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Genome engineering of plant photosynthesis for carbon sequestration

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Abstract

Faced with a changing climate, there is increased scientific interest in the use of biotechnology for capturing and storing atmospheric carbon. Plant genome engineering and editing, and specifically the improvement of photosynthesis, is an important avenue by which this might be accomplished at scale. In this review, we identify the main agronomic and agroecological opportunities and challenges for plant-based carbon dioxide removal and discuss the molecular and physiological adaptations required for implementation. Using examples from evolutionary genetics, comparative genomics, and metabolic modeling, we evaluate the theoretical basis for improving photosynthesis. Downstream of carbon capture itself, we discuss the potential of engineering plant physiology to drive biomass accumulation, improve nitrogen and water use efficiency, and facilitate stable carbon storage. Finally, we emphasize the outsized opportunity for using gene editing in improving photosynthesis and identify the outstanding technical frontiers in this field of study. We highlight how utilizing gene editing approaches, which in many countries is presently or imminently exempt from biosafety regulations, will enable researchers to better understand and improve plant-based carbon sequestration via enhanced photosynthesis, ultimately expediting the engineering of carbon crops.

Key Points

- Plant photosynthetic adaptations have evolved slowly to adapt to Earth's climate, and rapid adaptation is needed to keep pace with climate change
- Photosynthesis is theoretically and empirically inefficient, and the genetic basis of this inefficiency is in part known
- Gene-edited plants could be used for carbon dioxide removal; photosynthetic improvements would speed up this process
- Plant-specific genome editing technology is rapidly developing
- Genome editing can leverage decades of transgenic research to improve limits on existing carbon crop yields and open up new opportunities in alternative crops

Introduction

Earth maintains at least 99.9% of its carbon in its core, mantle, and crust, the remainder of which cycles between oceans, the atmosphere, and living organisms (Figure 1)¹. During 3.5 billion years of life on Earth, cycling carbon has dynamically transitioned between these reservoirs, modulating ocean temperature and surface climate, and prompting Earth's organisms to continuously evolve the mechanisms by which they live off of atmospheric carbon^{2–4}. Over the past 250 years, atmospheric CO₂ levels have steeply increased through the burning of fossil fuels, a large change that is unprecedented in the geological record⁵. This geochemical shift has altered Earth's carbon cycle, prompting changes in global mean surface temperatures, increasing the incidence of extreme weather events, accelerating biodiversity loss, and threatening global agricultural productivity⁶. Even under the most optimistic trajectories, combating these effects will involve not only decarbonization and emission reduction, but also the intentional sequestration and removal of atmospheric carbon (IPCC 2023).

Towards this latter aim, carbon dioxide removal (CDR) describes approaches that capture carbon from the atmosphere and sequester it in Earth's geochemical reservoirs. In considering different approaches for scaled CDR, land plant photosynthesis, which draws down 112–169 GtC (1GtC = 10¹⁵ g carbon) per year⁷, is an indispensable technology. Outside the energy sector, agriculture and agroforestry steward more managed carbon than any other human industry, and some of the most salient and inexpensive proposed interventions for CDR involve the propagation of genetically improved carbon crops. Leveraging state-of-the-art advances in CRISPR technology, functional genomics, artificial intelligence, and agroecology, decades of research on improving photosynthesis might be implemented at scale.

In this review, we identify and describe the agricultural domains wherein engineered plants might be used for CDR and make the case that engineering photosynthesis can be indispensable in ensuring the techno-economic feasibility of these approaches. While some previous efforts to genetically engineer and improve photosynthesis have been successful, the agricultural implementation of these approaches has been inhibited by the regulation of transgenic crops. Following the increasing global regulatory acceptance and rapidly progressing technical landscape of CRISPR gene editing⁸, we emphasize the potential for obtaining photosynthetic improvements with small, *cis*-genic mutations. We discuss the experimental and theoretical findings indicating that photosynthesis can be improved, and outline specific genetic targets that might be used for improving carbon crops. Downstream of carbon capture, we discuss the plant physiological and agroecological considerations related to soil carbon retention and biomass processing. We describe the state of the art in crop gene editing as it applies to amending the metabolic efficiency and physiology of carbon crops. In particular, we discuss current and outstanding technical developments in CRISPR reagent delivery, the use of machine learning and functional genomics in CRISPR target selection, and the imperative to align engineering efforts with regulation around biotechnology-derived crops. We believe the coming years present an unprecedented opportunity to translate decades of photosynthesis research in the field.

Section 1. Opportunities for plant-based carbon sequestration

As new carbon offset markets have generated demand for large-scale CDR,

technological approaches for gigaton-scale carbon sequestration have developed across disciplines⁹. In this budding field, using plants for scalable carbon sequestration is an attractive option, owing to their low costs of scalable propagation and the high ‘technology readiness level’ of agricultural CDR¹⁰. Land plants alone account for ~80% of Earth’s biomass^{11,12}, and the ecosystems they support constitute some of Earth’s most significant terrestrial carbon stores. By some estimates, land plant carbon reservoirs could be doubled in mass (from 450 GtC to ~916 GtC) by agroecological adaptation and forestry maintenance¹³. Land plants perform ~50% of global photosynthesis¹⁴, and compared to other photosynthetic organisms – photoautotrophic bacteria, algae, and aquatic plants – they are extraordinarily effective at converting CO₂ into stable biomass. However, of the estimated ~120 Gt CO₂ captured each year by photosynthesis in terrestrial ecosystems, to a first approximation the entirety is lost to plant (60 Gt/year) and microbial (60 Gt/year) respiration¹⁵. Even so, plants are central to agricultural and ecological management, and innovating on the ways they are grown, harvested, and processed could reduce these respiratory emissions and help transition the global carbon cycle from a resource-extractive model to a circular bioeconomy¹⁶. Across crops and cropping systems, opportunities to use gene-edited plants for CDR take three forms: 1) engineering existing agricultural crops to accelerate carbon sequestration into soil, 2) generating bespoke carbon crops for post-harvest carbon removal and storage, and 3) engineering trees and woody crops for accelerated agroforestry.

In terms of land area, the most scalable opportunities for plant CDR involve engineering crops to replenish and remediate soil organic carbon (SOC). Soil accounts for approximately 80% of all stored carbon on land¹⁷, and since the introduction of agriculture, an estimated 133 Gt of carbon has been lost from soils¹⁸. This loss is most pronounced in wild lands converted to cropland and pasture¹⁹ and the rate of loss is rising as a burgeoning human population increases the demand for food and fiber. Mechanistically, the loss of SOC is attributable to increased biomass allocation towards harvested plant material rather than soil, soil erosion from agricultural management, and the oxidative degradation of soil organic matter from agricultural processes²⁰. Warming climate conditions have exacerbated soil carbon loss by increasing microbial respiration²¹, and this effect is predicted to accelerate in response to climate warming^{22,23}. As such, modifying crops and crop systems to remediate SOC is a high-priority CDR opportunity in croplands and pasture, where improvements in soil carbon retention also stand to improve soil fertility and long-term agronomic yield²⁴.

Engineering efforts to improve carbon sequestration in food crops have operated on several aspects of plant physiology, including improving root depth and durability, improving intrinsic water and nitrogen use efficiency, and modifying root exudates to shift the physical and microbial constitution of soil beds¹⁵. In these approaches, photosynthetic efficiency improvements are valuable to ensure that biomass allocation to soil does not come at the cost of harvestable yield and the economic viability of the resultant plant. Excess biomass from enhanced photosynthesis can help mitigate the tradeoff between yield and soil carbon remediation. In addition to the sequestration potential of plants fortified for photosynthesis, genetic improvements to photosynthesis frequently pleiotropically improve abiotic stress tolerance and agronomic yield.

Beyond soil sequestration, engineering plants for the explicit purpose of farming carbon has been the focus of significant research. Under one approach termed biomass carbon removal and storage (BiCRS), purpose-grown carbon crops, as well as agricultural waste and forest litter, are processed for carbon storage. This can be accomplished via a number of

modalities, all of which involve conversion of above-ground biomass into a geologically stable form. For one example, pyrolyzing crude biomass produces bio-oil (which can be deposited into exhausted oil wells), carbon gas (which can be filtered to pure CO₂ and pumped into underground water and sediment deposits), and biochar (which can be used as an agricultural input) (Figure 1). From a crop engineering perspective, farming purpose-grown carbon crops for BiCRS has the potential to expand the range of crops and crop systems that can be used for plant-mediated carbon drawdown, as carbon crops can be selected for different characteristics and ecological preferences than crops grown for food and fiber. Many prospective carbon crops might not have been bred for biomass production, so increasing photosynthetic efficiency is an important lever by which they can be rapidly improved for cost-competitive carbon offset potential.

Engineering photosynthesis for CDR can also be applied to reforestation and afforestation, wherein forests are either restored or established from photosynthetically augmented trees. While genetic engineering in woody crops has been a longstanding interest in plant biology – particularly as it pertains to growth rate, biomass accumulation, and cell wall composition²⁵ – few studies have focused on increasing the carbon assimilatory potential of trees by improving photosynthesis²⁶. Leveraging advances in engineering photosynthesis at forest scale will be dependent on improvements in the approaches to genetically modify trees, which might also be an important technical competency in fortifying trees to fire, abiotic, and biotic stress. Beyond the current set of trees grown for carbon agroforestry (e.g., oaks, pines, poplar), potential exists to domesticate other woody plants or convert herbaceous crops to stable perennial forest plants. Although not all plants are woody, all or most flowering plants have the genetic repertoire for producing wood²⁷, highlighting the potential to adapt and reprogram secondary cell wall characteristics beyond what nature has evolved.

Across CDR opportunities, improving photosynthesis stands to improve the assimilatory potential and commercial viability of engineered carbon crops. Below, we explore the fundamental premises by which photosynthesis might be improved and identify the technologies that will enable their creation.

Figure 1. Large-scale opportunities for plant carbon capture and storage

Diagram of the major geological stores of carbon (grey), the ecological venues for plant CDR (blue), carbon-sequestering crops (green), and geological sinks for plant-derived biomass (yellow). Once biomass has been accumulated by farming, carbon cropping, or forestry, it can be used to augment soil carbon and/or be physically processed and stored geologically. A pyrolysis facility is provided as an example of a BiCRS process, where farm waste, purpose-grown biomass crops, and forest litter undergo thermochemical processing. The residual biochar (30-50 wt%) can be used as a soil and pasture amendment. Product gas (10-30 wt%) is composed of CO₂ and in small part syn-gas (CO, CH₄, and H₂) and can be passed into deep saline aquifers for mineralization. Oxidized bio-oil (20-40 wt%) is negligibly usable (biofuel 1-2 wt%, petroleum <0.1%) and can be stored in depleted oil fields using existing petroleum infrastructure. Outside physical processing, the same biomass sources can be buried into deep carbon reservoirs either on land or in the ocean. The carbon permanence, i.e., how long captured carbon remains sequestered without being re-released to the atmosphere, is indicated for each geological store.

Section 2. Inefficiencies and opportunities in engineering photosynthesis

Since their emergence on land ~500 Mya²⁸, plants have readily populated Earth's many diverse terrestrial ecosystems²⁹. Despite their indispensable role in supporting human life, plants are relatively inefficient compared to other human-engineered technologies in their

conversion of solar radiation into chemical energy. The maximum theoretical solar energy conversion efficiency of plant photosynthesis is just 4.6% for C3 plants and 6% for C4 plants^{30,31}, much less than the 18-40% efficiency of modern solar cells defined on similar terms³². Much of this inefficiency is attributable to the evolutionary, biophysical, and genetic constraints on photosynthetic evolution.

Plant chloroplasts evolved through the ancient endosymbiosis of a cyanobacterium and are a plant's engine for converting light into biochemical energy. In brief, chloroplasts are organelles in which photosystems absorb photons to drive photochemical charge separation at the reaction center (RC), initiating electron transfer down the photosynthetic electron transport chain (ETC) and culminating in the production of ATP and NADPH. These energy-rich molecules fuel the reductive Calvin-Benson (CB) cycle, where chemical energy is used to drive assimilation of inorganic CO₂ into plant biomass. Generally, photosynthetic efficiency is thought to be limited in both light harvesting and in the assimilation and utilization of carbon, resulting in less than 1% of total solar energy being converted into harvestable yield³³. The yield potential of crops is classically described by the following equation where $Y_p = \text{Energy}_{\text{light}} \times \epsilon_{\text{interception}} \times \epsilon_{\text{conversion}} \times \epsilon_{\text{partitioning}}$ ³³. Here, we explore the mechanisms that contribute to efficiency losses in each of these parameters and provide a quantitative understanding of their comparative impact on photosynthesis based on previous modeling efforts^{30,31}.

2.1 Limits to light harvesting

In terms of light harvesting, plants are limited in their ability to both intercept and utilize available light. In this process, the most significant limit to light capture is in the mismatch of the plant absorption spectrum with the total spectrum of available solar radiation. Plant light-harvesting antenna complexes (LHCs) are limited to a narrow range of the solar spectrum, defined as photosynthetically active radiation (PAR; 400-700 nm), that encompasses only 48.7%-of all available solar radiation³⁰ (Figure 2). Non-plant photoautotrophs demonstrate that this is not a hard biophysical limit for light capture. Cyanobacteria utilize far-red and near-infrared light (700-760 nm) using diverse chlorophylls, and some anoxygenic bacteria capture infrared light (800-1000 nm) with bacteriochlorophylls (Figure 2). Even between plants, variability in carotenoid and anthocyanin content drives efficiency differences in PAR absorption³⁴.

To quantify these differences, plants are evaluated in terms of the total interception efficiency of all PAR, described as $\epsilon_{\text{interception}}$. In a majority of land plants, this parameter is chiefly constrained by the weak or incomplete absorption of photons by light-harvesting pigments. As much as 4.9% of total solar energy is lost through the reflectance or transmittance of potentially harvestable photons^{31,35}. Green wavelengths (500-600 nm), in particular, are reflected as much as 50%, and those green photons that do pass through the leaf are captured with less than 20% efficiency³⁶. Thermodynamic limits related to charge separation at the RC further limit $\epsilon_{\text{interception}}$, as the average energy per mole of PAR photons (205 kJ) is generally higher than what is required to drive charge separation in the photosystems (176 kJ/mol for PSII, 171 kJ/mol for PSI). Much of the residual energy is thus lost as heat. Finally, $\epsilon_{\text{interception}}$ is strongly influenced by canopy architecture and leaf shape, which contribute to a plant's ability to make use of above-ground space for light harvesting. Above-ground architecture has been optimized in some plants by long-term human selection, but not in others³⁶. Given the great

diversity of plant evolutionary strategies and crop canopy environments, $\epsilon_{\text{interception}}$ in different plant species is estimated to range from 0.37 to over 0.9 (which translates to 18% to 43.8% of total solar radiation)^{31,37}, highlighting the potential to derive strategies for engineering light harvesting through inter-species comparison. While many modern crop plants have been improved to near-maximal efficiency in $\epsilon_{\text{interception}}$ ^{31,35}, mechanistic and genetic understandings of this efficiency are essential to rapidly gene edit novel and underdeveloped crops for CDR.

2.2 Limits to carbon capture and utilization

Downstream of light harvesting, the ratio of biomass energy produced relative to the light energy absorbed is described by the efficiency of energy conversion, $\epsilon_{\text{conversion}}$ ³¹. $\epsilon_{\text{conversion}}$ is limited by many processes in both the light and carbon reactions of photosynthesis, and compared to $\epsilon_{\text{interception}}$ has been comparatively unimproved by plant breeding³⁸. In C₃ photosynthesis, limits to $\epsilon_{\text{conversion}}$ are highly dependent on foliar light intensity and CO₂ concentration, and include the rate of rubisco carboxylation ($V_{c,\text{max}}$), the rate of RuBP regeneration (J), and the rate of light reaction-derived triose phosphate utilization (V_{TPU})^{39–41}.

CO₂ carboxylation is principally constrained by the enzymatic rate of CO₂ catalysis by rubisco, the rate of leaf CO₂ diffusion, and the rate of regenerating rubisco's substrate RuBP in the CB cycle. Rubisco itself is Earth's most abundant protein and the entry point for almost all inorganic carbon into biomass. Despite its abundance, rubisco is constrained by its catalytic mechanism for fixing CO₂, which is moderately slow ($k_{\text{cat,C}}=1\text{-}3\text{ s}^{-1}$ in C₃, $3\text{-}10\text{ s}^{-1}$ in C₄) and mistake-prone⁴². Rubisco also inherently reacts with O₂ to initiate a process called photorespiration, which competes with carbon fixation and costs extra energy to recycle downstream metabolic byproducts. Rubisco variants from across the tree of life indicate that there exist kinetic tradeoffs between carboxylation speed and specificity for CO₂⁴³. While estimates vary across species and environments, photorespiration is estimated to limit $\epsilon_{\text{conversion}}$ in plants by as much as 49% in C₃ plants ($\sim 6.1\%$ of intercepted solar radiation)³⁰. In the context of crop yield, this alone can account for a 50% reduction in agronomic productivity (i.e. Yp)⁴⁴.

Given rubisco's imperfect specificity for CO₂, and that CO₂ makes up a minute fraction of the atmosphere, $\epsilon_{\text{conversion}}$ in C₃ plants is limited by CO₂ conductance and diffusion in photosynthetically active mesophyll cells^{45,46}. Decades of experiments growing crops in elevated CO₂⁴⁷ and longitudinal studies of wild plant photosynthesis^{48–50} indicate that improving CO₂ saturation around rubisco can elevate biomass accumulation as much as 18%, largely owing to the suppression of photorespiration.

Beyond carboxylation, suboptimal expression and metabolic flux of certain CB enzymes⁵¹, mismatches between the stoichiometric production of ATP:NADPH and CB cycle demand⁵², as well as triose phosphate utilization (TPU) at high rates of photosynthesis⁵³ further limit $\epsilon_{\text{conversion}}$. In total, these inefficiencies contribute to a loss of 24.6% of all incident solar radiation in C₃ plants and 28.7% in C₄ plants³⁰. Understanding these differences, and paving a way forward for photosynthetic efficiency, is predicated on a deep understanding of the ways plants have adapted to modulate $\epsilon_{\text{conversion}}$ through evolution.

While $\epsilon_{\text{conversion}}$ determines the ratio of total solar energy harvested into biomass, $\epsilon_{\text{partitioning}}$ describes the portion of this total biomass partitioned into harvestable product, and is more commonly referred to as the harvest index (HI)³¹. Despite extensive optimization of $\epsilon_{\text{partitioning}}$ via modern breeding and domestication^{54,55}, this parameter may be further improved

via source-sink engineering (discussed in section 3). Together, understanding the mechanism underlying $\epsilon_{\text{interception}}$, $\epsilon_{\text{conversion}}$, and $\epsilon_{\text{partitioning}}$ provides a theoretical framework for optimizing photosynthesis to maximize use of total solar energy and contribute to the larger goal of carbon sequestration.

2.3 Evolutionary lessons in photosynthetic adaptation

Flowering vascular plants have been immensely successful on land since their emergence 150-250 Mya⁵⁶, and in the intervening geological periods the Earth has experienced wide swings in climate. This has largely been shaped by shifting atmospheric CO₂ concentrations, which in the history of terrestrial plant life have ranged from >2000 ppm at the Triassic-Jurassic boundary (~201 Mya) to 280 ppm prior to the Industrial Revolution⁵⁷. To adapt to changes in CO₂ concentrations, land plants evolved numerous changes in photosynthesis over millions of years⁵⁸. Strikingly, the modern rate of change in atmospheric CO₂ is orders of magnitude faster than what has been observed in previous geological eras⁵⁹. Thus, active human intervention will be crucial in adapting cultivated plants to a warming world with high CO₂.

One central axis of photosynthetic adaptation revolves around rubisco. During periods of low CO₂ availability in Earth's history, diverse photosynthetic organisms have evolved carbon concentrating mechanisms (CCMs), wherein specialized cellular structures, cell types, and metabolic pathways elevate CO₂ levels around rubisco. By minimizing oxygenation reactions, CCMs have allowed rubisco to evolve in the direction of increased carboxylation rate both in plants⁶⁰ and other microbial autotrophs⁶¹. Biophysical CCMs, which are found in autotrophic bacteria⁶², eukaryotic algae⁶³, and hornworts⁶⁴, operate by concentrating bicarbonate and converting it into CO₂ in a subcellular compartment housing rubisco. An alternative biochemical CCM in flowering plants is C4 photosynthesis, wherein CO₂ is initially fixed into a four-carbon compound in mesophyll cells before being transported to bundle sheath cells for localized decarboxylation around rubisco (Figure 2). C4 has evolved at least 62 times in flowering plants⁶⁵, with the most prolific adaptive radiation of C4 occurring in the past 30 million years, coincident with comparatively low levels of atmospheric CO₂⁶⁶.

C4 grasses like sorghum, miscanthus, switchgrass, sugarcane, and maize have all been developed as feedstocks for biomass farming⁶⁷ due in part to the hardiness, water use efficiency, and abiotic stress tolerance inherent to C4 plant physiology. Various research programs have sought to understand and implement the molecular mechanisms⁶⁸ by which C3 plants could be converted to C4^{69,70}. The set of gene families and tissue types in flowering plants is largely conserved between C3 and C4 plants, and C4 is thought to have evolved via the spatial differentiation, alteration in expression profile, and modification of protein sequence in various ancestral genes. In some cases, this may be only several hundred genes^{71,72}, comprising a relatively small portion of a plant's protein-coding genome. Multiple genetic and cell physiological mechanisms by which C4 has evolved from C3 have been described, as have various transitional species that provide insight into the sequence of events and functional contribution of individual components in the C4 syndrome⁷³. Molecular genetic studies of C4 and proto-C4 plants demonstrate that C4 evolution may be driven by *cis*-regulatory innovation^{74,75}, wherein alteration to promoter or mRNA UTR structure spurs changes in gene expression or cell specificity. While prior research in C3-to-C4 engineering has relied on

transgenesis, progress in understanding and engineering *cis*-regulation could enable the development of edited proto-C4 crops. Critically, C4 physiology is only competitive with C3 photosynthesis until ~500 ppm CO₂- projected to be reached in the middle of the 21st century (IPCC 2023), at which point the energy overhead of C4 metabolism outweighs the advantage of locally accumulating CO₂⁷⁶.

Crop domestication and improvement began approximately 10,000 years ago, and is owed to the contribution of diverse Neolithic human populations⁷⁷. Studies of diverse species and breeding populations indicate that photosynthesis has been improved, perhaps inadvertently, through selection on plants with higher yield potential and stress tolerance^{78–80}, although significant photosynthetic variation exists between and within crop species⁸¹. Leveraging natural genetic variation is a promising avenue for improving photosynthesis in certain species, but in some cases this diversity may be limited. While ~200-300 photosynthetic genes are mechanistically essential and conserved across all crops, their sequence diversification has likely been constrained by the highly oligomeric nature of photosynthetic complex assembly⁸². As such, observed variation in photosynthetic efficiency is commonly attributed to a diffuse network of thousands of non-canonical photosynthesis genes identified in genetic mapping studies⁸³. While these may afford some leverage for improving photosynthesis, the necessary genotypic diversity in ‘natural’ alleles is likely depleted in breeding populations. Domestication has in many cases led to the unintentional accumulation of deleterious alleles, commonly described as the “domestication-cost hypothesis”^{84,85}, as well as the loss of genetic diversity due to domestication bottlenecks⁸⁶. Through the introduction of new allelic diversity, gene editing presents an opportunity to improve photosynthetic function and reexamine various phenotypic trade-offs occurring during domestication.

Surveying the broad phylogeny of photosynthetic organisms reveals that the observed limits to plant photosynthesis are in many cases plant-specific. In the section below, we outline the specific genetic targets relevant to improving crop photosynthesis as revealed by genetic mapping, molecular genetic studies, and transgenic experimentation.

Figure 2. Theoretical and Evolutionary Insights for Improving Photosynthesis

Anatomy of C3 vs C4 leaves: In C4 plants, the CO₂-concentrating mechanism (CCM) is made possible by the C4 Kranz anatomy—the arrangement of bundle sheath and mesophyll cells in two concentric layers outside the vascular tissue. In the C4 mesophyll cell, CO₂ is initially fixed as oxaloacetate (OAA) by phosphoenolpyruvate carboxylase (PEPC) and converted into malate for transport into the bundle sheath cell. CO₂ is released via malate decarboxylation near the rubisco carboxylation site in the bundle sheath cell and subsequently incorporated into ribulose 1,5-bisphosphate (RuBP) by rubisco to form 3-phosphoglycerate (3PGA), which initiates the Calvin-Benson cycle. The glyceraldehyde-3-phosphate (GAP) that results after phosphorylation and reduction in the CB cycle is converted into sugars and exported to the vascular tissue. The pyruvate that results from malate decarboxylation is recycled back into the mesophyll cell where it is regenerated into phosphoenolpyruvate (PEP) by pyruvate phosphate dikinase (PPDK). In C3 leaves, Kranz anatomy is not present and mesophyll cells are differentiated into the palisade (vertically aligned) and spongy layers (loosely distributed horizontally).

Light harvesting: Plants rely on chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids to absorb solar energy, limiting the PAR spectrum to only 400-700 nm. Cyanobacteria and anoxygenic bacteria, on the other hand, have chlorophyll *d* (Chl *d*), chlorophyll *f* (Chl *f*), bacteriochlorophyll *a*, which enable them to absorb near-infrared light (700-760 nm) and infrared light (800-1000 nm). Light spectrum adapted from Cardona et al. (2018)⁸⁷.

Carbon assimilation: Leaf CO₂ uptake (*A*) can be described by the *A* versus *C_i* curve. In C3 plants, the *A/C_i* curve consists of 3 phases where it is limited by 1) rubisco carboxylation 2) rate of electron transport (*J_{max}*) and RuBP regeneration, and 3) triose phosphate utilization. In C4 plants, the *A/C_i* curve is only observed to have two phases

where it is 1) initially limited by PEPC carboxylation in the mesophyll cell and 2) by rubisco carboxylation in the bundle sheath cell.

Section 3. Engineering plant photosynthesis: molecular and physiological approaches

Despite extensive breeding efforts, recent stagnation in yield improvements underscores the need for engineering of crop photosynthesis and related processes^{88,89}. Given the inherent inefficiencies of photosynthesis described above, here we revisit many of the proven targets for photosynthetic improvement and highlight novel opportunities to improve $\epsilon_{\text{interception}}$ and $\epsilon_{\text{conversion}}$, targeting rate-limiting steps in the light and carbon reactions (Figure 3, Table 1). We consider the impact of fluctuating light environments in addition to steady-state photosynthesis and highlight the potential of multigene stacking for synergistic improvement of photosynthesis and stress tolerance.

3.1 Improving canopy structure, light use efficiency, and photoprotection

Agricultural carbon assimilation is heavily impacted by canopy architecture^{88,90,91}, which affects $\epsilon_{\text{interception}}$. Because biomass is known to scale almost linearly with $\epsilon_{\text{interception}}$ ³¹, optimizing light interception holds immense potential for improving photosynthesis. Plants have evolved oversized LHCs to maximize light harvesting and shade out competitors in nature⁹², but this strategy significantly reduces PAR penetration to the lower canopy layers under monocultural agronomic settings. This prevents most parts of the canopy from reaching the maximum rate of photosynthesis, and is one major reason why crop plant photosynthesis operates at less than one-third of the theoretical maximum^{31,93,94}.

Canopy architecture is shaped by canopy development and closure^{31,95}, leaf angle distribution⁹⁶, and canopy clumping³⁷, and its contribution to $\epsilon_{\text{interception}}$ might be improved via quantitative trait loci (QTLs) engineering^{96,97}. Engineering of a ‘smart canopy’, where antenna size is reduced in upper canopy leaves and preserved in shaded regions, has also been proposed as a strategy to maximize light use efficiency (LUE)⁹⁸ (Figure 3). Significant progress has been made towards this goal by reducing chlorophyll (Chl) content, which destabilizes LHCs and reduces antenna size. Chl reduction via knock-down of relevant biosynthetic enzymes improves biomass accumulation^{38,91,99,100}, and could be accomplished in other crops using endogenous mutations¹⁰¹. However, the precise degree to which Chl can be reduced without incurring deleterious effects seems species-specific^{99,102,103}, highlighting the necessity of ‘smart canopy’ engineering for true optimization of canopy photosynthesis (Figure 3). Proof-of-concept work in the unicellular green alga *Chlamydomonas reinhardtii* highlights how the addition of light-responsive sequences into the untranslated regions of Chl biosynthetic genes can enable realization of ‘smart canopies’¹⁰⁴.

Beyond the scope of standard PAR, spectral expansion of light absorption by introducing non-native LHC pigments such as red-shifted cyanobacterial Chls might also improve light capture and utilization^{105,106} (Figure 2). Site-directed mutagenesis of LHC proteins can result in shifts in absorption spectra, potentially allowing for absorption and utilization of far-red light^{91,98,107}. Given the current advances in protein design and computational modeling¹⁰⁸, it may be possible to identify key residues for precise mutagenesis. Notably, combining this with ‘smart canopy’ approaches would also allow for more efficient allocation of nitrogen resources,

allowing saved nitrogen to be redirected towards overexpression of other targets for multi-trait stacking⁹¹.

Non-photochemical quenching (NPQ) processes dissipate absorbed light as heat and are important for photoprotection, but NPQ can significantly limit photosynthesis under fluctuating light conditions¹⁰⁹. The relatively slow turning off of NPQ can lower observed $\epsilon_{\text{conversion}}$ by 9-11%^{90,110}. Preventing photoinhibition of photosynthesis by excess light is a major area of interest since the associated inactivation of the photosystem II reaction center protein D1 can result in hours of lost productivity (Figure 3)^{31,111,112}. Fortunately, this may be mitigated by D1 overexpression or generation of resistant forms of D1 via protein engineering and site-directed mutagenesis¹¹³⁻¹¹⁶. Beyond relieving photoinhibition, accelerating NPQ relaxation is a validated strategy to improve photosynthesis and crop productivity. Simultaneous overexpression of three key NPQ genes, *PsbS*, *VDE* and *ZEP*, has been shown to accelerate NPQ relaxation when overexpressed in the correct ratios¹¹⁷. However, the optimal ratio seems to vary across species, and the species-specific effects of altering NPQ are still not entirely understood^{118-120, 91,98,107, 118-120}. Gene editing of *PsbS* in rice to achieve overexpression provides a proof of concept for editing of NPQ dynamics¹²¹.

3.2 Improving photochemistry and electron transport efficiency

Even with optimized NPQ, dissipation of absorbed light is inevitable in full sunlight when photosynthesis becomes limited by downstream electron transport or carbon reactions⁹⁰. Despite serving as a key regulator in photosynthetic control to prevent PSI photodamage^{122,123}, the cytochrome *b₆f* complex is known to limit photosynthetic electron transport and thus carbon assimilation^{88,91,124}. Increased cytochrome *b₆f* accumulation via overexpression of its Rieske subunit (PetC) has been shown to improve photosynthetic efficiency in both C3 and C4 crops¹²⁵⁻¹²⁸. However, this target has not yet been stacked with other improvements that could further improve electron transport rate. The electron carriers plastocyanin (PC) and ferredoxin (Fdx), despite being less explored, represent promising candidates downstream of cytochrome *b₆f* that could be upregulated or modified via protein engineering to further enhance electron transport rate¹²⁹⁻¹³². ATP synthase, on the other hand, represents another promising target for stacking efforts since increased energy supply via its upregulation¹³³⁻¹³⁶ and improved redox properties for light-activation upon dark-light transitions are likely to offer benefits to crops grown in dense canopies¹³³⁻¹³⁶.

3.3 Improving carbon reactions

Rubisco catalysis rate and lack of specificity for carboxylation represent primary limitations to carbon fixation⁴⁵, and eliminating rubisco oxygenation alone is estimated to improve $\epsilon_{\text{conversion}}$ of C3 plants from 4.6% to 8.8%³⁰. Transgenic approaches to introduce CCMs or photorespiratory bypasses in C3 plants have shown potential in reducing photorespiration¹³⁷⁻¹³⁹, but these approaches have required the integration of multiple heterologous components, limiting their usefulness in terms of technical feasibility and regulatory burden. As such, engineering the rubisco protein itself is a high-priority opportunity for improving carbon assimilation. Recent efforts in rubisco mutational scanning demonstrate a novel method to alter rubisco's enzyme kinetic behavior^{42,140}. By assessing the fitness consequences of thousands or millions of rubisco variants in parallel, long-standing hypotheses about the biochemical

constraints⁴³ and evolvability¹⁴¹ of rubisco can be interrogated. During non-steady-state photosynthesis, rubisco is rapidly deactivated in the dark¹⁴² and slowly activated (≤ 30 minutes) during photoinduction^{143,144}, forgoing potential CO₂ assimilation¹⁴⁵. Protein engineering may be a valuable approach for altering these properties of rubisco as well.

Due to rubisco's inefficiencies, plants devote a significant portion of their leaf nitrogen to rubisco biosynthesis (20-30% in C₃, 5-9% in C₄)¹⁴⁶ to increase net carboxylation rate. Modeling suggests that increasing rubisco content by 20% could improve CO₂ assimilation without significant nitrogen costs^{88,147}. Improvements in photosynthesis have been obtained by overexpressing the native rubisco small subunit S (*rbcS*)^{88,148,149}, as well as its chaperones, rubisco assembly factors *RAF1* and *RAF2*^{150–152}. Rubisco activase, a heat-labile chaperone-like protein that displays a range of thermal sensitivities in different plant species, releases inhibitory sugar phosphates from rubisco's active site and is inhibited by ADP^{153–156}. It is likely that both shortcomings can be addressed with protein engineering^{140,157,158}.

Downstream of rubisco, optimizing the expression and regulation of CB enzymes provides another route to improve carbon assimilatory flux. For example, SBPase and FBPase limit metabolic flux and RuBP regeneration in the CB cycle⁵¹. Overexpressing these enzymes improves crop yield and photosynthesis^{159–162}, although species-specific differences may necessitate closer examination and fine-tuning of expression levels¹⁶³. Beyond upregulation, redox regulation of CB enzymes during dark-to-light transitions represents a relatively unexplored axis of photosynthetic engineering. The potential for altering redox regulation of SBPase and FBPase during light activation is only beginning to be appreciated^{164,165}, and combined protein engineering and overexpression efforts may synergistically boost carbon assimilation. Similar principles also apply to the CP12/GAPDH/PRK complex, which sequesters the CB cycle enzymes GAPDH and PRK in the dark (Figure 3). This complex can take up to minutes to dissociate upon illumination¹⁶⁶, and optimizing its dissociation kinetics via protein engineering could potentially increase integrated carbon assimilation (Table 1).

As more is discovered about CB cycle redox regulation, redox activators themselves present viable targets for photosynthetic gene editing (Figure 3, Table 1). Thioredoxin-f (Trx-f) and NADPH-dependent thioredoxin reductase C (NTRC) play roles in activating CB enzymes such as SBPase, FBPase, PRK, and GAPDH, and their constitutive overexpression is known to elicit biomass gains *in planta*^{167–170}. Systemic characterization of the fundamental drivers of CB cycle activation might provide valuable insights into systems-level engineering of carbon flux.

While validated C₃ gene targets like rubisco and the CB cycle enzymes are likely also good targets for improving C₄ photosynthesis, significantly less research has focused on improving C₄ crops¹⁴⁷. Based on recent modeling, C₄ photosynthesis is limited by both PEPC, which is responsible for the initial carboxylation reaction^{147,171}, and PPDK, which is responsible for regenerating PEP for PEPC carboxylation (Figure 2), and is especially limiting during photoinduction and at lower temperatures^{147,172,173}. Similar to other targets, overexpression and protein engineering efforts can again be integrated to relieve these bottlenecks.

3.4 Stomatal properties and gas exchange

Stomata are the pores through which CO₂ enters leaves, and stomatal limitations can affect carbon fixation at steady-state and during dark-light transitions in both C₃ and C₄ species^{90,174,175}. Stomatal conductance (g_s), which describes the rate at which CO₂ enters the leaf via

stomatal openings⁹⁰, critically determines the availability of mesophyll CO₂ and could limit carbon assimilation by as much as 10-15% during photoinduction^{45,176}.

Stomatal size, density, and kinetics are all known to influence g_s ¹⁷⁵ and have been validated as targets for improving photosynthesis in the past decade¹⁷⁷. Negative and positive regulators of stomatal density, EPIDERMAL PATTERNING FACTORS (EPFs) and EPF-LIKE proteins (EPFLs), have been targeted to improve carbon assimilation and intrinsic water use efficiency (WUEi), although optimizing one frequently comes at the expense of the other^{177–182}. However, a recent study targeting a paralog of EPFL9/STOMAGEN managed to fine-tune stomatal density while preserving photosynthetic efficiency¹⁸³, highlighting how paralog editing can be explored to attain desirable phenotypes.

The speed of stomatal opening is osmotically driven by ion transport and water flux in a process controlled by ion transporters across guard cell membranes¹⁷⁷. Optimizing stomatal kinetics via manipulation of ion transport therefore offers another avenue for improving assimilation while preserving WUEi^{184–187} (Figure 3). A recent study that modified a key K⁺ channel demonstrates the potential of precise protein engineering for relieving stomatal limitations¹⁸⁸, informing opportunities for how endogenous protein sequences could be modified to improve g_s .

Finally, beyond stomatal regulation, CO₂ encounters further resistance after entering the intercellular air space inside a leaf and before reaching the carboxylation site. Mesophyll conductance (g_m) describes the ease with which CO₂ reaches rubisco in chloroplasts. Improvements in g_m are estimated to increase C₃ photosynthesis by approximately 20%-35%^{31,45} and are especially important at higher temperatures¹⁸⁹. Manipulation of gene targets implicated in leaf anatomical structures and cell wall thickness therefore hold promise in improving g_m (Table 1), which could be stacked with g_s and other light and CB cycle enhancements to more holistically improve photosynthesis under dynamic environments^{190,191,192}.

3.5 Carbon allocation and source-sink dynamics

Source-sink dynamics are a critical aspect of whole-plant carbon assimilation and biomass conversion, affecting both $\mathcal{E}_{\text{conversion}}$ and $\mathcal{E}_{\text{partitioning}}$. Triose phosphate utilization (TPU) is the first step implicated in carbon partitioning and source-sink interactions, and is known to limit photosynthesis through feedback regulation^{39,173,193}. TPU limitation arises from low P_i availability to support ATP synthase activity due to phosphates being tied up in triose phosphates^{35,194}, and is especially relevant at lower temperatures^{195,196}. The rate of TPU (V_{TPU}) is primarily dependent on the rate of sucrose and starch synthesis, and upregulating enzymes involved in these processes is known to relieve TPU limitation¹⁹⁷. Once P_i is released from triose phosphates following sucrose synthesis, timely export of the sucrose is necessary to support sink development and prevent feedback inhibition of photosynthesis^{198,199}. To optimize phloem loading of sucrose at the source, the expression of SWEET and SUC/SUT sugar transporters, which mediate apoplastic sucrose efflux from leaves, has been upregulated through several strategies, including constitutive overexpression^{200–202}, tissue-specific overexpression^{203,204}, or knockdown of negative regulators²⁰⁵.

In sink tissues, sugars are unloaded from the phloem into developing sink organs by the same families of SWEET and SUC/SUT transporters and subsequently broken down by

invertases (INV) or sucrose synthase (SUS) for further utilization^{199,206} (Figure 3). Promising targets therefore include enzymes involved in sucrose unloading and sink organ metabolism (Table 1)^{207–209}, many of which have been validated to improve biomass and yield^{199,206,210,211, 207–209}. One recent study managed to achieve this via precise insertion of a heat shock element into the promoter of a cell wall invertase to overcome heat-induced source-sink disruptions²¹², highlighting the value of *cis*-regulatory element (CRE) engineering at native gene loci, which is particularly important for source-sink engineering because it is highly dependent on tissue-specific overexpression^{213,214}.

The intricate interplay between carbon and nitrogen metabolism cautions against considering carbon partitioning alone in the context of source-sink engineering. Nitrogen supply is known to have a direct impact on V_{TPU} ²¹⁵, as well as on partitioning and sink organ development¹⁹⁹. Beyond overexpression of targets implicated in nitrogen use efficiency (NUE)^{216,217} and enzymes involved in amino acid uptake and metabolism^{218–221}, it is also worth considering how genes implicated in leaf senescence, sucrose transport, and nitrogen remobilization could be spatiotemporally upregulated during sensitive stages of grain filling and sink organ development (Table 1)¹⁹⁸. Moving forward, this may be critical for multigene stacking efforts which will be necessary for approaching the theoretical maximum of yield and harvestable biomass²²².

Figure 3. Targets for Improving Photosynthesis

Photosynthetic targets in the light and carbon reactions of photosynthesis

Electron transport chain (ETC)

In the photosynthetic electron transport chain (ETC), light received by light harvesting antenna complex II (LHC II) is passed on to the reaction center in photosystem II (PSII) to drive charge separation where e^- split from H_2O initiates downstream electron transport. Upon lumen acidification in highlight conditions, the protonated Photosystem II subunit S (PsbS) induces a conformational change in PSII and initiates non-photochemical quenching (NPQ). Violaxanthin de-epoxidase (VDE) converts the xanthophyll pigment violaxanthin into zeaxanthin as part of the NPQ process, and zeaxanthin epoxidase (ZEP) converts zeaxanthin back to violaxanthin once excess light is no longer present. Plastoquinone (PQ) oxidizes the primary electron acceptor (not shown) at the reaction center (RC) (consisting of the D1 subunit), and passes the electron to the Cytochrome b_6f complex (Cyt b_6f). The Rieske subunit of Cyt b_6f (encoded by PetC) reduces plastocyanin (PC) for downstream electron transport to photosystem I (PSI). The electron is re-excited at PSI and then transferred to ferredoxin (Fdx) and subsequently ferredoxin-NADP⁺-reductase (FNR) where it reduces NADP⁺ into NADPH. The proton gradient generated by the electron transport process drives phosphorylation of ADP to ATP. The resulting ATP, along with NADPH, powers carbon reactions in the Calvin-Benson cycle.

Calvin-Benson Cycle (CB Cycle)

Rubisco accumulation factor 1 (Raf1) aids in the assembly of Rubisco, while Rubisco activase (Rca) activates Rubisco by releasing inhibitory sugars. In the CB cycle, rubisco catalyzes formation of 3-phosphoglycerate (3-PGA) through reaction with CO_2 . CO_2 is released from carboxylation of a C4 acid in C4 plants and arrives at the carboxylation site via diffusion from stomatal openings in C3 plants. 3-PGA is then converted into 1,3-bisphosphoglycerate (1,3-BPGA) via phosphorylation by ATP and subsequently reduced by GAPDH using NADPH. The resultant glyceraldehyde-3-phosphate (GAP) is either exported as sugars for downstream sink metabolism or recycled back into the regenerative phase of the CB cycle. In the regenerative phase, GAP is either converted into dihydroxyacetone-phosphate (DHAP) or fructose 1,6-bisphosphate (F1,6-BP), where it is subsequently converted to sedoheptulose 1,7-bisphosphate (S1,7-BP) by fructose 1,6-bisphosphate/sedoheptulose 1,7-bisphosphate aldolase, or to fructose-6-phosphate (F6-P) by fructose 1,6 bis-phosphatase (FBPase), respectively. S1,7-BP is then converted to sedoheptulose 7-phosphate (S7-P) by sedoheptulose 1,7-bisphosphatase (SBPase). F6-P is subsequently converted to xylulose-5-phosphate (Xyl5-P) via several steps and finally ribulose 5-phosphate (Ru5P). S7-P is also converted to Ru5P through several reactions and regenerated into ribulose 1,5-bisphosphate (RuBP) through phosphorylation by ribulose-5-phosphate kinase (PRK).

GAPDH and PRK are sequestered in the GAPDH₂-CP12₄-PRK₂ complex in the dark. This complex dissociates upon Trx-f and NTRC reduction in the light as part of photoinduction, allowing for activation of the CB cycle.

‘Smart Canopy’

A ‘Smart Canopy’ can be engineered via insertion of a light-responsive element into the untranslated regions (UTR) of chlorophyll biosynthetic genes or other genes implicated in pigment metabolism. This light-responsive element would theoretically recruit transcriptional or translational repressors under high light and lead to lower chlorophyll levels and smaller antenna complexes under highlight while allowing for increased chlorophyll accumulation and formation of larger antenna complexes under low light. This holds potential for maximizing light use efficiency in canopy environments.

Stomatal & Mesophyll Conductance

Stomatal patterning is determined by the Epidermal Patterning Factor (EPF) and EPF-like (EPFL), which are secreted proteins that play key roles in epidermal patterning, development, and morphology. Fine-tuning expression of these key genes enables precise control over stomatal density, which subsequently determines stomatal conductance (g_s) and influences mesophyll conductance (g_m). Optimizing the kinetics of stomatal opening and closing via modification of guard cell H⁺ ATPase and K⁺ channels holds further potential for improving CO₂ uptake and carbon assimilation.

Source-Sink Engineering

Sucrose is exported out of the source leaf via Sugars Will Eventually be Exported Transporters (SWEETs) and SUC/SUT transporters and loaded into the phloem for transport to the developing sink. At the sink, either the same SWEET or SUC/SUT transporters or members of the same family are responsible for unloading sucrose into the developing sink. The unloaded sucrose is further metabolized by cell wall invertases (CWIN), vacuolar invertases (VIN), and sucrose synthase (SUS) to support sink tissue development.

3.6 Multi-target stacking and transcriptome engineering

Optimization of photosynthesis may necessitate synergistic improvement of multiple traits, so many labs have pursued overexpression of upstream transcriptional regulators to achieve multi-gene engineering. Overexpression of *GLK*, *HYR*, *HY5*, *ZMM28* and *DOF1* transcription factors, for instance, has shown considerable promise in boosting photosynthetic efficiency, yield and sometimes both (Table 1)^{223–229}. However, while this approach enables efficient targeting and simultaneous alteration of expression levels, it falls short in offering further tunability because of inherent constraints in endogenous gene regulatory networks. Many of these regulators, for example, increase Chl accumulation by upregulating biosynthetic genes or LHCs^{223,230}, which as discussed above, can be suboptimal for canopy photosynthesis and contribute to inefficient allocation of nitrogen.

Moving forward, to minimize pleiotropic effects associated with generic overexpression, reconcile species-specific differences, and ultimately achieve precise transcriptome-level engineering, it is crucial to integrate computational modeling with *in-planta* validation to help inform precise and holistic engineering of carbon crops.

Table 1. List of photosynthetic and physiological gene editing targets in plants and the types of edits that could be used for their improvement.

Gene	Role	Type of modification	Type of edit	Editor	Citation
Chlorophyll a oxygenase (CAO)	Chlorophyll biosynthesis	knockdown / modification of enzyme catalytic activity	Promoter -LOF	Cas9, PE, BE	Friedland et al. (2019) ¹⁰³
Mg Chelatase (MgCh)			Promoter - LOF; protein sequence modification		Eid et al. (2021) ¹⁰¹ ; Gao et al. (2016) ²³¹

PsbS	NPQ and NPQ relaxation	overexpression	Promoter -GOF	Cas9, PE, BE	Kromdijk et al. (2016) ¹¹⁷ ; Garcia-Molina and Leister (2022) ¹¹⁹ ; De Souza et al. (2022) ¹¹⁸ ; Lehretz et al. (2022) ¹²⁰ ; Xin et al. (2024) ²³² ; Patel-Tupper et al. (2024) ¹²¹ ;
VDE		overexpression	Promoter -GOF	Cas9, PE, BE	
ZEP		overexpression	Promoter -GOF	Cas9, PE, BE	
PsbA/D1	Photosynthetic reaction center protein (prone to photodamage, implicated in ql)	overexpression, engineering resistance to photoinhibition	UTR modification Protein sequence modification	TALE-linked base editors	Huo et al. (2016) ²³³ ; Narusaka et al. (1999) ¹¹⁵ ; Mok et al. (2024) ²³⁴
PetC	Photosynthetic control	overexpression	Promoter -GOF	Cas9, PE, BE	Simkin et al. (2017) ¹²⁶ ; Ermakova et al. (2019) ¹²⁵ ; Ermakova et al. (2023) ¹²⁷
Plastocyanin (PETE1; PETE2)	electron transport	overexpression	Protein sequence modification	PE, BE	Pesaresi et al. (2009) ²³⁵
Ferredoxin (Fdx)	electron transport	overexpression	Protein sequence modification	PE, BE	Chang et al. (2017) ²³⁶
ATP Synthase Subunit D	ATP synthesis	overexpression	Protein sequence modification	PE, BE	Ermakova et al. (2022) ¹³³
ATP synthase γ subunit	ATP synthesis, redox regulation	Kinetic parameters	Protein sequence modification	PE, BE	Kohzuma et al. (2017) ¹³⁶
RbcS	rubisco carboxylation	Kinetic parameters, expression	CDS - base/prime edit; UTR uORF editing; Promoter - GOF	Cas9, PE, BE	Prywes et al. (2025) ¹⁴⁰ ; Salesse-Smith et al. (2025) ⁸⁸
Rubisco assembly factor (RAF1, RAF2)	rubisco assembly	overexpression	Promoter -GOF	Cas9, PE, BE	Salesse-Smith et al (2018) ¹⁵⁰ ; Eshenour et al. (2024) ¹⁵¹ ; Whitney et al. (2015) ¹⁵²
Rubisco activase (Rca)	rubisco activation	overexpression; thermostability	Protein sequence modification	PE, BE	Sparrow-Muñoz et al. (2023) ²³⁷ ; Degen et al. (2020) ²³⁸
Trx-f	Light redox activation	overexpression, redox activation	Promoter - GOF	Cas9, PE, BE	Sanz-Barrio et al. (2013) ¹⁶⁷ ; Guinea Diaz et al (2020) ¹⁶⁹
NTRC	Light redox activation	overexpression, redox activation	Promoter -GOF	Cas9, PE, BE	Teh et al. (2023) ¹⁶⁸ ; Kim et al. (2017) ¹⁷⁰ ; Guinea Diaz et al (2020) ¹⁶⁹
CP12	RuBP regeneration	redox activation	Promoter -GOF; potential protein sequence modification	Cas9, PE, BE	Teh et al. (2023) ¹⁶⁸ ; Li et al (2018) ²³⁹
GAPDH	RuBP regeneration	redox activation and dissociation kinetics	Potential protein sequence modification	PE, BE	Simkin et al. (2019) ¹⁴³ ; Gurrieri et al. (2021) ²⁴⁰ ; Marri et al. (2009) ²⁴¹
PRK	RuBP regeneration	redox activation and dissociation kinetics	Potential protein sequence modification	PE, BE	
SBPase	RuBP regeneration	overexpression and redox activation	Promoter -GOF; potential sequence modification to improve redox properties and activity	Cas9, PE, BE	Simkin et al. (2015) ²⁴² ; Ding et al. (2016) ¹⁶⁰ ; Driever et al. (2017) ²⁴³
FBPase	RuBP regeneration	overexpression and redox activation			Simkin et al. (2015) ²⁴²

PEPC	C4 PEP carboxylation	overexpression and protein modification	Promoter -GOF; potential protein sequence modification	PE, BE	Wang et al. (2014) ¹⁷³ , Sales et al. (2021) ¹⁴⁷
PPDK	C4 PEP Regeneration	overexpression and protein modification	Promoter -GOF; potential protein sequence modification	PE, BE	Wang et al. (2014) ¹⁷³ , Sales et al. (2021) ¹⁴⁷ , Wang et al. (2018) ¹⁷²
EPF	Stomatal density	Knockdown and fine-tune expression	Promoter allelic series	Cas9, PE, BE	Sakoda et al. 2020 ¹⁷⁹
EPFL9/STOMAGEN	Stomatal density	overexpress or fine-tune expression	Promoter allelic series		Tanaka et al. (2013) ²⁴⁴ , Karavolias et al. (2023) ¹⁸³
H ⁺ ATPases	Stomatal kinetics	overexpress or kinetic parameters	Promoter - GOF; potential protein sequence modification	Cas9, PE, BE	Wang et al. (2014) ¹⁸⁵ , Toh et al. (2021) ¹⁸⁶
K ⁺ ion channels	Stomatal kinetics	kinetic parameters	protein sequence modification	PE, BE	Huraruang et al. (2022) ¹⁸⁸
COTTON-GOLGI-RELATED (CGR)	mesophyll conductance	overexpression	Promoter -GOF	Cas9	Salesse-Smith et al (2024) ¹⁹¹
KRP1	mesophyll conductance	overexpression	Promoter allelic series/overexpression	Cas9	Lehmeier et al. (2017) ²⁴⁵
SWEET	Sucrose loading/unloading	Tissue/condition-specific overexpression	Promoter GOF or conditional GOF	Cas9, PE, BE	Chen et al. (2012) ²⁴⁶ , Schroeder et al. 2013 ²⁴⁷ , Yang et al. (2018) ²⁴⁸ , Sosso et al. (2015) ²⁴⁹
SUC/SUT	Sucrose loading/unloading	Tissue/condition-specific overexpression	Promoter GOF or conditional GOF	Cas9, PE, BE	Wang et al. (2015) ²⁰⁰ , Xu et al. (2020) ²⁰⁵ , Wang et al. (2020) ²⁵⁰ , Grant et al. (2021) ²⁵¹
CWIN	Sink metabolism	Tissue/condition-specific overexpression	Promoter GOF or conditional GOF	Cas9, PE, BE	Lou et al. (2024) ²¹² , Kawaguchi et al. (2021) ²⁵²
SUS	Sink metabolism	Tissue/condition-specific overexpression	Promoter GOF or conditional GOF	Cas9, PE, BE	Ahmed et al. (2020) ²⁰⁸
GLK	Chloroplast biogenesis	Tissue/condition-specific overexpression	Promoter GOF or conditional GOF	Cas9, PE, BE	Hernández-Verdeja et al. (2024) ²²⁴ , Ye et al. (2022) ²²⁵
HYR	Photosynthetic carbon metabolism	Tissue/condition-specific overexpression	Promoter GOF or conditional GOF	Cas9, PE, BE	Ambavaram et al. (2014) ²²³
HY5	Light-responsive growth	Tissue/condition-specific overexpression	Promoter GOF or conditional GOF	Cas9, PE, BE	Chen et al. (2016) ²²⁶
ZMM28	Photosynthetic efficiency, NUE	Overexpression	Promoter - GOF or conditional GOF	Cas9, PE, BE	Wu et al. (2019) ²²⁹
DOF1	NUE	Tissue/condition-specific overexpression	Promoter GOF or conditional GOF	Cas9, PE, BE	Yanagisawa et al. (2004) ²²⁸
DRO1	Deeper rooting, WUE	Tissue/condition-specific overexpression	Promoter GOF or conditional GOF	Cas9, PE, BE	Uga et al., 2013 ²⁵³
DREB1C	NUE	Overexpression	Promoter GOF	Cas9, PE, BE	Erpen et al. (2018) ²⁵⁴ , Kudo et al. (2019) ²⁵⁵

ALMT	Nutrient transporter	Overexpression	Promoter - GOF or conditional GOF	Cas9, PE, BE	Kawasaki et al. (2021) ²⁵⁶
NAC (NAC1/2/3)	Abiotic stress response	Overexpression	Promoter - GOF or conditional GOF	Cas9, PE, BE	Erpen et al. (2018) ²⁵⁴
WRKY	Abiotic stress response	Overexpression	Promoter - GOF or conditional GOF	Cas9, PE, BE	Erpen et al. (2018) ²⁵⁴

Gain-of-function, GOF; Loss-of-function, LOF; Prime Editing, PE; Base Editing, BE; Transcription activator-like effectors, TALE.

4. Engineering plant physiology for carbon sequestration

Downstream of light and carbon capture, the regulation of plant development, metabolism, growth habit, and ecological interactions are essential for the stable sequestration of photosynthesis-derived biomass. Here, we examine key opportunities for modifying plants for carbon sequestration. These approaches not only improve long-term carbon storage but also enhance nutrient availability, soil structure, and crop resilience. By integrating biological, chemical, and agronomic innovations, these strategies provide a comprehensive framework for improving soil carbon sequestration, agricultural productivity, and climate resilience.

4.1 Modifying roots for soil carbon storage

While carbon partitioning has traditionally been considered in the context of improving crop yield, redirecting fixed carbon to below-ground biomass for carbon sequestration comes with a different set of physiological and ecological considerations²⁵⁷. Deep roots are logically more efficient at depositing carbon in the subsoil/B-horizon (i.e. >1 m deep), where SOC is less common and less susceptible to microbial decomposition and erosion^{258,259}. Rhizodeposition, which refers to the release of organic molecules from plant roots, can consume up to 50% of total photoassimilate²⁶⁰ and make up 60-80% of SOC²⁵⁷. These rhizodeposits can take the form of root exudates, water-insoluble secretions such as mucilage, and remnants of dead root tissue. Increasing root diameter, root hair density, and rhizosheath mass can increase SOC input^{257, 261}, and increasing root depth by 5–20% has been estimated to enhance carbon sequestration by 30–130 kg C ha⁻¹ per year in croplands²⁶².

Although much remains to be understood, decades of research have identified key genes implicated in root development and architecture^{253,257,263–266}. Genetic targets like DEEPER ROOTING 1 (*DRO1*), a major rice drought-tolerance QTL, might be upregulated to lengthen roots and improve WUE by enabling access to subsoil water stores (Table 1)²⁵³. This sort of improvement in conjunction with genetic alterations to root cortical cell thickness, multiseriate cortical sclerenchyma, and stele diameter, all of which improve a plant's ability to grow in compacted soils, might allow access to deeper water and nutrient reserves while increasing SOC deposition^{267,268}.

4.2 Modifying cell walls for soil carbon storage and BiCRS

Besides below-ground root depth, cell wall composition is a key determinant of root carbon persistence. Modulating the incidence and stoichiometry of native cell wall polymers like cellulose, suberin, and lignin, as well as ectopically producing recalcitrant plant biopolymers like sporopollenin²⁶⁹, is speculated to enhance the long-term durability of deposited SOC^{270,271}.

While the assumed influence of these modifications is still largely theoretical, genetic targets for modification of cell wall synthesis are well understood. For example, overexpression of genes in the phenylpropanoid pathway can enhance root lignin accumulation²⁷², and plant root residue might be made more inert to microbial degradation and respiration by leveraging multiple biosynthetic pathways. Despite this potential, major methodological challenges in the quantification of SOC must be addressed, especially across short timescales and in deeper parts of the soil bed with appropriate spatial resolution^{273,274}. Development of fast, inexpensive, and distributed tools to measure carbon deposition is thus essential for the large-scale implementation of soil CDR.

Amending the cell wall composition of carbon crops is also useful for the BiCRS-based production of biochar²⁷⁵. The composition of plant biomass significantly influences biochar yield and stability, with lignocellulosic biomass (cellulose, hemicellulose, and lignin) determining both the efficiency of pyrolysis and the recalcitrance of the resulting biochar²⁷⁵. Lignin is the primary determinant of both, and genetic approaches to increase its production could be useful for improving the pyrolysis potential of agricultural residues and purpose-grown crops²⁷⁶. Conversely, reducing hemicellulose and ash content through pretreatment or genome editing could improve the efficiency of pyrolysis by minimizing the production of destabilizing volatile byproducts²⁷⁵. Using biochar as a soil amendment improves soil microbial ecology²⁷⁷, nutrient availability²⁷⁸, and moisture retention²⁷⁹, among other benefits.

4.3 Modifying plant-microbe interactions for soil carbon sequestration

SOC usually takes the form of the more labile particulate organic matter (POM) and the more recalcitrant mineral-associated organic matter (MAOM), which could persist for centuries to millennia^{280–282}. MAOM formation is significantly impacted by plant exudates and the presence of microbial communities. Microbial biomass and necromass collectively account for 50–80% of the stable SOC²⁸³, and their activities are essential for promoting conversion of labile C into MAOM^{284,285}. Modifying plant exudate profiles to promote plant-mycorrhizal and rhizobacteria symbiosis therefore presents an important engineering opportunity. Mycorrhizal fungi are known to direct up to 30% of plant photosynthates into below-ground biomass²⁸⁶, affect SOM deposition and decomposition²⁸⁵, and promote long-term soil carbon retention^{287,288}. Upregulating plant phosphate transporters²⁸⁹, downregulating negative transcriptional regulators²⁹⁰, and improving strigolactone biosynthesis²⁹¹ can be used to improve crop mycorrhizal symbiosis beyond modification of root anatomical traits²⁹².

Bacteria constitute another indispensable part of the microbial community and play a pivotal role in plant physiology and soil carbon stability²⁸⁵. Notably, rhizobacterial production of extracellular polymeric substances (EPS), which consist of polysaccharides, proteins and other biopolymers, facilitate binding of soil particles and stabilize SOC^{285,293,294}. Overexpressing nutrient transporters (Table 1)²⁵⁶, and modifying plant exudate content to increase flavonoids may therefore serve as an effective strategy to improve colonization of beneficial rhizobacteria and subsequently enhance SOC stability^{295–299}.

Beyond roles in soil carbon cycling, both mycorrhizal and rhizobacterial symbiosis are known to enhance plant abiotic and biotic tolerance through providing microbe-assisted stress resilience^{300–303}. Biotic and abiotic stresses can account for as much as 40% to 80% of crop yield and biomass losses^{304,305} and should be considered as part of the carbon equation.

Constitutive or conditional overexpression of stress-responsive transcription factors, such as DREB, NAC, and WRKY, can be stacked on top of genetic targets aimed at enhancing beneficial microbial interactions to further reduce C loss and improve carbon sequestration (Table 1)^{254,255}.

4.4 Agroecological considerations for soil carbon retention

The ability of soil to store carbon is influenced by climate, soil texture, nutrient content, and land use practices³⁰⁶. While most SOC is annually turned over by microbial respiration, strategic land management practices can counteract these losses and even enhance soil carbon sequestration. Nitrogen fertilizer use accounts for 2.1% of global greenhouse gas (GHG) emissions, owing in approximately equal parts to emissions from the Haber-Bosch process and microbial nitrous oxide (N₂O) production³⁰⁷. The amplitude of land-atmospheric carbon flux continues to increase as the climate warms, and recent evidence suggests that the increased global use of nitrogen fertilization³⁰⁷ is the largest contributor to this trend³⁰⁸. Enhancing plant NUE is thus paramount to scalable CDR. Research across crop species indicates that photosynthetic efficiency is tightly linked to NUE³⁰⁹, and reducing proteomic partitioning to photosynthesis-related gene products is a promising avenue for decreasing reliance on exogenous nitrogen. For example, an average 18.2% of nitrogen is partitioned into rubisco³¹⁰, and so increasing the speed and efficiency of carboxylation, and thus reducing the amount of rubisco required for photosynthesis, could markedly reduce crop nitrogen requirements³¹¹. Similarly, high NUE cultivars³¹² and first-principles models of proteomic nitrogen allocation⁵¹ provide insight into how NUE might be improved by rewiring photosynthesis.

Beyond crop engineering, CDR projects should consider the full spectrum of agroecological and technological possibilities to ensure the minimization of agricultural emissions. Optimizing the rate and timing of fertilization³¹³, the use of nitrification inhibitors^{314,315}, and the use of biochar all stand to lower the N₂O emissions inherent to modern industrial agriculture. Environment-specific land use practices like no-till farming³¹⁶, cover cropping³¹⁷, crop rotation (especially with perennial crops)³¹⁸, intercropping³¹⁹, and more have been found to increase soil carbon²⁵⁷, although the usefulness of each of these is highly constrained by the environment in which they are performed^{320,321}. In sum, any engineering effort for crop CDR should be considered in the full context of the resultant crop and its surrounding agronomic system(s).

5. Genome editing for scalable carbon dioxide removal (CDR)

Since their first application in plants^{322,323}, CRISPR tools have proven exceptionally useful in the analysis, improvement, and domestication of crop plants. Below, we delineate the ways in which CRISPR might be used most effectively for improving carbon assimilation and sequestration, the state-of-the-art tools and techniques transforming plant breeding, and the outstanding technical advances that will enable gene editing for carbon capture to be realized at scale (Figure 4).

Figure 4. Technological approaches for carbon crop gene editing

Beyond plant vector images (shown above), this is one we'd like to work out with the art editor.

5.1 Gene editing for photosynthetic engineering

Most prominent examples of CRISPR crop improvement have leveraged gene knockouts to alter traits such as pathogen resistance, secondary metabolism and nutritional profile, abiotic stress tolerance, yield and aboveground architecture, and toxic metabolite production³²⁴. Compared to other traits, the essentiality of most photosynthesis-associated genes complicates their consideration as good knockout targets, perhaps with the exception of genes regulating light-harvesting antenna size^{99,325} and paralogous genes that quantitatively contribute to a photosynthesis-related phenotype¹⁸³. Instead, some of the best opportunities in gene editing for engineering photosynthesis involve modulating the expression of specific genes and gene families. As mentioned in Section 3, stable overexpression of rate-limiting photosynthetic genes has been shown to improve carbon assimilation and agronomic yield. However, almost none of these improvements have been translated to crops in farmers' fields to date, largely due to regulations and expenses surrounding the commercialization of GM crops. To realize the potential of decades of transgenic plant research, we suggest developing core competencies in editing *cis*-regulatory elements to fine-tune expression in photosynthetic gene networks.

Modulating photosynthetic gene expression can be achieved by mutating *cis*-regulatory DNA to alter the expression of endogenous genes³²⁶. Gene editing promoters, introns, and untranslated regions (UTRs) at high-effect loci has been used to improve grain yield³²⁷, seed protein content³²⁸, plant height and grain size³²⁹, stomatal patterning³³⁰, and recovery from photoprotection¹²¹. Despite the proliferation of precision genome-editing tools, the use of *cis*-regulatory editing in crops is currently constrained by the relative dearth of information on how *cis*-regulatory sequences contribute to crop phenotype. Compared to mammals, plants are less dependent on distal enhancers³³¹, and thus immense focus has recently been placed on deciphering the form and function of plant promoters. Preliminary investigations mutagenizing promoters with CRISPR reveals that, at least for certain genes, sequence conservation and epigenetic signatures are informative in identifying high-effect *cis*-regulatory regions^{329,332}, although the throughput of these experiments is highly constrained by the transformation, regeneration, and screening of edited plants. To work around this, transient assays using protoplast transfection and *Agrobacterium* infiltration have been employed to screen the effects of thousands of promoter, terminator, and intron variants in parallel^{333–335}. In tandem, predictive machine learning-based language models have been demonstrated to infer the functional consequence of edits in noncoding DNA³³⁶, and the application of these tools to study plant gene expression is imminently useful for plant editing. The combination of these two approaches stands to accelerate the understanding of noncoding DNA, reduce the scale and complexity of promoter mutagenesis experiments, and lead to more informed efforts in engineering gene regulation.

Regardless of the potential of gene editing in its own right, its widespread implementation in plants is dependent on the involvement of crop breeders to introgress and evaluate new alleles in diverse germplasm. In many cases, CRISPR tools can be used to make the work of breeders - crossing, backcrossing, and refining germplasm - more efficient³³⁷. Significant allelic variation exists at many photosynthesis QTLs^{338,339}, but this diversity may be inaccessible to breeders due to the physical proximity of many photosynthetic genes and the extended linkage disequilibrium characteristic of cultivated crops³⁴⁰. This allelic diversity is

especially pronounced in the thousands of photosynthesis-related genes outside of those that encode the core proteins involved in light harvesting and carbon assimilatory processes⁸³. CRISPR tools engineered for targeted recombination^{341,342} and dividing-cell-specific CRISPR nuclease activity^{343,344,345} offer potential strategies to direct recombination events, which could be leveraged during meiosis to generate novel, photosynthetically optimized haplotypes from existing germplasm³⁴⁶. In an industrial breeding context, targeted recombination can be used to limit the number of biparental crosses required to stabilize a desired genotype³⁴⁷, greatly reducing the time and land area required to screen novel genetic architectures.

Besides regulatory permissiveness, a distinct advantage of gene editing compared to traditional transgenesis is that it facilitates the ‘stacking’ of multiple desired alleles within a single genotype. Traditional transgenesis is limited both by the size³⁴⁸ and stability³⁴⁹ of inserted genetic material, complicating the implementation of more complex strategies for genetic reprogramming. Stacking edited alleles will be essential to photosynthetic engineering efforts, as it allows for precise stoichiometric expression that is important for traits like photoprotection^{117,118}, and because significant improvements in light harvesting, electron transport, and carbon assimilation are only accessible through distinct genetic pathways³⁵⁰. To enable experimentation along this axis, high-efficiency multiplexed gene editing must be achievable in a broad range of species.

Table 2. Genome editing tools and techniques for plants

Potential applications	Tools	Delivery	Species	Advantage	Disadvantage	Citations
Gene knockout	CRISPR/Cas9 editing	Agrobacterium, Biolistics	Most plants	Simplify target design while ensuring high efficiency and enabling multiplexed mutations.	PAM requirement, NGG	Shan et al. 2013 ³²³ ; Jiang et al. 2013 ³⁵¹
	Genome editing by Cas12	Agrobacterium	Rice, Maize, Arabidopsis	Expand editing scope on AT-rich regions	PAM requirement; Cas12a (TTTV), Cas12b (TTV)	Tang et al. 2017 ³⁵² ; Ming et al. 2020 ³⁵³
	Genome editing by Cas9-NG, SpRY, and xCas9	Agrobacterium	Rice	Expanding editing scope	Editing efficiency varies in non-canonical PAM; Cas9-NG (NG), SpRY (NRN, NYN), xCas9 (GAD, GAA, NG, NAG)	Endo et al. 2019 ³⁵⁴ ; Ren et al. 2021 ³⁵⁵ ; Zhong et al. 2019 ³⁵⁶

Targeted knock-in/replacement	Base editing	Agrobacterium	Rice, Wheat, Maize, Soybean, Rapeseed, Poplar	Introduce single-nucleotide variants	Limited active editing windows with potential bystander edits	Jiang et al. 2020 ³⁵⁷ , Xu et al. 2020 ³⁵⁸ , Lin et al. 2020 ³⁵⁹ , Butt et al. 2020 ³⁶⁰
	Prime editing	Agrobacterium	Rice, Maize, Wheat, Tomato, Arabidopsis	Achieve precise nucleotide insertions	Efficient insertion of fragments smaller than 6 bp; additional InDel frequently introduced	Li et al. 2017 ³⁶¹ , Zong et al. 2017 ³⁶² , Lu and Zhu 2017 ³⁶³ , Van Vu et al. 2024 ³⁶⁴ , Cao et al. 2024 ³⁶⁵ , Gupta et al. 2024 ³⁶⁶
	Transposase-assisted target-site integration	Agrobacterium	Arabidopsis, Soybean	Efficiently insert large DNA fragments	Integration of undesired flanking sequencing	Liu et al. 2024 ³⁶⁷
	HDR-mediated targeted gene insertion	Agrobacterium, Biolistics	Rice, Maize	Effectively incorporate large DNA fragments	Occurs with low efficiency	Dong et al. 2020 ³⁶⁸ , Peterson et al. 2021 ³⁶⁹ , Zhou et al. 2025 ³⁷⁰
Fine-tuning gene regulation	CRISPR activation	Agrobacterium	Arabidopsis, Rice	Simultaneously upregulate transcription	Challenging to fine-tune transcription precisely	Pan et al. 2021 ³⁷¹ , Casas-mollano et al. 2023 ³⁷²
	CRISPR interference	Agrobacterium	Arabidopsis, Rice, N. benthamiana, Tomato	Silence both exogenous and endogenous RNAs	Mediated by the endogenous RNA interference pathway	Aman et al. 2018 ³⁷³ , Zhang et al. 2019 ³⁷⁴ , Sharma et al. 2022 ³⁷⁵
	Cis-regulatory element (CRE) editing	Agrobacterium, Biolistics	Tomato, Rice, Maize	Fine-tuning transcription by manipulating CRE	Challenging to predict the correlation between genotype and phenotype	Rodríguez-Leal et al. 2017 ³²⁶ , Liu et al. 2017 ³²⁷ , Song et al. 2022 ³⁷⁶ , Want et al. 2025 ³²⁸ , Lou et al. 2024 ²¹²
	DNA methylation manipulation	Agrobacterium	Arabidopsis, N. tabacum, Cassava	Manipulate DNA methylation	Complexity associated with epigenetic regulation	Gallego-Bartolomé et al. 2018 ³⁷⁷ , Papikian et al. 2019 ³⁷⁸ , Veley et al. 2023 ³⁷⁹

Plastid genome editing	TAL effector cytosine base editing	Agrobacterium, Protoplast transfection	Rice, Arabidopsis, Rapeseed, Lettuce	Substitute nucleotide in plastid genome	Lack of selective marker in monocots	Li et al. 2021 ³⁸⁰ , Nakazato et al. 2021 ³⁸¹ , Kang et al. 2021 ³⁸²
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Clustered regularly interspaced short palindromic repeats, CRISPR; Homology-directed repair, HDR; Transcription activator-like, TAL.

5.2 Technical frontiers

Even if genetic targets are identified, the use of CRISPR tools is limited by the low throughput, long timescales, and issues with somaclonal variation that are endemic to plant transformation and regeneration. While the species- and cultivar-specific bases of these limitations is still poorly understood^{383,384}, some of the most exciting developments in CRISPR effector delivery stand to improve or altogether obviate the process of *in vitro* regeneration. Recent advances in morphogenic transformation, wherein developmental genes like *WUSCHEL*, *BABY BOOM*, *GRF-GIF*, *WOX5*, and *REF1* are used in *Agrobacterium*-mediated transformation, has greatly expanded the number of plant species and cultivars in which genome-editing techniques can be applied^{385–388}. The use of morphogenes has also expanded the range of tissues that can be used for somatic embryo formation³⁸⁹, unlocked transformation in new species and genotypes³⁹⁰, and produced strategies wherein *Agrobacterium*-mediated transformation can be used on non-embryonic explants to edit by *de novo* meristem production³⁹¹.

In some cases, reliance on plant regeneration may make it difficult or impossible to generate transgene-free edited plants. This is the case in many vegetatively propagated species where integrated transgenes cannot be segregated away in subsequent generations (e.g. banana, cassava, grapevine, fruit trees, sugarcane), species where tissue culture techniques are uncommon or have yet to be developed (e.g. pigeonpea, forest trees), and species where mature plants must be edited due long generation times (e.g. grapes, many tree species). It may be possible to remove these barriers through the use of CRISPR reagents optimized for delivery *in planta*. In preliminary studies, attaching CRISPR RNAs to systemically trafficked phloem mobile RNAs allows for the precise editing of meristematic and reproductive tissues, producing stably edited and transgene-free progeny in sexually reproducing species. Early examples of this approach^{392–394} demonstrated the use of RNA viruses and phloem-mobile RNA signals to deliver editing reagents and generate edits in somatic and germline tissues of *Nicotiana benthamiana*. While these approaches hold great potential in other crops³⁹⁵, the widespread application of these techniques has been hindered by the relatively small cargo size of RNA viruses (most or all of which are too small to express both the guideRNA and its cognate CRISPR effector) and the relative dearth of well-characterized vectors based on plant RNA viruses.

In regards to cargo size limits, recent reports have described highly active miniature CRISPR tools, some of which rely on overlapping protein and RNA coding regions to reduce the size of CRISPR cassettes³⁹⁶. Application of compact type-V CRISPR tools has shown potential in editing via RNA viruses^{392,393}, and this approach will benefit from further development and

discovery of minimal gene-editing systems. This potential highlights the importance of discovering or domesticating novel phloem-mobile viruses³⁹⁷ and of understanding the molecular determinants of phloem RNA transmission³⁹⁸, meristem permeability, and phloem loading across crops. Many RNA viruses and viroids are stably transmitted between generations via episomal retention in gametes³⁹⁹, and comprehensive investigation of the intergenerational (vertical) and environmental (horizontal) transmission of viral vectors is needed to mitigate plant-pest risk.

In perennial carbon crops and woody trees, viral editing might be indispensable for generating stable edits in mature plants⁴⁰⁰. In annual, sexually reproducing crops, viral administration could eliminate the incidence of somaclonal variation and need for T-DNA segregation inherent to modern transformation pipelines. Beyond viruses, crops could also be edited via graft-mobile RNA molecules that deliver effectors in conjunction with tRNA and viral-derived signal sequences⁴⁰¹. While the determinants of RNA transmission between graft and scion is still poorly understood, advances in this space could open up considerable opportunities in crops grown commercially as grafts (e.g., tomatoes, melons, apples, citrus).

Beyond delivery, plant gene editing has benefitted from the continual development of CRISPR-derived tools for precision genome editing (Table 2). Advances in plant tool development have resulted in a proliferation of useful Cas9 constructs optimized for target search, editing activity, and plant expression^{354–356}. Cas12/type-V editors have emerged as versatile alternatives to Cas9, allowing access to previously inaccessible loci and editing outcomes by way of their unique protospacer adjacent motif (PAM) requirements and ability to induce double-stranded breaks (DSBs) with single-stranded overhangs, respectively^{352,353}. SPRY-Cas9, an engineered variant of Cas9 without PAM requirements, allows for editing agnostic of the target sequence⁴⁰², and its continued optimization in plants³⁵⁵ has the potential to unlock new abilities for precision editing. Prime editing and base editing have further expanded the genome-editing toolbox by enabling highly precise modifications of sequences with improved efficiency^{357–363}. Advances in transposase-assisted target-site integration (TATSI) provide a promising strategy for the targeted integration of large DNA fragments into plant genomes³⁶⁷. In terms of precise gene regulation, notable progress has been made in regulating the expression of endogenous genes using RNA-guided CRISPR activation systems^{371,372}, and epigenetic editing tools^{377–379}. Collectively, these developments present substantial potential for precise, efficient, and flexible genetic modifications in plant biotechnology.

Several core components of the photosynthetic apparatus are encoded on the plastid genome, which presents a unique challenge in genome engineering. The plastid genome is contained in tens of discrete, double-membraned organelles per cell, and is maintained at a copy number that is tens to hundreds of times higher than that of the nuclear genome⁴⁰³. As the plastid is not known to import RNA, and because the plastidial protein import machinery involves unfolding polypeptide cargo, the delivery of CRISPR ribonucleoproteins encoded by nuclear or episomal constructs has yet to be achieved. Despite this, recent progress has been made using TAL effector-based base-editing systems to edit the plastid genome, obviating the need for RNA import³⁸¹. These tools have been used to improve rubisco catalytic efficiency via targeted substitution of amino acids of the *RbcL*⁴⁰⁴ gene, and they offer immense potential to modify other plastid-encoded genes involved in photosynthetic electron transport and ATP synthesis. Understanding the genetic determinants of plastid DNA copy number and

recombination^{405,406} stands to be immensely useful in improving the speed and efficiency of plastid DNA modification.

5.3 Generating new carbon crops

Looking forward, among the most impactful applications of gene editing will be in the rapid domestication of plants for applications such as CDR. Many genes and mutations associated with crop domestication are known⁴⁰⁷, and CRISPR has been used both to rapidly recapitulate ancient domestication processes⁴⁰⁸ and to domesticate heretofore wild plants⁴⁰⁹. The application of these principles to generate new carbon crops presents an exciting opportunity for creating bespoke carbon sequestration systems. For example, immense global focus has been placed on the breeding of deep-rooted perennial grasses to replace annual wheat⁴¹⁰ and on the identification of climate-hardy alternatives to common varieties⁴¹¹. The use of gene editing could broadly accelerate the refinement of crops like these to fit novel agronomic niches.

Future Directions

Progress in plant gene editing is propelled by a coalescence of recent advancements across biotechnology. The introduction of CRISPR as a tool for crop improvement has dramatically transformed plant scientists' ability to tune plant phenotypes, and its global usefulness is dependent on the continued development of biochemical tools and delivery methods for editing, genomic resources between and within species, and predictive AI models trained on diverse datasets from plants (e.g., data on chromatin modification, gene expression and protein production, metabolites and chemical constitution, single-cell data). Many photosynthetic trait improvements require changes in gene expression or activity, so the development of high-throughput assays (single cells, pooled transfection or transduction) and computational tools (genomic language models, genetic foundation models, protein structure and function models) to understand how to edit *cis*-regulatory DNA, paralog-rich gene families, and protein sequences will be instrumental in photosynthesis research. There is presently much attention on the development of genomic language models to understand the structure and configuration of DNA, and the usefulness of these in understanding plant transcriptional regulation is yet to be fully realized. Application of genomic language models might also be useful in the annotation of newly sequenced genomes, in estimating fitness differences between crop accessions, and in understanding complex biological datasets for plants. Immense genomic resources exist in some crops, such as maize, rice, and tomato, while entire crop-bearing clades of plants have yet to be sequenced. Public genomic resources that heavily sample within and between species are critical to improving some applications of machine learning in gene editing and basic biology.

As outlined in this review, improving photosynthesis is an important lever for enabling novel CDR approaches, but the benefits of tuning light and carbon capture across crop species can be appreciated by the global community at large. Photosynthetic efficiency is a major determinant of yield and stress tolerance, and its improvement by gene editing is an intriguing approach for improving under-resourced and orphan crops. The development of CRISPR viral delivery methods could obviate the need for tissue culture, somatic embryogenesis, and transgene segregation, allowing regulatorily permissible editing in recalcitrant, clonally

propagated, and mature or established crops. Discoveries of plant viral vectors to suit a range of species, ways to alter the phloem-transmissibility and meristem penetrance of RNA, and gene-editing tools (nucleases, base and prime editors) that can be expressed on size-constrained RNA viruses all stand to enable low-cost, distributed crop improvement efforts. Major morphogene-based transformation approaches rely on the use of proprietary genetic approaches and patent-protected sequences, which emphasizes the need for discovery and dissemination of open-source approaches for embryogenesis in under-resourced crop species.

Looking forward, almost all applications of gene editing in CDR are dependent on a number of economic and social incentives, and will rely on technological innovation outside plant genetics. The establishment of robust public and private carbon offset markets could significantly incentivize the development of photosynthetically enhanced carbon crops, but the path to accrediting carbon sequestration at scale is still a hurdle. Measuring soil carbon presents a technical challenge in the verification and dispensation of carbon credits, emphasizing the need for reliable measurement methodologies, standardized protocols, and advanced technologies capable of accurately monitoring soil carbon dynamics. While BiCRS stands to have numerous benefits, its application is constrained by high capital investment requirements and significant transportation costs. Identifying low-cost carbon cropping and biomass processing practices that minimize transportation costs is essential. Finally, the widespread adoption and scaling of gene-edited carbon crops is contingent upon biosafety regulations that differentiate gene editing from traditional genetically modified organism (GMO) designations, thereby exempting them from costly, burdensome, and time-intensive regulatory processes⁸. Clear and harmonized international standards governing the field-testing and commercialization of gene-edited crops will be critical given that many agricultural commodities rely on global trade for profitability.

In sum, the coming years present an outsized opportunity to leverage contemporary biotechnological tools to improve photosynthesis. Harnessing CRISPR-mediated gene editing to enhance crop photosynthetic performance offers a dual promise: simultaneously addressing global food security and enabling scalable, nature-based solutions for CDR. Ultimately, the strategic integration of photosynthesis-enhanced crops into agricultural systems not only represents an important stride toward sustainable agriculture but also provides an innovative pathway toward meeting urgent climate mitigation goals.

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Author contributions

E.D.G. and F.Z.W. researched data, discussed content, created figures, and wrote the review with equal contribution. A.J. contributed significantly to research, writing, and review/editing of the manuscript. K.K.N. and D.F.S. contributed to discussion, conceptualization and review/editing of

the manuscript throughout the writing process. J.S. contributed to research, writing and review/editing of the manuscript. P.G.L. contributed to discussion and review/editing of the manuscript before submission.

Competing interests

The authors declare no competing interests.

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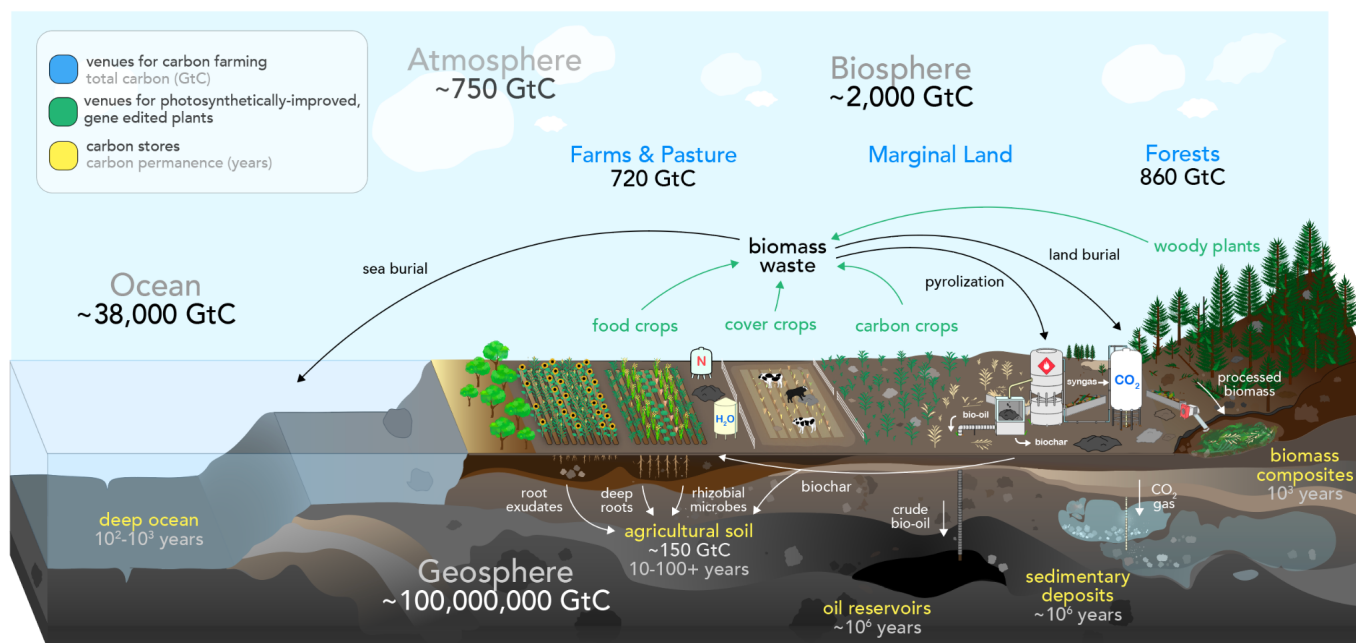


Figure 1. Large-scale opportunities for plant carbon capture and storage

Diagram of the major geological stores of carbon (grey), the ecological venues for plant CDR (blue), carbon-sequestering crops (green), and geological sinks for plant-derived biomass (yellow). Once biomass has been accumulated by farming, carbon cropping, or forestry, it can be used to augment soil carbon and/or be physically processed and stored geologically. A pyrolysis facility is provided as an example of a BiCRS process, where farm waste, purpose-grown biomass crops, and forest litter undergo thermochemical processing. The residual biochar (30-50 wt%) can be used as a soil and pasture amendment. Product gas (10-30 wt%) is composed of CO₂ and in small part syn-gas (CO, CH₄, and H₂) and can be passed into deep saline aquifers for mineralization. Oxidized bio-oil (20-40 wt%) is negligibly usable (biofuel 1-2 wt%, petroleum <0.1%) and can be stored in depleted oil fields using existing petroleum infrastructure. Outside physical processing, the same biomass sources can be buried into deep carbon reservoirs either on land or in the ocean. The carbon permanence, i.e., how long captured carbon remains sequestered without being re-released to the atmosphere, is indicated for each geological store.

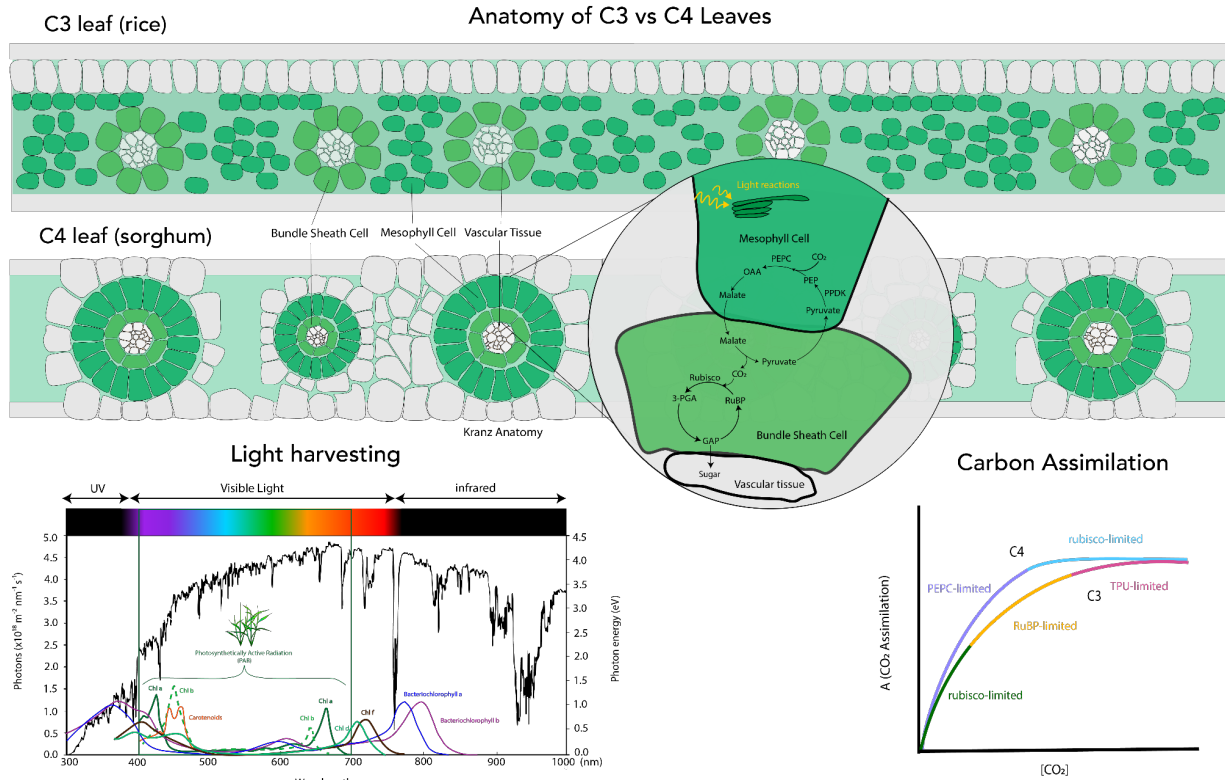


Figure 2. Theoretical and Evolutionary Insights for Improving Photosynthesis

Anatomy of C3 vs C4 leaves: In C4 plants, the CO_2 -concentrating mechanism (CCM) is made possible by the C4 Kranz anatomy—the arrangement of bundle sheath and mesophyll cells in two concentric layers outside the vascular tissue. In the C4 mesophyll cell, CO_2 is initially fixed as oxaloacetate (OAA) by phosphoenolpyruvate carboxylase (PEPC) and converted into malate for transport into the bundle sheath cell. CO_2 is released via malate decarboxylation near the rubisco carboxylation site in the bundle sheath cell and subsequently incorporated into ribulose 1,5-bisphosphate (RuBP) by rubisco to form 3-phosphoglycerate (3PGA), which initiates the Calvin-Benson cycle. The glyceraldehyde-3-phosphate (GAP) that results after phosphorylation and reduction in the CB cycle is converted into sugars and exported to the vascular tissue. The pyruvate that results from malate decarboxylation is recycled back into the mesophyll cell where it is regenerated into phosphoenolpyruvate (PEP) by pyruvate phosphate dikinase (PPDK). In C3 leaves, Kranz anatomy is not present and mesophyll cells are differentiated into the palisade (vertically aligned) and spongy layers (loosely distributed horizontally).

Light harvesting: Plants rely on chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids to absorb solar energy, limiting the PAR spectrum to only 400-700 nm. Cyanobacteria and anoxygenic bacteria, on the other hand, have chlorophyll *d* (Chl *d*), chlorophyll *f* (Chl *f*), bacteriochlorophyll *a*, which enable them to absorb near-infrared light (700-760 nm) and infrared light (800-1000 nm). Light spectrum adapted from Cardona et al. (2018)⁸⁷.

Carbon assimilation: Leaf CO_2 uptake (A) can be described by the A versus C_i curve. In C3 plants, the A/C_i curve consists of 3 phases where it is limited by 1) rubisco carboxylation 2) rate of electron transport (J_{max}) and RuBP regeneration, and 3) triose phosphate utilization. In C4 plants, the A/C_i curve is only observed to have two phases where it is 1) initially limited by PEPC carboxylation in the mesophyll cell and 2) by rubisco carboxylation in the bundle sheath cell.

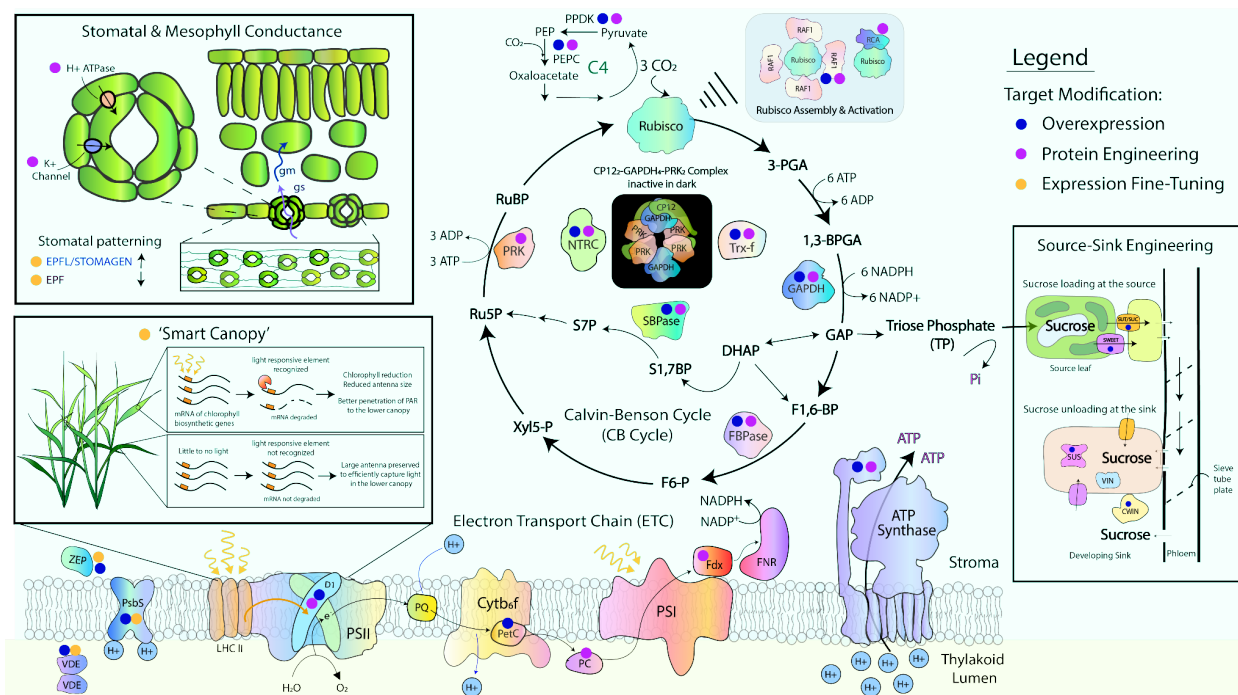


Figure 3. Targets for Improving Photosynthesis

Photosynthetic targets in the light and carbon reactions of photosynthesis

Electron transport chain (ETC)

In the photosynthetic electron transport chain (ETC), light received by light harvesting antenna complex II (LHC II) is passed on to the reaction center in photosystem II (PSII) to drive charge separation where e^- split from H_2O initiates downstream electron transport. Upon lumen acidification in highlight conditions, the protonated Photosystem II subunit S (PsbS) induces a conformational change in PSII and initiates non-photochemical quenching (NPQ). Violaxanthin de-epoxidase (VDE) converts the xanthophyll pigment violaxanthin into zeaxanthin as part of the NPQ process, and zeaxanthin epoxidase (ZEP) converts zeaxanthin back to violaxanthin once excess light is no longer present. Plastoquinone (PQ) oxidizes the primary electron acceptor (not shown) at the reaction center (RC) (consisting of the D1 subunit), and passes the electron to the Cytochrome b_6f complex (Cyt b_6f). The Rieske subunit of Cyt b_6f (encoded by PetC) reduces plastocyanin (PC) for downstream electron transport to photosystem I (PSI). The electron is re-excited at PSI and then transferred to ferredoxin (Fdx) and subsequently ferredoxin-NADP $^+$ -reductase (FNR) where it reduces NADP $^+$ into NADPH. The proton gradient generated by the electron transport process drives phosphorylation of ADP to ATP. The resulting ATP, along with NADPH, powers carbon reactions in the Calvin-Benson cycle.

Calvin-Benson Cycle (CB Cycle)

Rubisco accumulation factor 1 (Raf1) aids in the assembly of Rubisco, while Rubisco activase (Rca) activates Rubisco by releasing inhibitory sugars. In the CB cycle, rubisco catalyzes formation of 3-phosphoglycerate (3-PGA) through reaction with CO_2 . CO_2 is released from carboxylation of a C4 acid in C4 plants and arrives at the carboxylation site via diffusion from stomatal openings in C3 plants. 3-PGA is then converted into 1,3-bisphosphoglycerate (1,3-BPGA) via phosphorylation by ATP and subsequently reduced by GAPDH using NADPH. The resultant glyceraldehyde-3-phosphate (GAP) is either exported as sugars for downstream sink metabolism or recycled back into the regenerative phase of the CB cycle. In the regenerative phase, GAP is either converted into dihydroxyacetone-phosphate (DHAP) or fructose 1,6-bisphosphate (F1,6-BP), where it is subsequently converted to sedoheptulose 1,7-bisphosphate (S1,7-BP) by fructose 1,6-bisphosphate/sedoheptulose 1,7-bisphosphate aldolase, or to fructose-6-phosphate (F6-P) by fructose 1,6 bis-phosphatase (FBPase), respectively. S1,7-BP is then converted to sedoheptulose 7-phosphate (S7-P) by sedoheptulose 1,7-bisphosphatase (SBPase). F6-P is subsequently converted to xylulose-5-phosphate (Xyl5-P) via several steps and finally ribulose 5-phosphate (Ru5P). S7-P is also converted to Ru5P through several reactions and regenerated into ribulose 1,5-bisphosphate (RuBP) through phosphorylation by ribulose-5-phosphate kinase (PRK).

GAPDH and PRK are sequestered in the GAPDH₂-CP12₄-PRK₂ complex in the dark. This complex dissociates upon Trx-f and NTRC reduction in the light as part of photoinduction, allowing for activation of the CB cycle.

'Smart Canopy'

A 'Smart Canopy' can be engineered via insertion of a light-responsive element into the untranslated regions (UTR) of chlorophyll biosynthetic genes or other genes implicated in pigment metabolism. This light-responsive element would theoretically recruit transcriptional or translational repressors under high light and lead to lower chlorophyll levels and smaller antenna complexes under highlight while allowing for increased chlorophyll accumulation and formation of larger antenna complexes under low light. This holds potential for maximizing light use efficiency in canopy environments.

Stomatal & Mesophyll Conductance

Stomatal patterning is determined by the Epidermal Patterning Factor (EPF) and EPF-like (EPFL), which are secreted proteins that play key roles in epidermal patterning, development, and morphology. Fine-tuning expression of these key genes enables precise control over stomatal density, which subsequently determines stomatal conductance (g_s) and influences mesophyll conductance (g_m). Optimizing the kinetics of stomatal opening and closing via modification of guard cell H⁺ ATPase and K⁺ channels holds further potential for improving CO₂ uptake and carbon assimilation.

Source-Sink Engineering

Sucrose is exported out of the source leaf via Sugars Will Eventually be Exported Transporters (SWEETs) and SUC/SUT transporters and loaded into the phloem for transport to the developing sink. At the sink, either the same SWEET or SUC/SUT transporters or members of the same family are responsible for unloading sucrose into the developing sink. The unloaded sucrose is further metabolized by cell wall invertases (CWIN), vacuolar invertases (VIN), and sucrose synthase (SUS) to support sink tissue development.

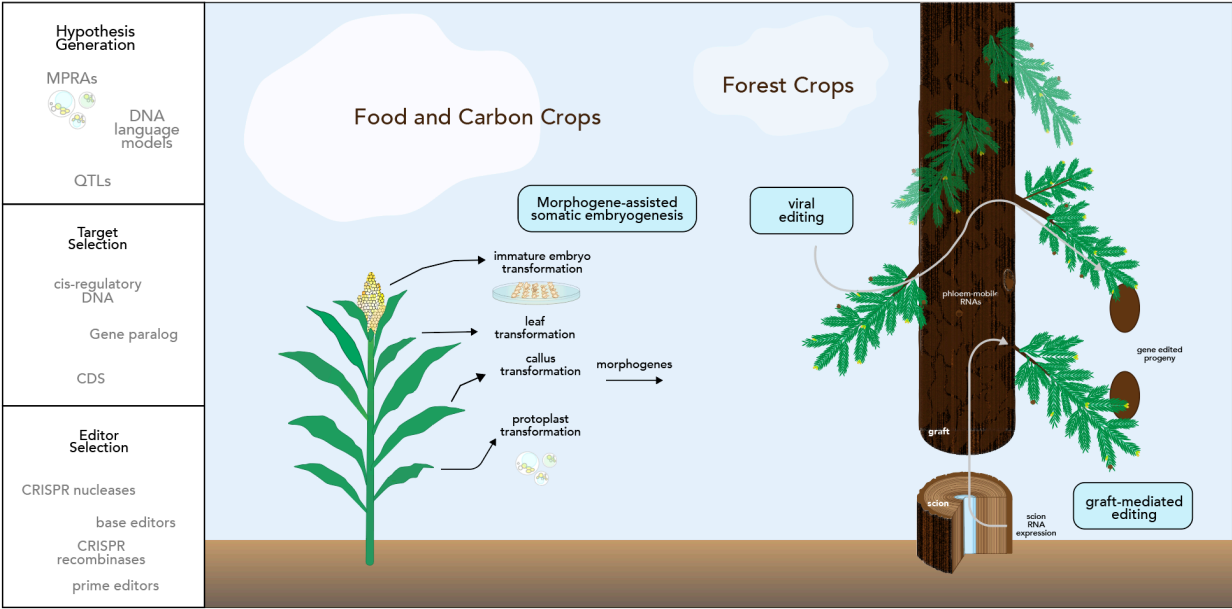


Figure 4. Technological approaches for carbon crop gene editing
Beyond plant vector images (shown above), this is one we'd like to work out with the art editor.