rice plants with overproduced or decreased Rubisco content. *Soil Sci. Plant Nutr.* **64**, 352-359. doi:10.1080/00380768.2018.1433455

Suganami, M., Suzuki, Y., Tazoe, Y., Yamori, W. and Makino, A. (2021). Co-overproducing Rubisco and Rubisco activase enhances photosynthesis in the optimal temperature range in rice. *Plant Physiol.* **185**, 108-119. doi:10.1093/plphys/kiaa026

Suzuki, Y., Miyamoto, T., Yoshizawa, R., Mae, T. and Makino, A. (2009). Rubisco content and photosynthesis of leaves at different positions in transgenic rice with an overexpression of *RBCS. Plant Cell Environ.* **32**, 417-427. doi:10.1111/j.1365-3040.2009.01937.x

Tanaka, Y., Adachi, S. and Yamori, W. (2019). Natural genetic variation of the photosynthetic induction response to fluctuating light environment. *Curr. Opin. Plant Biol.* **49**, 52-59. doi:10.1016/j.pbi.2019.04.010

Taylor, S. H., Gonzalez-Escobar, E., Page, R., Parry, M. A. J., Long, S. P. and Carmo-Silva, E. (2022). Faster than expected Rubisco deactivation in shade reduces cowpea photosynthetic potential in variable light conditions. *Nat. Plants* **8**, 118-124. doi:10.1038/s41477-021-01068-9

Tholen, D. and Zhu, X. G. (2011). The mechanistic basis of internal conductance: a theoretical analysis of mesophyll cell photosynthesis and CO_2 diffusion. *Plant Physiol.* **156**, 90-105. doi:10.1104/pp.111.172346

Tsai, Y. C. C., Liew, L., Guo, Z., Liu, D. and Mueller-Cajar, O. (2022). The CbbQO-type Rubisco activases encoded in carboxysome gene clusters can activate carboxysomal form IA Rubiscos. *J. Biol. Chem.* **298**, 101476. doi:10.1016/j.jbc.2021.101476

Turmo, A., Gonzalez-Esquer, C. R. and Kerfeld, C. A. (2017). Carboxysomes: metabolic modules for CO_2 fixation. *FEMS Microbiol. Lett.* **364**, fnx176. doi:10.1093/femsle/fnx176

Walker, B. J., Vanloocke, A., Bernacchi, C. J. and Ort, D. R. (2016). The costs of photorespiration to food production now and in the future. *Ann. Rev. Plant Biol.* **67**, 107-129. doi:10.1146/annurev-arplant-043015-111709

Xi, Y., Yin, L., Chi, Z. Y. and Luo, G. (2021). Characterization and RNA-seq transcriptomic analysis of a *Scenedesmus obliqnus* mutant with enhanced photosynthesis efficiency and lipid productivity. *Sci. Rep.* **11**, 11795. doi:10.1038/s41598-020-79139-8

Xu, M., Bernát, G., Singh, A., Mi, H., Rögner, M., Pakrasi, H. B. and Ogawa, T. (2008). Properties of mutants of *Synechocystis* sp. strain PCC 6803 lacking inorganic carbon sequestration systems. *Plant Cell Physiol.* **49**, 1672-1677. doi:10.1093/pcp/pcn139

Yin, X. and Struik, P. C. (2017). Can increased leaf photosynthesis be converted into higher crop mass production? A simulation study for rice using the crop model GECROS. *J. Exp. Botany* **68**, 2345-2360. doi:10.1093/jxb/erx085

Zhu, X.-G., Long, S. P. and Ort, D. R. (2010). Improving photosynthetic efficiency for greater yield. *Ann. Rev. Plant Biol.* **61**, 235-261. doi:10.1146/annurev-arplant-042809-112206

Zhu, X.-G., Ort, D. R., Parry, M. A. J. and von Caemmerer, S. (2020). A wish list for synthetic biology in photosynthesis research. *J. Exp. Bot.* **71**, 2219-2225. doi:10.1093/jxb/eraa075