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Review

# Feeding from the sun—Successes and prospects in bioengineering photosynthesis for food security

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## SUMMARY

There is an urgent need for increased crop productivity to reduce food insecurity and improve sustainability. Photosynthesis converts sunlight energy into carbohydrates, providing the source of nearly all of humanity's food. Photosynthesis is a key target for improvement, owing to inherent inefficiencies in the biochemical process. Over the last decade of advancements in bioengineering, strategies to increase the efficiency of photosynthesis were tested with proven enhancements to crop yields in field trials. Simple strategies like increasing the content of photosynthetic proteins have reliably increased photosynthesis and productivity in crops, as have more complex strategies such as bypassing photorespiration. While insertion of carbon-concentrating mechanisms into $C_3$ plants remains an engineering challenge, modeling suggests that achieving that would have the greatest gain for crop improvement. This review discusses the many successes in improving photosynthesis achieved over the past decade and quantifies the potential for future engineering targets to increase crop productivity.

## INTRODUCTION

The last 10 years have seen a progressive decline in the availability of food at the global level such that today 8.2% of the global population, ∼700,000,000 people, faced hunger in 2024.[1] This is driven in part by a continually widening gap between crop production and demand.[2] A meta-analysis of 57 detailed global food scenario quantitative projections predicted that 35%–56% more crop production will be needed by 2050.[3] Decreasing demand and waste would lower this increased requirement; however, current global trends for these factors continue in the opposite direction, and this is not anticipated to change substantially going forward.[4] Crop losses due to increased intensity and frequency of extreme climatic events will add to the gap between production and demand.[4,5] Despite climate change and new pest and disease challenges, plant breeding, coupled with improved agronomy, continues to result in annual increases in production for several food crops,[6] but new innovations need to be added to this pipeline if projected future demand is to be met.

Photosynthesis converts the energy of the sun with $CO_2$ and water into the chemical energy for crop growth and yield, and indeed almost all life on the planet. Directly, or indirectly, it is the source of all our food. Despite this fundamental link, only recently has it gained attention as a means to decrease the supply gap in the global food system. Equally, it provides a means to supply feedstocks, including forestry, to meet the increasing demand for non-food bioproducts with less land. Two factors make photosynthesis a potentially important means to increase yield. First, the efficiency of the process, even in our best crops, falls well below the theoretical maximum, suggesting considerable room for improvement.[7] Secondly, the process is much the same across all food, feed, forestry, herbage, and biomass crops, such that a means to improve photosynthesis will likely apply to most crops, with further adjustments needed to maximize benefits in different environments.[7,8]

The only significant variation in photosynthetic biochemistry across the major crops is $C_3$ versus $C_4$. $C_3$ refers to crops in which $CO_2$ is first combined with $C_5$ ribulose-1,5-bisphosphate (RuBP) to form two molecules of $C_3$ glycerate-3P (G3P), catalyzed by RuBP carboxylase/oxygenase (Rubisco). G3P is recycled to RuBP through the Calvin-Benson-Bassham cycle (CBBc). With each turn of the cycle, additional C enters the anabolic pathways, forming all organic constituents of the plant. $C_3$ crops include rice, wheat, barley, potato, legumes, and all tree crops. In $C_4$ crops, $CO_2$ is first combined with phosphoenolpyruvate (PEP) to form the $C_4$ dicarboxylate, oxaloacetate, catalyzed by PEP carboxylase. This reaction occurs in the mesophyll, which surrounds a photosynthetic bundle sheath where Rubisco is confined. Dicarboxylates diffuse to the bundle sheath, where decarboxylation provides a high $CO_2$ concentration ($[CO_2]$), which competitively inhibits the oxygenation activity of Rubisco and resulting losses of assimilate through photorespiration, while promoting carboxylation and onward metabolism in the CBBc. The resulting $C_3$ product of decarboxylation diffuses back to the mesophyll, where it is metabolized to PEP at the cost of 2 ATP, completing the $C_4$ photosynthetic cycle. This, in effect, serves as an energy-driven $CO_2$ concentration mechanism. As a result, $C_4$ crops are generally among the most productive and include maize, sorghum, millets, sugarcane, tropical pasture grasses, and the bioenergy/bioproduct crop *Miscanthus*. Despite their small number, they constitute a major part of the global food and bioproduct supply.[6]

10 years ago, when the predecessor to this article was published, the assessment that photosynthetic efficiency could be improved in crops was an unproven hypothesis based largely on metabolic and biophysical mathematical modeling.[8] The models showed credible means to lessen this gap. Given the varying complexities of what would be involved, we forecast how long it might take to achieve test of concept for different bioengineering manipulations.[8] Skepticism that any improvement in crop photosynthetic efficiency could be achieved was a prevailing view then, with the assumption that natural and breeder selection would have already maximized efficiency.[9,10] Since then, bioengineered improvements to photosynthetic efficiency leading to increased productivity have been shown through at least four different approaches at the level of single-site test-of-concept field trials.[11–13] Generally, these have been achieved in a shorter time frame than we forecast.[8] Major strides have also been made toward achievement for manipulations that we anticipated would require a long-time horizon.[14–16] What has changed to facilitate this acceleration? Resequencing of large number of crop cultivars and relatives, breakthroughs in transformation and DNA editing in crops, high-throughput phenotyping of photosynthesis and crop development, 3D reconstruction of key photosynthetic proteins from sequence, atomistic simulation of protein function, and *in silico* mutation coupled with directed evolution of *Escherichia coli* strains engineered to be dependent on photosynthetic proteins have all contributed to recent success.[17–23]

Here, we review what has been achieved in genetic improvement of photosynthesis over the last 10 years and how new molecular developments have and are accelerating this. We also tabulate what has now been achieved and what looks probable over the near future.

## RUBISCO

Nearly all carbon in plants is assimilated through Rubisco. Despite being the most abundant protein in the leaf and the world,[24] it is often limiting to light-saturated photosynthesis. It appears that to minimize the costly oxygenase activity leading to photorespiratory losses of carbon and energy, Rubisco in plants has evolved to have a higher affinity for $CO_2$ to maximize discrimination against the far more abundant $O_2$. However, high $CO_2$ affinity slows catalysis, partially compensated by the production of large amounts of Rubisco.[25] The advent of the Farquhar-von Caemmerer-Berry (FvCB) model[26,27] has shown us that at past atmospheric $[CO_2]$, Rubisco content in $C_3$ leaves was strongly limiting to light-saturated photosynthesis. As $[CO_2]$ rises, RuBP regeneration co-limits photosynthesis. In $C_4$ crops, the opposite is occurring. As $[CO_2]$ rises, the concentration in the bundle sheath increases, and the content of Rubisco is increasingly limiting to $C_4$ photosynthesis. As Rubisco limits both $C_3$ and $C_4$ photosynthesis in leaves under light-saturated conditions, the simple approach of increasing Rubisco content is a strategy for increasing photosynthesis and crop yield.[28] Recently, transgenic upregulation of Rubisco content has been proven to be effective in increasing productivity of $C_3$ rice[12] and $C_4$ sorghum[13] in field trials. In addition to transgenic approaches to enhance Rubisco content, increased expression of the *BOOSTER* (*BSTR*) gene in poplar enhanced Rubisco expression, resulting in greater photosynthesis, plant height, and biomass.[29] *BSTR* was discovered from high-throughput screening of genetic variation in photosynthesis, opening up the possibility for discovery of novel approaches to increase Rubisco. Additionally, gene editing approaches that target promoter regions or upstream open reading frames could potentially enhance Rubisco expression.[28] However, Rubisco is a major sink for nitrogen, and a more sustainable approach will be to develop more efficient Rubisco, rather than simply more.

### Engineering a better Rubisco

Improving Rubisco carboxylation has long been a target for increasing photosynthesis and crop productivity. Despite abundant sequence, structure, and catalytic data, attempts to rationally engineer catalytic improvements to the enzyme have been largely unsuccessful. Instead, random mutagenesis and directed evolution have been the go-to strategies to assess the potential to engineer Rubisco and identify residues influential to catalysis. Screening Rubisco activity has relied heavily on Rubisco-dependent *E. coli* (RDE) strains that enable activity-based selection from random mutant libraries. These strains have been effective in isolating variants exhibiting universal kinetic improvements.[30,31] Mutations affording these improvements are often distant to the active site and difficult to interpret from a structure-function perspective, explaining earlier rational design failures. More advanced RDE strains feature genetic deletions in central carbon metabolism that can be alleviated by Rubisco activity.[32,33] Tuning the RDE growth environment during selection can even enable the kinetic properties of library variants to be estimated by growth rate alone.[34] One of the most attractive features of metabolically dependent strains is an absence of false positives from off-target mutations that frequently circumvent the selection circuit of earlier RDE platforms.

Directed evolution has focused almost exclusively on prokaryotic isoforms, as recombinant production of plant Rubisco was achieved relatively recently.[35,36] Engineering improvements to plant Rubisco are essential to translational improvements in crops. However, directed evolution is complicated by the numerous molecular chaperones needed to support plant enzyme production and maintenance in RDE. Most of these chaperones are essential to produce meaningful amounts of the plant isoform in *E. coli* and are poorly compatible with non-cognate Rubisco partners.[37] For example, monocot enzymes from cereal crops are completely dependent on monocot-specific chaperonin (CPN60) complexes.[38] Directed evolution of Rubisco in the presence of chaperones improves mutational tolerance but restricts functional outcomes,[39] suggesting a need for coevolution of the entire system to explain the distinct cognate chaperone-Rubisco partnerships observed in plant lineages. The recent finding that plant Rubisco chaperones can alter the catalytic properties of the assembled enzyme in a recombinant system further reinforces their importance in any engineering context.[40]

Directed evolution campaigns that include the chaperone suite may be challenging but are now accessible thanks to a rapid advancement in mutagenesis technologies that can be paired with high-throughput Rubisco selection platforms. The use of *in vivo* mutagenesis methods allows for much larger libraries of random mutants to be generated and can operate in tandem with RDE selection.[41] These advancements provide the groundwork for developing a continuous directed evolution platform for Rubisco capable of screening huge libraries of genetic diversity in high throughput.

A more extreme alternative is to replace Rubisco with a different carboxylase. Although all carbon is assimilated through Rubisco in aerobic photosynthetic organisms, other carboxylases lacking lability to $O_2$ and with a high $k_{cat}$ can be found in some chemoautotrophs. At least five other autotrophic $CO_2$ assimilatory pathways have been identified.[42] A predicted autotrophic pathway using a coenzyme A (CoA)-dependent carboxylase and 16 further enzymes to complete the cycle was assembled and, *in vitro*, shown to be some 5 times more efficient than the Rubisco-based CBBc.[43] However, the CBBc is integral with multiple key anabolic pathways leading to all constituents of the plant, and re-engineering these, while not out of the question, would be a far larger task than any of the other bioengineering targets discussed in this article.

## Engineering a better Rca

Rubisco only has value if it is active when needed. Despite being a major limitation to photosynthesis, only about 80% of Rubisco is active when photosynthetic rates are maximal.[44] Further, its slow induction on shade-sun transitions imposes a further major limitation.[45–48] Achieving greater activation of Rubisco at both steady state and induction is therefore another means to increase crop photosynthetic efficiency. Rubisco binds inhibitors and requires its molecular chaperone, Rubisco activase (Rca), to change the enzyme's conformation to remove inhibitory sugar-phosphates from catalytic sites. The activity of Rca is regulated in response to irradiance levels via the energetic balance and redox status of the chloroplast.[49] This regulation is a key determinant of photosynthetic light induction, and inefficient regulation of Rubisco activity limits daily photosynthetic productivity in crop leaves that experience variable irradiance with many transitions between shade and full sun.[45,48,50] Furthermore, Rca is thermolabile and progressively loses its ability to maintain Rubisco activity as temperatures rise above the optimum for a given plant species.[51] Thus, Rca has emerged as another key target to improve the productivity and climate resilience of field crops where leaves are frequently exposed to variable light regimes and increasingly threatened by heatwaves.[44,49,52–55]

Significant progress has been achieved toward genetic engineering of Rca in crops since overexpression of a thermostable Rca in *Arabidopsis* was shown to increase photosynthesis and biomass under heat stress.[56,57] In rice, Rca overexpression increased photosynthesis, but only when accompanied by Rubisco small subunit (RbcS) overexpression to avoid downregulation of Rubisco abundance.[58,59] Both inter- and intraspecies diversity in the regulatory properties and temperature response of Rca isoforms have been reported.[60–67] In wheat and cowpea, heat increased expression of more thermotolerant Rca isoforms, but their protein abundance remained low or undetectable.[62,66,67] While in rice and wheat, the more thermotolerant isoforms were slower at activating Rubisco,[61,62,64,66] cowpea thermotolerant Rca isoforms quickly and effectively activated Rubisco at a broad temperature range, providing a route to enhance thermotolerance in crops via genetic engineering.[67]

Significant developments in AI-based protein structure prediction, machine learning (ML)-directed functional protein design,[68–71] molecular dynamics simulations incorporating docking and subtle local fluctuations in residue contacts,[72–76] and high-throughput directed evolution systems[77] provide an exciting opportunity to accelerate the rate of progress in engineering of proteins such as Rca. Experimentally determined Rca protein structures (Figure 1) enabled modeling the similar—yet distinct—mechanisms of Rca-Rubisco interaction.[78–82] The structural flexibility and polydisperse nature of Rca have hindered crystal X-ray diffraction and cryoelectron microscopy (cryo-EM) structural studies,[82] and current predicted Rca structures lack the disordered N- and C-terminals involved in the Rca-Rubisco interaction. Specific amino acid residues have been identified through detailed experimental studies with crop Rca isoforms.[62,66] Now the greatest promise lies in combining computational tools to develop a plant Rca-Rubisco interaction model and use this in conformational dynamics simulations to design superior Rca variants for experimental testing. A recent large ML-directed screen of Rca proteins identified thermotolerant synthetic variants with minimal mutations[83] using a deep generative model to capture key patterns of amino acid mutations coupled with a high-throughput assay to assess the ability of hundreds of Rca variants to hydrolyze ATP after a heat shock. The most resilient proteins had lower rates of activity at optimal temperatures and will need testing for Rubisco

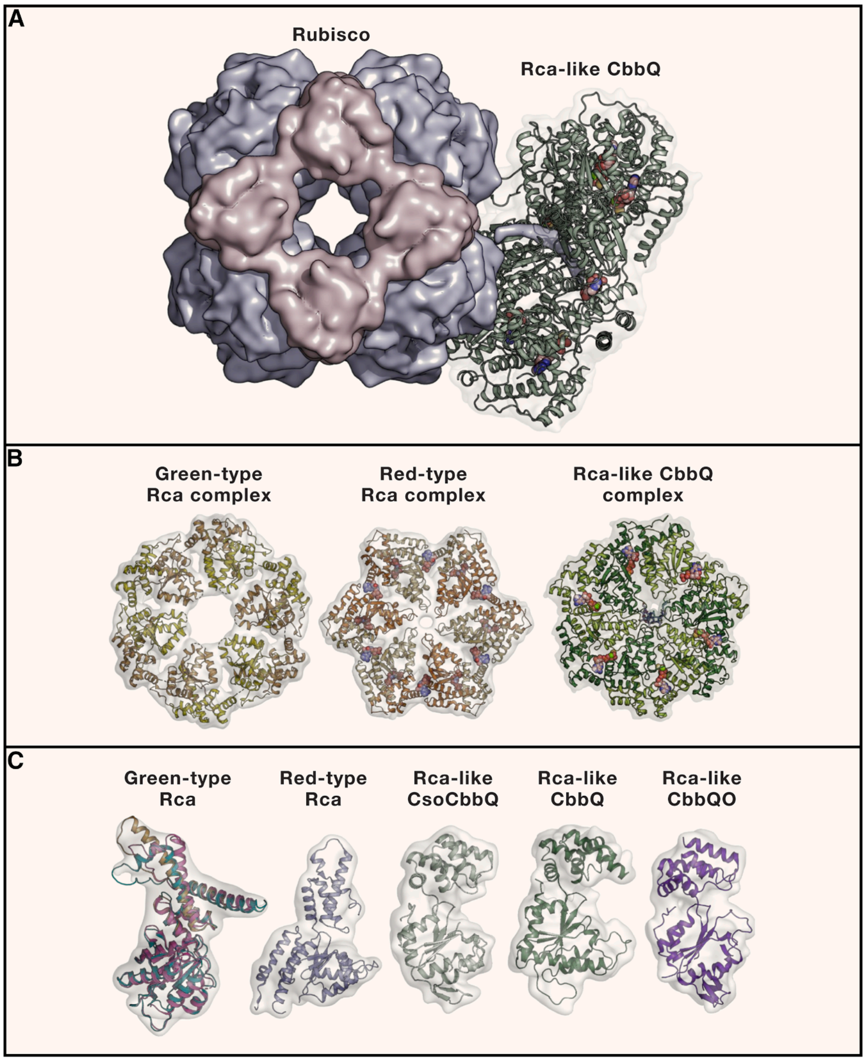

**Figure 1. Variation in the structure of Rubisco and Rca**

(A) Three-dimensional protein structure of Rubisco interacting with Rubisco activase (Rca) from *Nostoc* sp. strain PCC7120.[78]

(B and C) (B) Three-dimensional protein structures of Rca hexamers and (C) Rca monomers from green-type Rcas, red-type Rcas, and Rca-like proteins. Structures were experimentally determined using crystal X-ray diffraction and/or cryo-EM.[84–87]

reactivation efficiency,[66] yet this is an exciting step toward gene editing of crop Rca thermotolerance.

## RuBP REGENERATION AND ALTERNATIVE ASSIMILATION PATHWAYS

### RuBP regeneration in the CBBc

Carboxylation of RuBP catalyzed by Rubisco produces two molecules of 3-phosphoglycerate (3-PGA), which is metabolized through the CBBc to supply the carbon intermediates for many metabolic pathways and to regenerate RuBP. As noted earlier, the widely used and validated FvCB model of steady-state photosynthesis[26,27] shows that at past atmospheric [$CO_2$], Rubisco strongly limited light-saturated photosynthesis. As [$CO_2$] continues to rise, co-limitation by the rate of regeneration of the $CO_2$ acceptor molecule at Rubisco, RuBP, occurs.[88] Application of evolutionary algorithms to complete dynamic models of photosynthetic and photorespiratory metabolism indicated several steps with strong metabolic control over RuBP regeneration. The CBBc enzyme sedoheptulose-1:7-bisphosphatase (SBPase) was predicted to have strong limitation, rising with [$CO_2$].[89,90] Transgenic upregulation of SBPase in tobacco and soybean resulted in higher rates of leaf photosynthetic $CO_2$ assimilation and yield when grown under open-air elevation of [$CO_2$] to the levels predicted for mid-century.[91,92] Inevitably decreasing metabolic control at one point in the regeneration of RuBP increases control elsewhere. Other key control points included fructose-1:6-bisphosphatase (FBPase) and electron transport. Combined transgenic overexpression of SBPase, FBPase, and algal cytochrome $c_6$, shown previously to accelerate electron transport when introduced into *Arabidopsis*,[93] significantly increased photosynthetic $CO_2$ uptake rates in tobacco grown in the field at current [$CO_2$] and resulted in a 39% increase in dry-matter productivity.[94] Given the need for multiple upregulation of proteins involved in RuBP regeneration, identification of master regulators that could achieve this through a single upregulation would present an ideal way forward, and MYB-related and b-ZIP transcription factors appear prominent among these.[95,96]

### Photorespiratory bypasses

Oxygenation of RuBP by Rubisco produces one molecule of 3-PGA and one molecule of 2-phosphoglycolate (2PG). The plant photorespiratory pathway metabolizes two molecules of 2-PG to 3-PGA through reactions in the peroxisomes and mitochondria, eventually re-entering the CBBc for RuBP regeneration in the chloroplast. This pathway costs the loss of one molecule of $CO_2$ in the mitochondria for every two molecules of 2-PG plus 7 ATP and 4 NAD(P)H equivalents.[97] This loss of carbon previously assimilated and the huge energetic cost impose a major cost on net $C_3$ crop photosynthesis and productivity, estimated at 36% and 20% for US soybean and wheat[98] and considerably more for hot semi-arid regions.[99]

Foundational work in *Arabidopsis* demonstrated that glycolate metabolism could be diverted from photorespiration into alternative metabolic pathways (i.e., bypasses) to regain carbon in the

CBBc without the large energy losses imposed by the native photorespiratory pathway. The first such bypass relocated glycolate metabolism to the chloroplast with the expression of three microbial enzymes and demonstrated enhanced photosynthetic performance and biomass.[100] The second moved photorespiratory glycolate oxidation to the chloroplast, linked to a synthetic malate decarboxylation cycle, again releasing $CO_2$ at the site of Rubisco.[101] The observed benefits of these pathways have been difficult to reconcile with our current understanding of photorespiration and $C_3$ photosynthesis.[102,103] However, more than a decade later, nine unique bypasses have been expressed across seven additional species.[97,104–113] A systems-level comparison shows that each bypass design confers distinct advantages in carbon export or energetic efficiency depending on environmental conditions, underscoring the importance of moving beyond steady-state interpretations of metabolism.[114] Critically, observed benefits have moved beyond controlled conditions into replicated single-location field trials, demonstrating yield increases of 20%–37% in tobacco,[97,115] 30% in potato,[116] and 19%–37% in rice.[105,107,108,113] Yield increases from multiyear and multilocation field trials of 13%–69% in potato[110] and 17%–29% in rice[108] provide even stronger support for stable yield gains across differing environmental and management conditions.

Over the ∼15 years required to translate these targets into commercial crop cultivars, global mean temperatures are expected to reach ∼1.6°C–2.0°C above pre-industrial levels, increasing the frequency of extreme heat stress during the growing season.[4] Rates of photorespiration increase with temperature due to declines in Rubisco specificity for $CO_2$ relative to $O_2$, increasing the yield penalty to $C_3$ crops.[98,99] Consequently, alternative pathways to photorespiration offer a thermoprotective benefit against yield losses, particularly in tropical environments.[117] One glycolate oxidation bypass engineered into tobacco chloroplasts reduced the yield penalty associated with 5°C of canopy warming by 19%.[115] When transformed into potato, the same pathway conferred increased photosynthetic capacity and daily carbon assimilation during naturally occurring heatwaves.[116] Therefore, engineering photorespiration offers an opportunity to increase both yield potential and resilience to global temperature increase. Further optimization may confer greater improvements, as the largest benefits to crop growth under heat stress conditions are predicted to come from synthetic carbon-fixing pathways such as the tartronyl-CoA pathway, though this remains to be thoroughly tested.[114,118]

## CCMs

The Rubisco oxygenase reaction and energy expenditure in the subsequent photorespiratory reactions are limitations to productivity, especially in environments that support high rates of photorespiration. $O_2$ competes with $CO_2$ for Rubisco's active sites. Concentrating C at the site of Rubisco achieves two things. First, it increases $[CO_2]/[O_2]$, suppressing photorespiration and allowing the use of Rubiscos with lower specificity and substantially higher $k_{cat}$. Second, it increases $[CO_2]$ concentration, causing faster carboxylation rates. Together, these factors greatly decrease the amount of resource-expensive Rubisco required for assimilation.[99] High $[CO_2]$ at Rubisco has been achieved through different evolutionary mechanisms in plants, cyanobacteria, and green algae.

One means to increase $[CO_2]/[O_2]$ in the leaf is to increase mesophyll conductance ($g_m$). This term describes the ease with which $CO_2$ may diffuse from the intercellular leaf air spaces to Rubisco. It includes a series of barriers to diffusion, including the cell wall, cell membrane, cytoplasm, chloroplast envelope, and stroma, to eventually reach Rubisco, and is also affected by interconversion with bicarbonate. During photosynthesis these diffusion limitations cause a significant drawdown in $[CO_2]$ at Rubisco to about 70% of that in the intercellular air spaces. This slows the rate of carboxylation and allows increased oxygenation. One seemingly simple solution would be to engineer or select for increased $g_m$.[119,120] Since diffusion here is in the liquid pathway, it has the added attraction that any gain in photosynthesis would not result in more water loss; hence, it would have the benefit of higher water use efficiency (WUE).[121] Despite many attempts at different points in the diffusion pathway, this has proved challenging.[122] Recently, however, success was achieved in tobacco by transgenically increasing the porosity and decreasing the width of the cell. This significantly increased leaf $g_m$, WUE, and $CO_2$ uptake by about 10% in a replicated field trial.[123]

## Engineering CAM, C2, and $C_4$ into $C_3$ crops

Beyond mesophyll conductance, plants have evolved different strategies to achieve much greater increases in $[CO_2]$ at Rubisco. These include crassulacean acid metabolism (CAM), C2, and $C_4$ photosynthesis. Each of these has the attraction that they have already evolved multiple times from $C_3$ plant ancestors,[124] suggesting the plausibility of engineering these into $C_3$ crops to minimize photorespiration. CAM fixes $CO_2$ into dicarboxylates at night, which are decarboxylated during the day to provide a high $[CO_2]$ when stomata in these plants are closed so also minimizing water loss.[125] It parallels $C_4$ photosynthesis using temporal separation of PEP carboxylation and dicarboxylate decarboxylation, rather than spatial separation. Work is proposed to engineer CAM into crops, which would have particular benefit for hot semi-arid regions and salinized soils with strong diurnal temperature variation.[126,127] C2 photosynthesis is a weak carbon-concentrating mechanism (CCM) that has evolved in over 50 species, including monocots and dicots, to lower photorespiration. The pathway increases net $CO_2$ assimilation by delivering and re-assimilating $CO_2$ released by photorespiration back to the chloroplast.[128] Key innovations for evolving a functional C2 cycle include enhancing the number and activity of chloroplasts and mitochondria in bundle sheath cells and increasing the symplastic connections between the mesophyll and bundle sheath cells. A second step to engineering a C2 shuttle is restriction of glycine decarboxylase (GDC) activity to the bundle sheath cells such that the $CO_2$ it releases must diffuse back through the photosynthetic mesophyll cells. Constitutive overexpression of the GLK transcription factor in rice achieved some of the anatomical changes needed for the C2 shuttle,[129] and transcription factors and regulatory changes required to restrict GDC to bundle sheath cells have been elucidated,[130–132] suggesting that engineering C2 photosynthesis is within reach. Although the benefits of operating a C2 shuttle in rice were modeled to improve photosynthetic rates modestly (<10%), the improvements were consistent across a wide range of environmental conditions.[133]

Engineering the $C_4$ photosynthetic CCM into $C_3$ species like rice has greater potential to increase photosynthesis in high-light and high-temperature conditions than C2 photosynthesis.[133] However, engineering $C_4$ photosynthesis in rice or wheat is a formidable challenge that international consortia have been tackling for over 25 years.[134,135] These projects have articulated the evolutionary requirements and anatomical and biochemical adjustments required for a functional $C_4$ cycle. Partially functional $C_4$ cycles have been inserted into rice,[136,137] although tissue-specific expression of the decarboxylase and changes in anatomical features like bundle sheath cell size and increased vein density are still needed to fully support $C_4$ photosynthesis. Exciting recent evidence that bundle-sheath-specific expression of $C_4$ photosynthetic genes is associated with pre-existing DNA sequences that control gene expression (*cis*-code) found in $C_3$ leaves suggests a rational engineering pathway for cell-specific expression of $C_4$ genes.[138] Whether the anatomical bottlenecks in engineering $C_4$ CCMs in species like rice or wheat can be overcome remains to be seen but appears closer with this discovery.

## Engineering carboxysomes into crops

In the aquatic environment the bulk of inorganic carbon available for photosynthesis is the relatively membrane-impermeable bicarbonate ($HCO_3^-$). Dominating many aquatic niches are cyanobacteria, ancient microbes that share a common ancestor with plant chloroplasts.[139] To make use of $HCO_3^-$ as their source of $CO_2$ for Rubisco, cyanobacteria possess a highly efficient CCM that boosts photosynthesis[140] (Figure 2). This system is bipartite, requiring membrane-bound $HCO_3^-$ transport proteins and carboxysomes: proteinaceous icosahedral microcompartments that encapsulate relatively fast Rubisco and carbonic anhydrase (CA) to convert bicarbonate into $CO_2$.[140] Two types exist: α-carboxysomes and β-carboxysomes, each with distinct protein compositions and assembly pathways. By mimicking this CCM in crop chloroplasts, there is enormous potential to enhance $CO_2$ fixation and reduce photorespiration. A chloroplastic CCM also has the potential to improve water and nitrogen use efficiency (NUE).[8] Cyanobacterial CCMs, like pyrenoids (discussed below), would appear to bring the benefits of $C_4$ photosynthesis to $C_3$ crops, but without the need to engineer dimorphic photosynthetic tissues.[8]

Carboxysomes have been successfully assembled in crop chloroplasts, despite initial concerns this would be more challenging. These complex structures (100–600 nm in diameter) require 10 or more genes encoding proteins (depending on their species origin), required in tightly regulated stoichiometries.[141] While generation of β-carboxysomes within chloroplasts has been challenging,[142,143] α-carboxysomes from two species have been generated in tobacco that are structurally isomorphic with those of the source organism but lack some minor but likely essential proteins.[15,16,140] This work has relied mainly on plastid transformation, a logical approach given the complexity of expressing and assembling carboxysome proteins from nuclear genes. However, this highlights a requirement for more widespread capability to transform $C_3$ crop chloroplasts.[144]

To function, carboxysomes require the concentration of substrate $HCO_3^-$ in the stroma.[145–147] In cyanobacteria this is achieved via plasma membrane $HCO_3^-$ pumps and the absence of CAs outside of the carboxysomes. Approaches to mimic this have focused on targeting $HCO_3^-$ transporters to the chloroplast inner envelope membrane (IEM), so that stromal $HCO_3^-$ can be actively accumulated. Transporters have been successfully targeted to the chloroplast IEM,[148–153] but thus far no improvements in plant productivity have been attributed to increased chloroplastic $HCO_3^-$.

These efforts mark incremental, yet remarkable progress toward $HCO_3^-$ transport into $C_3$ chloroplasts. Indeed, initial efforts to solve this engineering challenge were focused on protein targeting,[149,151] but the challenge has shifted to achieving transporter function in plants. Recent work suggests that directed evolution may help to enable $HCO_3^-$ transport in heterologous systems.[146] Progress toward this element of the CCM strategy has been slower than expected, highlighting the need for better understanding of $HCO_3^-$ transporter biology.[147]

Establishing and maintaining a chloroplastic $HCO_3^-$ pool is essential for CCM function.[147] To this end, the essential step of eliminating stromal CA enzymes has been achieved in both *Arabidopsis*[154] and tobacco,[155] creating foundational plant lines for building and testing CCM components. Engineering carboxysomes into crops remains promising, but key hurdles must be overcome.[156] These include optimizing $HCO_3^-$ transporter function, ensuring functional carboxysome biogenesis, and integrating the system into tractable crop genomes.

## Engineering pyrenoids into crops

Another promising approach for engineering a CCM into crops is based on the pyrenoid, an organelle found in the chloroplasts of nearly all eukaryotic algae.[157–159] The pyrenoid offers several engineering opportunities. As with carboxysomes, it functions at the single-cell level, eliminating the need for engineering leaf morphology and organelle localization associated with C4 and C2 mechanisms.[160] Additionally, pyrenoid-based CCMs may not require chloroplast envelope transporters,[14] which, as noted above, have posed challenges in carboxysome-based CCM engineering. Moreover, components of the pyrenoid-based CCM are natively encoded in eukaryotic nuclear genomes and targeted to chloroplasts,[152] which is compatible with established crop nuclear genome engineering approaches. This contrasts with carboxysome systems, whose prokaryotic origins preferably require chloroplast genome engineering, which is yet to be achieved in monocots,[161] or re-engineering all components for chloroplast targeting from the nucleus.

Despite these opportunities, a challenge of the pyrenoid-based CCM is that it remains relatively poorly understood, especially in comparison to the extensively studied carboxysome-based CCM. Pyrenoid-based CCMs are convergently evolved; thus, while general principles are thought to be similar between lineages, the specific genes that mediate functions in each lineage are different. The best-understood pyrenoid is that of the

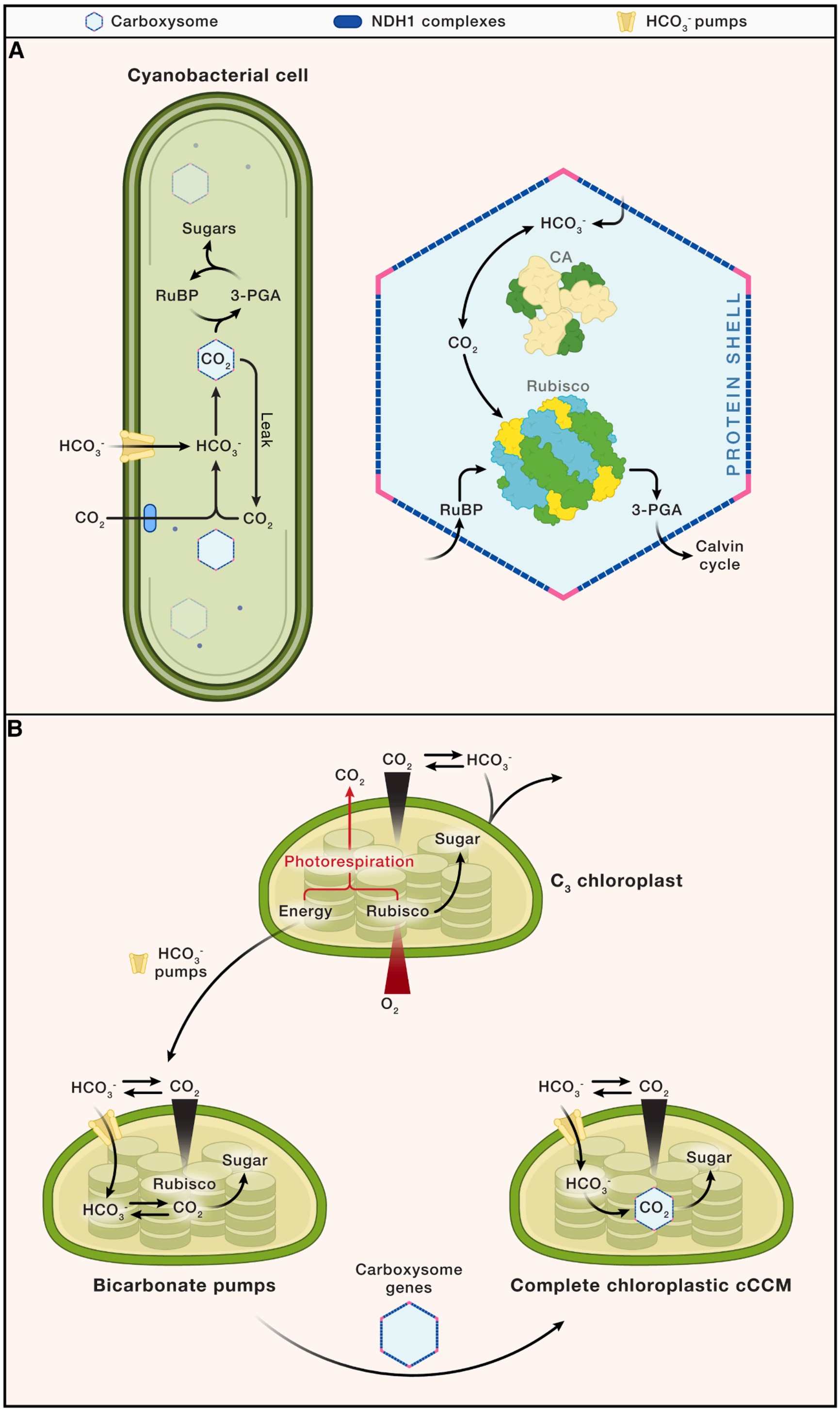

**Figure 2. Incorporation of a cyanobacterial carboxysome into plants**

(A) Schematic of a cyanobacterial cell featuring a $CO_2$-concentrating mechanism. Rubisco is enclosed within the carboxysome, where elevated $CO_2$ levels are maintained by active bicarbonate ($HCO_3^-$) uptake via membrane transporters and conversion by a carboxysomal CA. A specialized NDH-1 complex recaptures $CO_2$ that leaks from the carboxysome, converting it back into bicarbonate.

(B) A typical $C_3$ plant chloroplast, where Rubisco reacts with both $CO_2$ and $O_2$, leading to photorespiration. Engineering efforts have independently introduced bicarbonate transporters pump to increase $CO_2$ concentration in the carboxysome. The bottom shows a typical $C_3$ chloroplast where both $CO_2$ and $O_2$ react with Rubisco. Bicarbonate pumps and carboxysome genes into $C_3$ plants, and the latter enabling α-carboxysome assembly, have been successfully inserted into $C_3$ plants to engineer α-carboxysomes in plant chloroplasts (bottom).

model green alga *Chlamydomonas reinhardtii*, benefiting from an extensive parts list[162–164] and molecular characterization of many of the key components. This depth of understanding, together with the evolutionary closeness of *Chlamydomonas* to land plants, has made this system a focus of current plant-engineering efforts.

Pyrenoids comprise three core substructures: a Rubisco-containing matrix, specialized $CO_2$-delivering membranes, and $CO_2$ leakage barriers. The pyrenoid matrix forms by phase separation of Rubisco with a linker protein,[165–167] This has been reconstituted in the model plant *Arabidopsis*,[168] a breakthrough step in realizing pyrenoids in higher plant chloroplasts. $CO_2$-delivering membranes traverse the matrix, and recent studies identified two key components, enabling partial reconstitution in plants.[169] These membranes will need to be functionalized for $CO_2$ delivery, which is expected to require expression of $HCO_3^-$ transporters[170] and targeting of a CA. Finally, modeling indicates that although $CO_2$ leakage barriers around the pyrenoid—comprised of starch, membranes, and/or proteins—are not essential for function, they will be crucial for maximizing energetic efficiency.[14] Encouragingly, starch has been successfully recruited to the matrix in plant cells using *Chlamydomonas*-derived proteins.[171]

Current efforts focus on assembling a minimal, functional pyrenoid-based CCM in plants using characterized components.[158] This objective is supported by efforts to discover novel components critical to membrane biogenesis and $CO_2$ delivery and exploring pyrenoids across photosynthetic lineages to identify components that may simplify engineering[172,173] or improve function.[174,175] Establishing a pyrenoid in a model plant should facilitate the transfer of this engineered system into diverse crops.

## CROP LEAF CANOPIES

Optimizing light utilization is a key target for improving crop photosynthesis and yield. The ancestors of most of our major food crops grew largely as isolated plants in semi-arid and nutrient-limited environments, such that they would have evolved as plants in which most or all leaves were exposed to full sunlight and shading was rare. Breeding and agronomic improvements have resulted in dense monotypic stands where leaf area index (LAI), the area of leaf above a unit area of land, can often exceed 5 or 6. In effect, modern agriculture has taken plants adapted to photosynthesizing in full sun and created a situation where most leaves are shaded. Such high-density stands are a recent construct of modern agriculture but would have given little time for predominantly sun plants to become more adapted to shade.[48] Despite such stands being a key part of higher yields, there are several lines of evidence that modern crop canopies are far from optimized for maximizing photosynthetic carbon gain. In a crop field, high-light levels at the top of a canopy progressively decrease to near darkness at the bottom of a dense canopy.[176] This presents a conundrum, as light energy is present in excess for optimal photosynthesis at the top of the canopy and deficient at the bottom of the canopy. While visible light (400–700 nm) is strongly absorbed, including green light, near-infrared (NIR, >700 nm) is largely transmitted such that while starved of visible light, the lower crop canopy is bathed in NIR. Some cyanobacteria contain chlorophylls d and f, which can utilize NIR to almost 800 nm in their photosynthesis. Given the structural similarity of these chlorophylls to those of plants and deep knowledge of the biosynthetic pathways, engineering these into crops appears feasible. While this has not been achieved, a simulation that incorporated these into a digital twin of an actual soybean crop throughout its growth period in the field suggested they could result in a 26% increase in crop photosynthetic carbon gain over the growing season.[177]

### Modifying crop canopy architecture and composition

Both structural and biochemical approaches for improving light use in crop canopies have seen promising results over the past decade. Shoot architecture, defined by the three-dimensional aerial display of stems, leaves, and reproductive structures, together with pigment composition and concentrations determine the spatial patterns of light interception and carbon gain within the canopy.[11,178,179] Domestication and selection dramatically shaped shoot architecture in crops. In the Green Revolution, semidwarf, high-yielding varieties were selected for less stem and more seeds also responding favorably to nitrogen fertilizer without lodging. This resulted in a more compact leaf canopy and more intense shading, further exacerbated by subsequent increases in LAI.[180] In parallel, many genes that regulate plant height and tiller number have been described and manipulated to mitigate the effects on canopy light distribution.[181–183]

Leaf angle, the angle between the vertical stem and the midrib of the leaf blade, is a critical trait to maximize photosynthesis and enable high planting density in cereals.[184,185] More erect leaf angles at the top of the canopy improve light distribution, allowing more light to reach lower canopy leaves, while decreasing the heat load on the upper leaves and lowering the need for dissipation of excess energy as non-photochemical quenching of chlorophyll fluorescence (NPQ). Such modifications are associated with greater seed yields in rice,[186] wheat,[187] and maize.[188,189] There is significant genetic variation in leaf angle in cereals, and many genes controlling leaf angle are known, which enables transgenic approaches to optimize leaf angle throughout the canopy.[184,185,190] Downregulation of liguleless transcription factors (*lg1* and *lg2*) in sorghum reduced leaf angle, improved canopy light distribution, and increased both yield and WUE.[191] Recently, the *leaf angle architecture of smart canopy 1* (*lac1*), which encodes a brassinosteriod C-22 hydroxlase, was identified in maize. *lac1* mutants have erect leaves in the upper canopy, less erect leaves in the middle, and flat lower canopy leaves owing to an interaction of phytochrome photoreceptors, the transcription factor RAVL1, and *lac1*, which together regulate brassinosteroid levels.[189] This transition from erect leaves at the top of the canopy to horizontal at the base is projected to be optimal for maximizing crop photosynthetic efficiency. *lac1* mutants achieve this ideotype of the so-called "smart canopy." An alternative, or complementary, approach to enabling a better distribution of light through the canopy is to utilize the deep knowledge of chlorophyll biosynthetic and packaging pathways to reduce visible light absorption, which is typically 80%–90%, in the upper leaves.[99,176]

Erect upper canopy leaves in maize enable higher planting density and LAI. In a test of 146 maize varieties, high yield was associated with high LAI and leaf area duration, a measure of the longevity of the plant canopy over time.[192] When the canopy is developing, high LAI maximizes light interception and competitive advantage against weeds.[180] However, there is a trade-off between the size of the canopy and the respiratory burden, so optimal canopies maximize light interception at maturity with as low an LAI as possible for intercepting the available light, thus conserving resources.[180] In broadleaf crops like soybean or cowpea, LAI may be too high for optimal photosynthesis and productivity in current and future atmospheric environments.[193,194] A simple way to modulate LAI may be to reduce leaf area. In broadleaf crops like soybean, lanceolate leaf shape decreases LAI[195] and can improve light distribution throughout the canopy.[196] As for other architectural traits, the genes controlling leaf shape have been well-described. For example, the *GmJAG1* gene, encoding a JAGGED-like transcription factor, determines leaflet shape in soybean. A single-nucleotide polymorphism within the ethylene-responsive element binding factor-associated amphiphilic repression (EAR) motif of *GmJAG1* causes an amino acid substitution, which disrupts its repressor function and results in a lanceolate leaf phenotype.[197,198] When the lanceolate leaf phenotype was introduced into soybean, a significant reduction in LAI was obtained with no penalty to yield.[199] This approach of decreasing leaf area without changing the number of nodes where reproductive structures form should potentially enhance photosynthetic, water use, and nitrogen efficiency.[199] Canopy architecture is a genetically complex trait and interacts with the environment, so different architectures will almost certainly be needed to optimize yield in different growing regions.

### Accelerating adjustment of NPQ to sun-shade transitions

In full sun the light energy absorbed by leaves is typically in excess of that which can be used in photosynthesis and can result in photooxidative damage and inhibition of photochemical efficiency.[200,201] To prevent this, light-harvesting photosynthetic pigment antennae invoke intricate feedback de-excitation mechanisms, observable as NPQ. In crops, pronounced asymmetry exists between the rapid induction versus much slower rate of relaxation of NPQ, providing an inbuilt memory to anticipate future stress conditions. However, this memory may be too conservative for the situation in farmers' fields, where nutrients and water are more abundant[202] than in natural ecosystems. Indeed, early modeling work predicted that slow NPQ relaxation could represent a significant loss of canopy $CO_2$ assimilation of up to 30%.[48,203] Major progress has been made in the last decade to put this hypothesis to the test.[204–208] Initial attempts focused on overexpressing the thylakoid pH sensor photosystem II subunit S (PsbS), which upon acidification of the lumen promotes induction of energy-dependent quenching (qE), a rapidly relaxing form of NPQ. PsbS overexpression in the crop plant rice manifested a trade-off between the benefits of enhanced protection[204] and the disadvantages of competition with $CO_2$ assimilation.[205] The latter may go beyond energetic demands, since PsbS overexpression unexpectedly gave rise to dampened stomatal opening[206] due to its impact on chloroplast-derived $H_2O_2$ signals,[207] providing a new strategy to reduce crop water use.[208]

A subsequent strategy targeted both qE and qZ,[209] a more sustained form of NPQ associated with the abundance of zeaxanthin (Zx), via combined overexpression of violaxanthin de-epoxidase (VDE), zeaxanthin epoxidase (ZEP), and PsbS. Proof of concept of this VPZ strategy was first demonstrated in tobacco, which showed enhanced photochemical efficiency and biomass productivity,[210] and subsequently in soybean, where benefits also extended to enhanced seed yield.[211] Whereas both of these studies evaluated phenotypes in field-grown plants, similar VPZ attempts in potato[212] and *Arabidopsis*[213] grown under greenhouse conditions or artificial light regimes were less successful, replicating some of the impacts on NPQ kinetics but not translating into growth or yield. As noted in the study of potatoes, a challenge is balancing the increases in VDE and ZEP such that relaxation on sun-to-shade transitions is accelerated without raising NPQ in high light, which would compete with $CO_2$ assimilation.[212] This is difficult to obtain with simple addition of transgenes with promoters and may be more successful with newer technologies allowing tuned gene expression.[214]

The VPZ results demonstrate that re-epoxidation of Zx is key to accelerate NPQ relaxation,[215] but questions remain with regard to the precise mechanisms underlying Zx-dependent quenching. Significant progress has been made in determining the relative contributions of quenching sites on monomeric and trimeric LHCII,[216] but the relative importance of Chl-Zx charge or exciton transfer mechanisms[217–219] versus allosteric effects of Zx to promote aggregation of LHCII trimers[219] is still subject to debate.

While further understanding could advance targeted NPQ engineering strategies, mining the existing allelic variation in crop germplasm provides an alternative route. In an early study in rice, the strongest quantitative trait locus (QTL) was associated with *cis*-genic variation upstream of PsbS.[220] However, more recent work in maize,[221,222] sorghum,[29,223–225] and poplar[29] uncovered a plethora of novel targets. To facilitate rapid translation, multiplexed editing strategies targeted to *cis*-genic non-coding regions can create novel non-GMO expression level variants[225] in any crop of choice to enable faster relaxation of NPQ via QTL stacking or genomic prediction strategies.

## MAXIMIZING AND ACCELERATING GENETIC IMPROVEMENTS IN PHOTOSYNTHESIS

Table 1 and Figure 3 summarize the bioengineered manipulations to improve photosynthetic efficiency achieved or underway and what might be expected going forward. In the 10 years since the prior review of the topic,[8] six of the forecast potential improvements have now been realized, four with double-digit increases in productivity or yield in at least a single replicated field trial of multiple events. This progress is just the first step toward placing these improvements in farmers' hands. These strategies must deliver repeatable increases in more years and across different environments and be tuned to maximize their potential in different environments and genetic backgrounds. One manipulation, photorespiratory bypasses, has made the most progress in this respect in that it has been demonstrated in at least 3 crops, including different locations and multi-year trials (see above). Where transgenes are involved, deregulation must follow, and then the manipulation must be bred into elite cultivars that are adapted to different climate zones and that are acceptable and suited to regional markets.

To achieve a stronger basis for comparison of the proposed photosynthetic manipulations, the potential benefit of each of the suggested improvements was tested using a 3D soybean model that estimates canopy photosynthesis on a clear sky day.[28,226] Fourteen changes were substituted into the leaves, and total photosynthesis for the day was calculated for a single elite soybean cultivar at one point of growth (supplemental methods), so clearly results will likely differ for other cultivars, days, and species. For example, a 5% improvement in canopy photosynthesis was predicted for manipulation 15 (Table 1), yet for another elite cultivar, using the same manipulation, the improvement was 11.5%,[226] reflecting differences in canopy form. Each manipulation required several assumptions (Table S1), which, in the case of strategies requiring more discovery (U in Table 1), may not hold up. However, this modeling provides a common framework for comparisons. As such, it indicates that by far the greatest prize would come from integrating any one of the CCMs into C3 crop leaves. By comparison, the predicted gain in daily canopy $CO_2$ assimilation for the achieved manipulations is largely single digit, with the exception of canopy architecture. In most cases, however, the observed increase in productivity or yield exceeds the predicted increase in canopy photosynthesis (Table 1). The predicted increase is for a critical time point, early seed fill. If photosynthetic efficiency is improved throughout the lifetime of the crop, this could result in more reserves and reproductive initials, so gains could be cumulative. Given that annual yield improvements currently achieved in our most intensively bred crops are 1%–2%, a 5%–10% improvement on top is substantial. Further, the improvements achieved so far involve fewer genes than CCMs, and critically, they appear additive such that gene stacking of

**Table 1. The major ongoing or achieved genetic improvement strategies for improving crop photosynthetic efficiency**

| Manipulation | | Type | Predicted canopy daily % gain c/e | Obs. leaf Psn. % Inc | Avg obs % gain in biomass prodn (p) or yield (y) | Time scale | Additional benefits |
|---|---|---|---|---|---|---|---|
| 1 | increase BS Rubisco content $C_4$ | Syn, Ed, B | 10/10 | So 14[13] | So 15(p)[13] | A | $C_4$ |
| 2 | increase Rubisco content $C_3$ | Syn, Ed, B | 8/7 | R 14[12] | R 6(p),[12] R 8(y)[12] | A | $C_3$ |
| 3 | increase mesophyll conductance | Syn, Ed, B | **5**/4 | T 8[123] | T 4(p)[123] | A | $C_3$ >>WUE: >NUE |
| 4 | add algal $CO_2$/HCO3 pumps | Syn | 3/1 | – | – | U | $C_3$ >WUE: >NUE |
| 5 | convert $C_3$ crops to $C_4$ | Syn | 49/32 | – | – | U | $C_3$ >>WUE; >NUE |
| 6 | convert $C_3$ crops to C2 | Syn | NS | – | – | U | $C_3$ >WUE; >NUE |
| 7 | add cyanobacterial carboxysome system | CSyn | 68/49 | – | – | U | $C_3$ >>WUE; >NUE |
| 8 | add algal pyrenoid system | Syn | 82/60 | – | – | U | $C_3$ >>WUE; >NUE |
| 9 | more efficient Rubisco engineered | CSyn, Syn | 11/7 | – | – | M | $C_3$ >WUE; >NUE |
| 10 | more rapid induction on shade-sun transition with improved Rca | Syn, Ed, B | 6/4 | – | – | S | $C_3$,$C_4$; >WUE; additive with all |
| 11 | synthetic photorespiratory bypasses | Syn | 9/9 | T 17,[97]<br>P 14,[110]<br>R 15[108] | T 22(p),[97]<br>P 33(y),[110]<br>R 29(y)[108] | A | $C_3$ >WUE |
| 12 | faster RuBP regeneration | Sys, Ed, B | 4/8 | T 10[94] | T 38(p)[94] | A | $C_3$,$C_4$ >WUE; additive with all |
| 13 | optimize canopy architecture | Sys, Ed, B | 21/18 | – | So 27(p),[191]<br>S 7(y)[199] | A | $C_3$,$C_4$ >>WUE; >NUE additive with all |
| 14 | extend usable spectrum of crop photosynthesis into NIR | Syn | 19/19 | – | – | U | $C_3$,$C_4$; >WUE; additive with all |
| 15 | more rapid relaxation of NPQ on sun-shade transitions | Syn, Ed, B | **5/5** | S 20,[211]<br>T **15**[210] | S 16(y),[211]<br>T 18(p)[210] | A | $C_3$,$C_4$; >WUE; additive with all |

Manipulation: change being undertaken.

Type of genetic manipulation: CSyn, synthetic addition of foreign genes to the chloroplast or plastid genome; Syn, synthetic addition to the nuclear genome; Ed, up- or downregulation by editing the upstream region of existing genes; and B, improvement tractable by breeding given adequate molecular markers and variation within germplasm.

Predicted daily canopy photosynthetic $CO_2$ assimilation at current 2025 [$CO_2$] c = 420 ppm and for 2050, e = 500 ppm. Predictions were made by substituting each manipulation into a digital twin of a soybean crop canopy of an elite cultivar (LD11-2170) at early seed fill on a clear sky day at an air temperature of 25°C.[28,226] NS, not simulated.

Obs. leaf Psn. are the average observed increases in leaf photosynthetic $CO_2$ uptake in field trials, where the manipulation has been achieved.

Avg obs % gain is the observed average increase across different transformation events in terms of commodity product yield (y) or shoot dry biomass (p) from field trials for R, rice; S, soybean; So, sorghum; and T, tobacco.

Timescale is an estimate of time to test of concept in one cultivar in at least one replicated field trial: A, achieved; M = 5–10 years; S, short term <5 years; and U, uncertain, as more discovery will be needed for implementation, but likely within 20 years.

Additional benefits indicated whether the manipulation will benefit $C_3$ and/or $C_4$ crops, is additive to other changes, and will improve water use efficiency (WUE) and/or nitrogen use efficiency (NUE).

Strategies discussed in the manuscript that could increase canopy photosynthesis are illustrated in Figure 3. The potential increase in daily carbon gain by incorporating each strategy into soybean is estimated in this table.

manipulations 2, 3, 11, 12, 13, and 15 of Table 1 could increase photosynthetic carbon gain by 52%.

Increasing photosynthetic efficiency can only have value if the crop is able to utilize the additional photosynthate in the harvested material, i.e., there is sufficient sink to utilize the additional source. In particular, there is potential to utilize additional photosynthate in the harvested product. Growth of $C_3$ crops under elevated [$CO_2$] artificially increases net photosynthesis by decreasing photorespiration and accelerating carboxylation. This provides a test of whether crops can allocate an increased photosynthate supply to greater productivity and yield. Results of such experiments have shown increased productivity to varying degrees in almost all $C_3$ crops tested, with the largest increases in root crops.[227] Growth of different cultivars of rice and soybean in the field under free air $CO_2$ enrichment (FACE) showed that the most recent cultivars are capable of fully utilizing increases in photosynthesis in increased yield up to at least 30%, contrasting with older cultivars.[228] This suggests that the intensive breeding of these two crops has largely removed sink limitation. It is uncertain that crops that have received less attention can respond as strongly without making more effort to improve their yield potential.

Manipulations 1 through 11 all concern increasing Rubisco carboxylation rates, either by increasing the amount of active

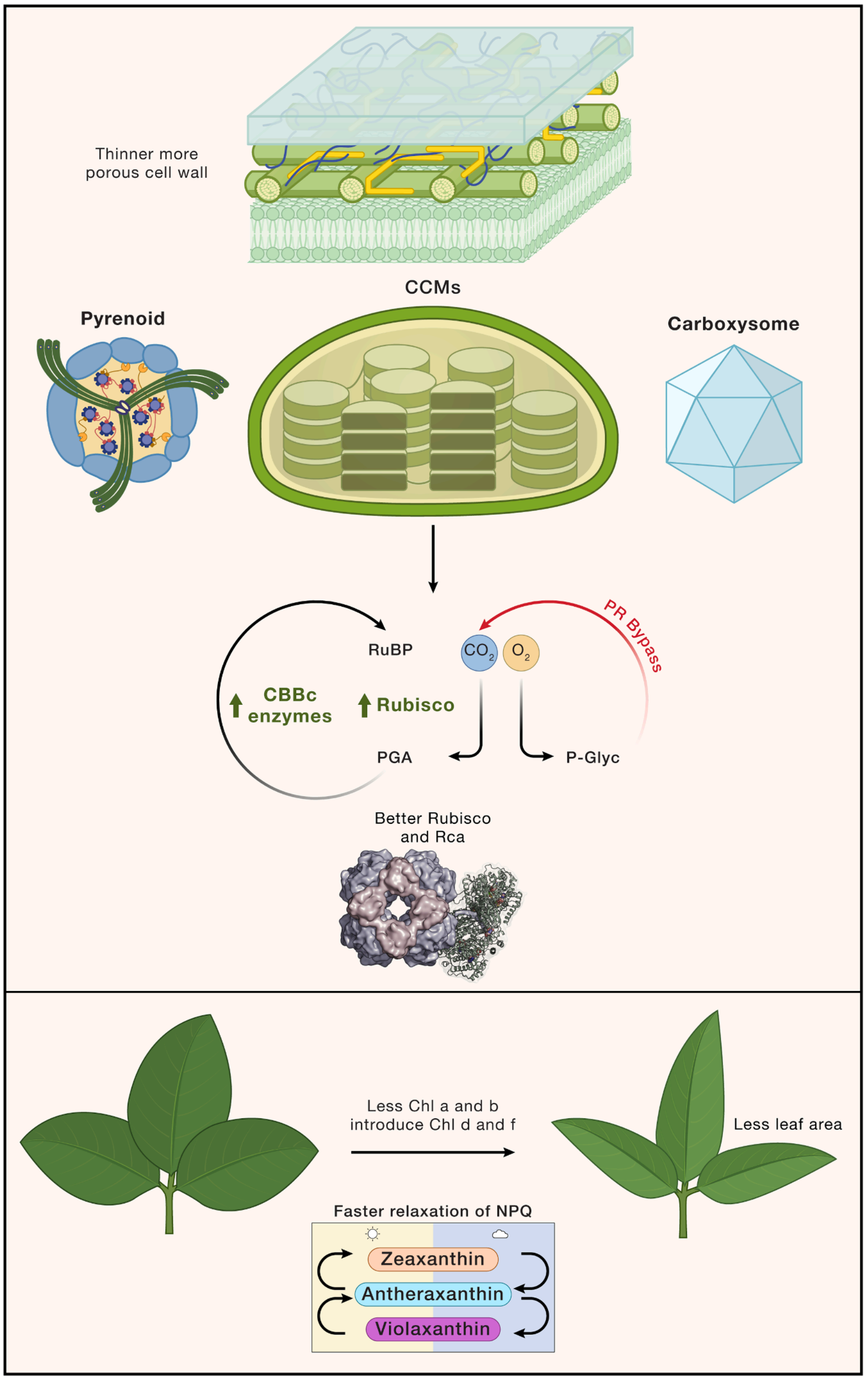

**Figure 3. Different bioengineering strategies to increase photosynthesis**
Strategies discussed in the manuscript that could increase canopy photosynthesis are illustrated in the figure. The potential increase in daily carbon gain by incorporating each strategy into soybean is estimated in Table 1.

enzyme, making the enzyme more efficient, inhibiting oxygenation, or decreasing the energetic costs of photorespiration resulting from oxygenation (Table 1). Except for manipulation 1, the predicted increase in canopy photosynthesis is slightly less at elevated [$CO_2$] (e = 500 ppm), reflecting increased competitive inhibition of oxygenase activity by rising [$CO_2$]. The predictions were made for a constant 25°C. However, with the concomitant rise in temperature, which favors oxygenase activity, gains might be similar to or lower than those predicted for current conditions.[99]

While it might be tempting to suggest focus should be on the predicted large prizes that CCMs could deliver, the smaller gains already achieved by other manipulations are much further along the pipeline toward seed for farmers and are potentially additive. In particular, the combined benefits of faster RuBP regeneration (manipulation 12) and increased Rubisco carboxylation (manipulations 1–11) may be required to realize enhanced canopy photosynthesis[229] of $C_3$ crops under future climate conditions, as only manipulation 12 is predicted to significantly increase canopy photosynthesis at elevated [$CO_2$] (Table 1). Manipulations 5 through 8 are not additive, since achieving one CCM would largely nullify the other. However, given the promise and future need, all four should be pursued vigorously, given the uncertainty in what may be needed to effectively install these systems. Further, it may be that different crops may be more amenable to one or the other of these CCMs. Manipulations 3 through 11 all potentially increase NUE, since here more carboxylations will be achieved per unit of Rubisco. For example, to match the maximum carboxylation rates of wild-type plants, plants containing carboxysomal CCMs require only ∼37%–58% of leaf N for carboxylation compared with wild type, enabling energy and N to be redirected to bicarbonate uptake.[229]

Manipulations that have increased biomass productivity or yield in the field have generally resulted considerably earlier than was predicted 10 years ago,[8] reflecting ever-improving technologies for genetic transformation, DNA editing, and high-throughput phenotyping. What can we expect going forward? Given the rapid progress in discovering the genes needed, it seems highly likely that with continued commitment at least one of the CCMs (5–8, Table 1) will reach the successful test-of-concept stage within 10 years, i.e., be demonstrated in a crop in at least one field replicated trial. Similarly, utilization of longer wavelength chlorophylls to extend the spectrum of solar radiation used by crops might be achieved in this time frame.

Several steps following the test of concept are needed for the achievements made or anticipated to make a difference in food, feed, and bioproduct supply. Although transgenic crops are gaining increased acceptance, still in 2023, only 27 of the world's 195 countries planted transgenically modified crops.[230] Even in these countries, gaining approval for planting a single crop with one modified trait is a lengthy and expensive process.[231] Except for photorespiratory bypasses, all of the achieved improvements in Table 1 could potentially be achieved by altering the expression of the target genes through precise editing of important *cis*-regulatory elements within the promoter, for example, by CRISPR-CAS9.[232] Such editing is increasingly, but not universally, accepted as equivalent to conventional breeding, and so this would shorten the time and cost of bringing these to market.[233] Advantaged traits, whether obtained with transgenes, edits, or existing alleles discovered within the germplasm, will only have value if bred into regionally appropriate and accepted elite cultivars. This requires a substantial plant breeding effort, and one challenged by current limited public domain capacity.[4] The advents of speed breeding, which reduces generation time, genomic selection, and high-throughput phenotyping of photosynthetic traits in the field could considerably accelerate breeding for photosynthetic efficiency.[234] These manipulations will be present throughout the life of the plant, so initial selection can be made with photosynthetic probes at the seedling stage, greatly reducing the amount of material that will need to be grown out.[210] Simulation models that use phenology, flowering time, and photosynthetic properties as their major inputs and predict the potential yield under different growth regions can narrow the appropriate manipulations and stacking options.[235] All this will depend on adequate numbers of breeders trained in, and equipped with, these technologies and successful integration of breeding and photosynthetic phenotyping.

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We dedicate this work to the memory of Dr. A.M.-C. and Dr. S.P.L., whose vision, insight, and passion for plant science profoundly shaped this research and continue to inspire all of us.

## AUTHOR CONTRIBUTIONS

S.P.L. outlined the topics for the review. All authors contributed to the text, figure development, and editing of the manuscript.

## DECLARATION OF INTERESTS

The University of Illinois, Lancaster University, and Princeton University have current and pending patent applications associated with the proposed improvements to photosynthetic efficiency.

## SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cell.2025.10.033.

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