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Review

Meeting the Global Food Demand of the Future by Engineering Crop Photosynthesis and Yield Potential

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Increase in demand for our primary foodstuffs is outstripping increase in yields, an expanding gap that indicates large potential food shortages by mid-century. This comes at a time when yield improvements are slowing or stagnating as the approaches of the Green Revolution reach their biological limits. Photosynthesis, which has been improved little in crops and falls far short of its biological limit, emerges as the key remaining route to increase the genetic yield potential of our major crops. Thus, there is a timely need to accelerate our understanding of the photosynthetic process in crops to allow informed and guided improvements via in-silico-assisted genetic engineering. Potential and emerging approaches to improving crop photosynthetic efficiency are discussed, and the new tools needed to realize these changes are presented.

An Emerging Yield Gap

Nothing is more important to human health and well-being than an adequate supply of food in terms of nutrition and calories. Although a significant proportion of the global population has suffered malnutrition over the last 50 years, it has been the result of failures in access to food, not in its global production. Indeed, over this period, we have seen surpluses of the major crops, which make shortages a very distant concern for most of the population. The most important primary foodstuffs, in terms of millions of metric tons (Mt) produced in 2013, were maize (1,018 Mt), paddy rice (746 Mt), wheat (713 Mt), and soybean (276 Mt) (Food and Agriculture Organization of the United Nations, 2015). These four crops account for about two thirds of calories consumed globally (Ray et al., 2013). Moreover, the average global yield per unit area of land (t/ha) for each of these crops has more than doubled since 1960, as illustrated for rice and wheat (Figure 1). So why bother worrying about food security now? One reason is that these global surpluses in staple crops have influenced the progressive decline in spending on plant science research and crop improvement, evident at the global level (Beintema and Elliott, 2009). However, this shift in funding may be myopic in the face of current global population and food consumption trends. Notably, the global population is expected to increase from just over 7 billion today to 9.5 billion by 2050, a 35% increase (USCB, 2015). An increasing proportion of the population will be urban, resulting in diets shifting increasingly from staples to processed foods, fortified with more meat and dairy products, which require large amounts of primary foodstuffs to produce. For example, 10 kg of feed is required to produce 1 kg live cattle (Smil, 2000). Thus, an increase in urban population will result in an increased demand for high-quality animal products, requiring an increase in crop production that is substantially faster than that estimated based solely on the projected population growth. This trend is expected to continue, and it is predicted that the world will need 85% more primary foodstuffs by 2050, relative to 2013 (Ray et al., 2013).

So is our current rate of increase in crop yields sufficient to meet this rising demand? It doesn't seem to be the case. If current rates of crop yield improvement per hectare are simply maintained into the future, supply will fall seriously below demand by 2050 (Figure 1; Ray et al., 2013). The resulting rise in global food prices may have the largest impact in the poorest tropical countries, which have the highest population increases. A compounding factor is that improvement in subsistence crops in these tropical countries is even slower than in our four leading crops. For example, the global average increase in yield per hectare of cassava, a major staple for sub-Saharan Africa, between 1960 and 2010 was 63%. This is less than half of the 171% increase for wheat over the same period (Figure 1). The problem is further compounded by the fact that the rate of improvement in yield of even our major crops in some areas of the globe is stagnating or even moving into reverse (Long, 2014; Long and Ort, 2010; Ray et al., 2012). Indeed, China, India, and Indonesia are the world's largest producers of rice, where yields per hectare across these countries increased by an average of 36% between 1970 and 1980 but only by 7% between 2000 and 2010 (Long, 2014). When faced with such numbers, one may rightfully ask: why are yield improvements stagnating?

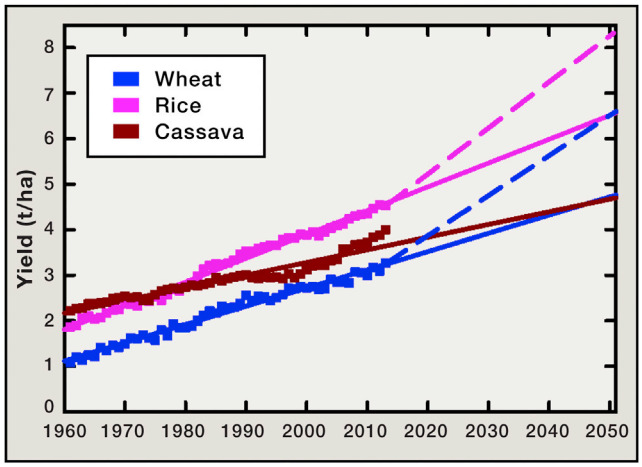

Figure 1. Annual Average Global Yields of Cassava, Rice, and Wheat from 1961 to 2013

Annual average yields for the entire globe in metric dry tons per hectare for each year from 1961 to 2013 for cassava, rice, and wheat (Food and Agriculture Organization of the United Nations, 2015). Solid lines are the least-square linear regressions fitted to these data and projected forward to 2050. The broken lines indicate the projected demand for rice and wheat, after Ray et al. (2013). The original data for cassava were provided as wet weight and are corrected here to dry weight, assuming a 70% water content.

Stagnation in Yield Improvement and Photosynthesis

The gains of the Green Revolution were achieved largely through improved genetics coupled with the enhanced agronomy and crop protection that allowed realization of the higher genetic yield potential. We can begin to understand these gains by defining them in mathematical terms. Yield potential (Y_p) is the mass of harvested material per hectare of land that a genotype of a crop can achieve in a given environment in the absence of biotic and abiotic stresses. Improved Y_p was achieved during the Green Revolution, in particular by selecting genotypes that partitioned more of their biomass into the harvested product. For example, the selection of dwarfed genotypes of wheat resulted in more biomass in the grain and less in the stem. This proportion of a plant's biomass that is invested into the harvested product, e.g., the grain of rice, is termed the partitioning efficiency or harvest index (ε_p). To a first approximation, the yield potential of a given genotype is then the product of the solar radiation received over the growing season by a unit area of land (Q) and the efficiencies with which the crop intercepts that radiation (ε_i), converts the intercepted radiation into biomass energy (ε_c), and then partitions the biomass into the harvested part of the plant (ε_p):

$$Yp = Q.\varepsilon_i.\varepsilon_c.\varepsilon_p..... \quad \text{(Equation 1)}$$

With reference to this equation, the Green Revolution increased ε_i and ε_p. In fact over the past 50 years, harvest index (ε_p) has almost doubled in the major grain crops and now stands at ~0.6 for modern cultivars of rice, wheat, and soy (Long et al., 2006b; Zhu et al., 2010). However, if these plants are to retain the structural components of the stems and ear or pod casings to support the seed at harvest, there is little prospect of further genetic improvement for this component of the equation. Similarly, interception efficiency (ε_i), that is the proportion of the visible sunlight that is intercepted by the crop over the growing season, has reached 0.8–0.9 for modern crop genotypes. Again, this suggests that this determinant of yield potential is also very close to its biological limits (Zhu et al., 2010). The one area in which there has been little or no improvement is in conversion efficiency (ε_c) of visible solar energy, which remains at about 0.02, and roughly one-fifth of the theoretical efficiency of 0.1 for C3 crops such as wheat and rice or 0.13 for C4 crops such as maize and sorghum (Zhu et al., 2008, 2010). Indeed, as it is clear that 50 years of conventional plant breeding has greatly improved ε_i and ε_p but not ε_c, this component of the equation appears to be a promising focus for further enhancement of yield potential.

Conversion efficiency depends on the efficiency of the process of photosynthesis, net of respiratory losses by the crop. Concern over global climate change motivated many studies of the effects of elevated CO_2 on crop production and photosynthesis. CO_2 is a limiting substrate for photosynthesis in C3 crops, so the primary effect is to artificially boost photosynthetic rate. Invariably, this results in increased yield (Ainsworth and Long, 2005; Kimball, 1983; Long et al., 2004, 2006a), demonstrating that there would be a clear benefit to yield if total crop photosynthesis could be increased genetically in crops (Long et al., 2006b). Yet, this also begets the question: if photosynthesis has such a strong influence on crop yield, why have traditional breeding and selection for higher yield delivered no or very small improvements in photosynthetic efficiency? There are several reasons for this effect. Within a crop species and its relatives, there is huge variation in ε_i and in factors affecting ε_p, such as the proportion of biomass invested in leaves during vegetative growth, rates of leaf growth, size of leaves, and leaf longevity. This has provided breeders with much variation in selecting for improved ε_i and ε_p. By contrast, the process of photosynthesis is highly conserved, not only within a crop species, but across a wide range of plants. Further, directed efforts have screened for germplasm with high light-saturated photosynthetic rates at the leaf level, and selection here has often been at the expense of other traits. For example, selection for higher light-saturated rates of leaf photosynthesis alone has often indirectly selected for lower total leaf area, offsetting any advantage at the crop level (Long et al., 2006b). This approach also ignores the fact that about half of crop carbon gain occurs under light-limited conditions (Long, 1993). How can we then approach increasing photosynthetic efficiency, and why might this be a timely strategy for a second Green Revolution when it was not for the first one?

Three factors make improving overall crop photosynthetic efficiency a possibility today. The first one is based on our understanding of the photosynthetic process. In the 50 years since the start of the first Green Revolution, knowledge of the photosynthetic process has exploded. From light capture by pigment molecules to production of storage carbohydrates; this fundamental process for all life on Earth is now understood in great detail. For higher plants, some algal species, and photosynthetic prokaryotes, not only is every step known, but the structures of the key proteins have been unraveled to high resolution to reveal the mechanism of their action, while the genes coding for the key components have been characterized. This includes

the recent isolation and characterization of the phycobilisome antenna complex and photosystems I and II from Synechocystis (Liu et al., 2013). This knowledge has facilitated the generation of kinetic models describing every discrete step of the entire processes of both C3 and C4 photosynthesis (Wang et al., 2014; Zhu et al., 2013). As a result, photosynthesis is undoubtedly the best known of all plant processes, and its similarity across all crops can be an advantage since what improves efficiency in one crop is likely to do so in another. The second factor lays in the emergence of high-performance computing (HPC). The rapid growth of computational power and new software tools has allowed the simulation of photosynthetic kinetic models of the complete process and application of optimization routines (Zhu et al., 2007, 2011). Not only can the metabolic pathways and their cellular organization be represented in silico, but there is now the opportunity to integrate them into realistic representations of the whole canopy of a crop, facilitating predictions of optimal distribution of resources at the sub-cellular, cellular, leaf, and whole-crop level (Drewry et al., 2014; Song et al., 2013; Tholen et al., 2012; Tholen and Zhu, 2011). HPC allows the in silico investigation of thousands of permutations of up- and downregulation of the genes and proteins involved in photosynthesis, or the impacts of the potential addition of foreign genes and pathways, to identify the best targets for practical manipulation (McGrath and Long, 2014; Xin et al., 2015). Finally, the third factor is the advance in genetic engineering. Genome editing and synthetic biology, once confined in the public domain to model species, is now becoming increasingly routine for a wide range of crops (Barampuram and Zhang, 2011). Combined, these three factors allow an informed and directed approach to engineering improved photosynthetic efficiency.

Does such a comprehensive strategy work? As an example, using this three-pronged approach, Zhu et al. (2007) predicted from applying evolutionary algorithms in silico that, for a given investment of resources into photosynthetic carbon metabolism, there should be significant re-allocation of resources between the proteins involved to maximize efficiency. This was predicted to deliver a 60% increase in photosynthetic efficiency, and the largest single change was an increase in investment in the enzyme sedoheptulose-1:7-bisphosphatase (SBPase). The benefit of upregulation of this enzyme was also predicted to increase as atmospheric CO_2 levels rise (Zhu et al., 2007). Subsequent upregulation of this enzyme in tobacco was shown to substantially increase the productivity of a field crop of tobacco, and this increase in photosynthesis was greater under an open-air elevation of CO_2 in the field (Rosenthal et al., 2011).

If such gains can be achieved, then why has natural evolution not already optimized the system? First, evolution in the wild selects for survival and fecundity and not directly for productivity. Second, the carbon dioxide concentration of the atmosphere averaged over the past 25 million years—and in which the ancestors of our crop plants evolved—was about 220 μmol mol^{-1} (Zhu et al., 2004b). Today, it is almost double that concentration, and most of that increase has occurred in the last 100 years, which is a too-short period of time for our crops to become adapted. Therefore, our challenge is to identify new targets and develop strategies to achieve these predicted gains.

Targets for Increasing Crop Photosynthetic Efficiency

From our fundamental understanding of photosynthesis, what are the likely targets for systems and synthetic improvement of efficiency in crops? As noted above, the achieved net photosynthetic efficiency of our crops falls far short of the theoretical; so what are the weak links in the process? Photosynthesis can be divided into two stages, sometimes referred to as the light and dark reactions. The light reactions concern the capture of light energy by chlorophyll and associated pigments, water splitting, and electron transport on the chloroplast membrane reducing NADP and providing the proton gradient that powers phosphorylation of ADP. In the dark reactions, the resulting NAPDH and ATP power the Calvin cycle, which assimilates carbon dioxide and reduces it to carbohydrate. Examination of the steps involved in this process shows that a minimum of eight photons are required for the assimilation of one molecule of CO_2 and release of one molecule of O_2 from water splitting (Blankenship et al., 2011). Analyses of the actual photon requirement of leaves of a wide range of plants and crops and of young and old leaves show that, under low light and in the absence of other stresses, the photosynthetic apparatus of most leaves comes very close to the theoretical requirement of eight photons (Björkman and Demmig, 1987; Long et al., 1993). This shows that the primary processes can already operate close to maximum efficiency. However, as absorbed light is increased, the efficiency of photon use declines. This is because of limitation in either electron transport or capacity to utilize the ATP and NADPH produced. There are nevertheless two apparent ways in which the efficiency of light capture and energy transduction could still be increased for field crops; the first one is by engineering pigments that could utilize more of the sunlight's spectrum, and the second one is by overcoming light saturation of the downstream photosynthetic processes. First, the pigment systems of plants, like the green algae from which they evolved, can only effectively use the visible spectrum, with a very small extension into the near infra-red and UV-A spectra. This means that more than half of solar energy is unavailable (Zhu et al., 2008). Other algae and some photosynthetic bacteria use pigments that are able to capture and utilize longer wavelengths of near infra-red radiation. Re-engineering the photosystems and their collection antennae that drive electron transport could raise the maximum efficiency by allowing use of another ~20% of the available solar energy (Blankenship et al., 2011). This would be particularly valuable in the lower levels of crop leaf canopies in which carbon assimilation will rise linearly with increased efficiency of light capture (Long, 1993). Another approach suggested as beneficial from modeling is to reduce the antenna size of the photosystems in upper canopy leaves. The antennae are the chlorophyll molecules that capture light energy and feed it to the photosystem centers (PS1 and PSII) that drive electron transport, delivering the NADPH and ATP that power carbon dioxide assimilation. Modeling suggests that these antennae are too large, trapping more light energy than they may use. This may have an evolutionary origin because, in the wild, an individual that can trap more light in its upper leaves denies light to competing plants underneath, even if it cannot itself use the light. But, in a crop monoculture, it is disadvantageous, and reducing antenna size could save resources and allow more light to reach lower leaves (Ort

et al., 2011). Decreasing the antenna size will also decrease loss of absorbed light energy in the form of heat and fluorescence for leaves under both high and low light levels (Zhu et al., 2005; Blankenship and Chen, 2013). Chlorophyll-a-oxidase has been reported to be related to antenna size (Masuda et al., 2003) and can thus be a target for manipulation to increase light capture efficiency and assimilation.

Downstream limitations of assimilation also exist, where in high light, i.e., between full and approximately one-third of full sunlight (i.e., 550–2,200 μmol m^{-2} s^{-1} visible photons), the photosynthetic apparatus is capturing more light energy than it may utilize and is saturated. If chlorophyll molecules remain in an excited state, the excitation energy can be transferred to oxygen, producing a range of oxidizing radicals. These in turn can destroy the photosynthetic apparatus (Aro et al., 1993; Long et al., 1994). Plants protect themselves against excess radiation by changes within the apparatus induced through the de-epoxidation of the xanthophyll pigment violaxanthin to zeaxanthin. These and associated changes result in dissipation of absorbed excess energy harmlessly as heat (Ahn et al., 2008; Müller et al., 2001; Niyogi, 1999). However, in a crop canopy, photosynthetic cells can pass rapidly from high light to low light as a cloud passes the sun or as the continuous change in solar angle can abruptly place a cell in one leaf in the shade of another leaf. Suddenly then, a photosynthetic cell is transferred from light saturation to light limitation, and here dissipation of absorbed light energy as heat will lower the efficiency of photosynthesis. Modeling of the dynamics of these light fluctuations shows that this could cost up to 30% of potential assimilation (Zhu et al., 2004a). Again, there are algal systems that can relax this heat dissipation far more rapidly, offering synthetic opportunities to overcome this loss. Improved understanding of the mechanism from model plants such as *Arabidopsis* indicates additional systems opportunities to enhance this recovery of photosystem II (PSII) efficiency. This is through up- regulation of genes coding for enzymes involved in inter-conversion of intermediates of the xanthophyll cycle and the interaction of these intermediates with the PSII complex (Murchie and Niyogi, 2011).

What are the limitations in the "dark" reactions of photosynthesis? Application of a widely validated steady-state biochemical model of photosynthesis (Farquhar et al., 1980, 2001) to crop leaves shows that, at light saturation, the process in vivo is typically co-limited by capacity for carboxylation and capacity for regeneration of the acceptor molecule for carboxylation, ribulose-1:5 bisphosphate (RubP) (Long et al., 2004). Capacity for carboxylation in C3 crops is determined by the activity of a single enzyme, RubP carboxylase/oxgenase (Rubisco). Rubisco typically represents 50% of the soluble protein in the leaf and is the most abundant protein on the planet (Spreitzer and Salvucci, 2002). Why is such an abundant enzyme limiting? Rubisco can catalyze both the carboxylation and oxygenation of RubP. If RubP is oxygenated, then a two-carbon compound, phosphoglycolate, is formed. Plants metabolize this product through a complex pathway involving peroxisomes and mitochondria to regenerate phospho-glycerate (PGA; Figure 2). PGA is a C3 intermediate of the Calvin cycle, but it is produced here at the cost of the loss of a molecule of CO_2 and the use of a significant amount of reductive and phosphorylating capacity generated by the light reactions (Figure 2) (Farquhar and Caemmerer, 1982). This process of oxygen consumption and CO_2 release is termed photorespiration. Photorespiration imposes a large penalty on net photosynthetic efficiency, which increases with temperature. This is because the specificity of Rubisco for CO_2 declines with temperature, so loss due to photorespiration rises from ~30% in cool climates to more than 50% in hot climates (Long et al., 2006b). To combat photorespiration, it appears that Rubisco in plants has evolved to become more specific for CO_2, but achieving specificity in evolution appears to have been at the expense of speed of catalysis. Indeed, the catalytic rate of Rubisco from plants is one of the slowest of any enzyme-catalyzed reactions at ~3.7 per active site per second (Parry et al., 2013; Tcherkez, 2013; Zhu et al., 2004b). Modern forms of Rubisco therefore represent a compromise between specificity (τ) and catalytic rate (k_{cat}). However, this compromise appears optimized for the past atmospheric CO_2 concentration of about 220 μmol mol^{-1} and not today's concentration of 400 μmol mol^{-1} (Zhu et al., 2004a). Computer simulation of crop canopies suggests that engineering a Rubisco optimized to today's atmosphere requiring a higher kcat, even at the expense of a lower specificity, could increase photosynthetic carbon gain by a crop canopy by up to 30% for the same total amount of enzyme (Zhu et al., 2004a).

Carbon dioxide is a competitive inhibitor of the oxygenation reaction of Rubisco. Evolution has exploited this in some photosynthetic organisms by the addition of structures to compartmentalize Rubisco and pathways that concentrate CO_2 in that compartment. C4 photosynthesis is one solution that has evolved independently over 60 times (Sage et al., 2012). In C4 plants, which include the crops maize, sorghum, sugarcane, and grain amaranth, Rubisco is isolated to an inner green bundle sheath surrounding the leaf veins. In these plants, carbon dioxide is first captured by carboxylation of phosphoenolpyruvate (PEP) to form a C4 dicarboxylate in an outer photosynthetic tissue or mesophyll and is then transferred to the inner tissue that it surrounds, the bundle sheath. Here, it is decarboxylated, releasing pyruvate that is then recycled back to the outer tissue, where it is phosphorylated back to PEP to complete the cycle (Long and Spence, 2013). In essence, this C4 cycle serves as a light-energy-driven CO_2 concentrating mechanism, which largely eliminates photorespiration (Sage et al., 2012; von Caemmerer and Furbank, 2003). The additional energy required by the C4 cycle is, in most circumstances, less than would be lost in photorespiratory metabolism (Long and Spence, 2013). C4 plants generally have higher rates of photosynthesis and include the most productive crops and plants known (DeLucia et al., 2014; Long and Spence, 2013; Piedade et al., 1991). Indeed, one approach to increasing photosynthetic efficiency in C3 crops such as wheat and rice is to convert them to C4 plants. A major effort is underway to achieve this in rice; however, it requires many changes in both anatomy and expression of Calvin cycle enzymes, as well as inserting and expressing the genes of C4 photosynthesis (von Caemmerer et al., 2012). Nevertheless, the fact that this process has successfully and independently evolved over 60 times in nature suggests that this is achievable, although it will require further understanding of the genetic basis of the dimorphic photosynthetic tissue and localization of components of the C4 and Calvin cycles.

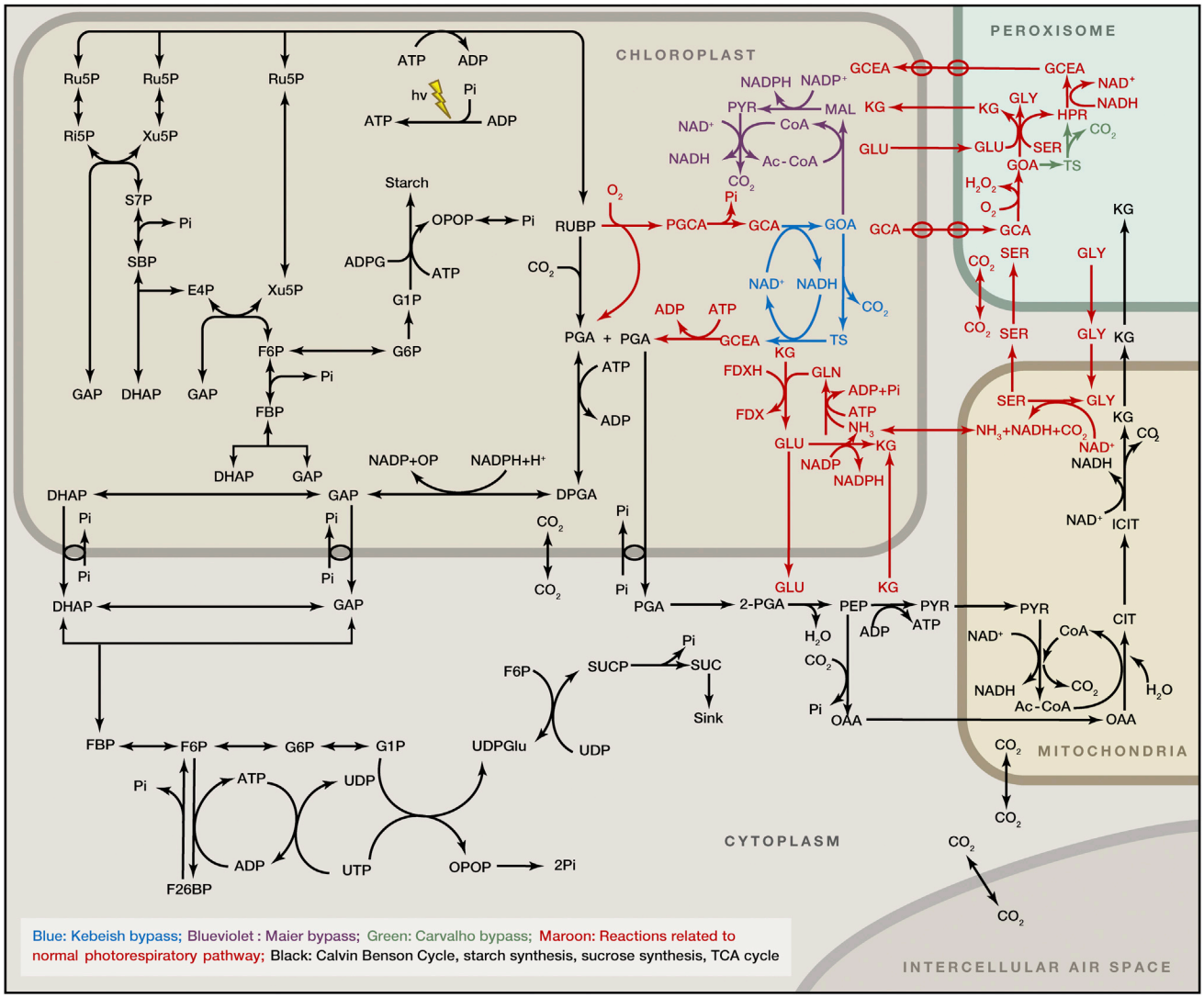

Figure 2. Schematic Representation of Photosynthetic C3 Metabolism, with Both the Native and Potential Synthetic Pathways of Photorespiratory C2 Metabolism

The C3 photosynthetic or Calvin cycle and pathways to immediate carbohydrate products in the cytosol are indicated in black. Pathways for C2 metabolism are indicated as follows: maroon, the native photorespiratory C2 pathway; blue, the synthetic bypass described by Kebeish et al. (2007); violet, the bypass described by Maier et al. (2012); and green, the bypass described by Carvalho et al. (2011) in green. Abbreviations: ADPG, ADP-glucose; AcCoA, Acetyl- Coenzyme A; CIT, Citric acid; CoA, Coenzyme A; DHAP, Dihydroxyacetone-phosphate; DPGA, 1,3- bisphosphoglycerate; E4P, Erythrose 4-phosphate; F6P, Fructose 6-phosphate; FBP, Fructose 1,6- bisphosphate; FDXH, Reduced ferrodoxin; FDX, Oxidized ferrodoxin; F26BP, Fructose 2,6-bisphosphate; G1P, Glucose 1-phosphate; G6P, Glucose 6-phosphate; GAP, Glyceraldehyde 3-phosphate; GCA, Glycollate; GCEA, Glycerate; GLU, Glutamate; GLN, Glutamine; GLY, Glycine; GOA, Glyoxylate; HPR, Hydroxypyruvate; ICIT, Isocitric acid; KG, a-Ketoglutarate; MAL, Malate; OAA, Oxaloacetic acid; OPOP, Pyrophosphate; PGA, 3-Phosphoglycerate; 2-PGA, 3-Phosphoglycerate; PGCA, 3-Phosphoglycollate; PYR, Pyruvate; Ri5P, Ribose 5-phosphate; Ru5P, Ribulose 5-phosphate; RuBP, Ribulose 1,5-biphosphate; S7P, Sedoheptulose 7-phosphate; SBP, Sedoheptulose 1,7- bisphosphate; SER, Serine; TS, tartronic semialdehyde; T3P, Triose phosphate; UDPGlu, Uridine Diphosphate Glucose; SUC, Sucrose; SUCP, Sucrose phosphate; UDP, Uridine 5′-diphosphate; UTP, Uridine-5′-triphosphate; Xu5P, Xylulose 5-phosphate; and Pi, phosphate. This image is redrawn and adapted from Xin et al. (2015).

Cyanobacteria, the ancestors of modern day crop chloroplasts, use a different method of concentrating CO_2 at Rubisco. These prokaryotes actively uptake bicarbonate into their cells. Within the cells, both Rubisco and carbonic anhydrase are localized within icosahedral protein shell bodies termed carboxysomes. Here, carbonic anhydrase catalyzes the formation of CO_2, serving to concentrate CO_2 around Rubisco to a sufficient level to minimize oxygenation and photorespiration (Badger and Price, 2003; Price, 2011). This appears simpler than converting a C3 plant to a C4 because it does not require the creation of two distinct photosynthetic tissues but instead requires the addition of bicarbonate and carbon dioxide pumps to the chloroplast membrane and production of carboxysomes within the chloroplast. A diffusion-reaction model of this system suggests that addition of these basic components would increase photosynthesis as much as 60%, whereas addition of the bicarbonate

pumps alone would increase photosynthesis in C3 leaves by ~9% (McGrath and Long, 2014). Ideally these components would be coded for and synthesized within the chloroplast by transformation of the remnants of the cyanobacterial DNA that persists in modern-day chloroplasts (Martin et al., 2002). However, transformation of chloroplast DNA has so far only succeeded in a few plants, notably tobacco and potato, but as yet not in any cereal (Scharff and Bock, 2014). An alternative may be to code for these components by nuclear transformation with transit peptides and membrane transporters. Many eukaryotic algae also include inorganic carbon concentrating mechanisms to suppress the oxygenase activity of Rubisco and photorespiration (Meyer and Griffiths, 2013). These also require bicarbonate transporters in the cell and chloroplast membrane. Within the chloroplasts of these algae, Rubisco concentrates in a region, typically surrounded by starch, and is termed the pyrenoid (Giordano et al., 2005). Pyrenoids appear to function similarly to carboxysomes, although the dynamic nature of their structure and genetics are less well understood, despite important recent advances (Meyer and Griffiths, 2013; Mitchell et al., 2014; Engel et al., 2015). So although transferring a eukaryotic-concentrating mechanism to other eukaryotes may appear more tractable, more genetic information is needed to understand how this might be engineered. Rubisco is not the only carboxylase in nature, and there are at least five known pathways of carbohydrate synthesis from CO_2 that use other carboxylases (Fuchs, 2011). However, only one of these, the 3-hydroxypropionate (3-HPA) bicycle, appears oxygen insensitive (Mattozzi et al., 2013). These existing pathways or totally synthetic pathways could be introduced into higher plants (Bar-Even et al., 2010). A challenge here, though, is that the intermediates of the Calvin cycle are integrally linked to much of essential plant metabolism. Introduction of such a different pathway would have complex effects on the whole of plant metabolism and may require successful re-engineering far beyond carboxylation.

A further opportunity to address the cost of photorespiration is to engineer a more efficient pathway for metabolism of the first product of the oxygenase reaction, phosphoglycolate. Plants and green algae use a single energy-consuming and protracted pathway involving the chloroplast, peroxisome, and mitochondrion, with the release of both carbon dioxide and ammonia in order to recover PGA that is then re-assimilated into the Calvin cycle. This is shown by the red intermediates in Figure 2. Prokaryotes have at least three simpler pathways for metabolism of phosphoglycolate to PGA (Carvalho et al., 2011; Maier et al., 2012; Maurino and Peterhansel, 2010; Xin et al., 2015). One that involves just three enzyme-catalyzed steps has been engineered into the chloroplast of *Arabidopsis* with an improvement in net photosynthetic efficiency (Kebeish et al., 2007). The three pathways and how they could be engineered into crop photosynthetic carbon metabolism are illustrated in Figure 2. A simulated energy balance analysis of the complete photosynthetic system with addition of each of these pathways has, in fact, predicted that this three-step pathway is the only one that would actually increase net photosynthetic efficiency (Xin et al., 2015), demonstrating the power of in silico analysis in directing practical manipulations.

As noted above, metabolic control of CO_2 assimilation is commonly shared between Rubisco and capacity for regeneration of RubP. When account is taken of the Calvin cycle, electron transport, photorespiratory metabolism, and transfer of intermediates of the Calvin cycle to storage and transport carbohydrates, starch, and sucrose, more than 60 reactions are involved. Representation of this system in silico and application of an evolutionary algorithm has indicated several pressure points, including SBPase, which, if upregulated, could increase photosynthesis by 60% without additional resources (Zhu et al., 2007). Further gains may also be achieved by altering the arrangement, amount, and color of leaves in a crop canopy (Drewry et al., 2014; Long et al., 2006b; Zhu et al., 2010). In full sunlight, the uppermost leaves of most crop canopies capture most of the incoming sunlight, which is more than they can use, whereas the lower leaves receive far less light than they could utilize; likely an evolutionary hold over. The wild ancestors of our crop plants were subject to selection as individuals growing in a competitive environment. By capturing most light on their upper leaves, even when this could not be effectively used, competitors growing below were denied this light (Zhu et al., 2010). But in a monoculture of genetically identical crop plants, this strategy is disadvantageous. By making upper leaves more vertical and lighter in color, light can be more evenly distributed, allowing up to a 60% increase in carbon gain by a canopy of the same total leaf area per unit ground area, while achieving improvements in water use efficiency (Drewry et al., 2014; Zhu et al., 2010). Finally, respiration is the other component determining the net efficiency with which crops convert intercepted solar radiation into biomass. Far less is known about plant respiration, in particular, whether it can be decreased without impacting growth and maintenance processes (Costa et al., 2014; Logan, 2007; Millar et al., 2011; Peckmann et al., 2012; Sweetlove et al., 2010). Early genetic work with ryegrass suggested that lines with lower respiration did have higher productivity (Robson, 1982), although this has not subsequently been confirmed in other crops. However, it clearly represents an area in need of far more investment.

Table 1 summarizes the major possible methods of improvement of photosynthetic efficiency that are currently apparent, the likely timescale, and the potential gain. Although some are clearly more tractable at the present time than others, we have insufficient knowledge to favor one approach over another. Indeed, the problem represented by Figure 1 is large enough to suggest that we should be actively and urgently pursuing all of these approaches, and although the potential improvements are not necessarily additive, they are not antagonistic.

Facilitating Translation of Research Opportunities to Crops

Some of the most tractable methods to improve photosynthetic efficiency include systems/synthetic biology, genetic engineering, and computational modeling strategies as part of a new Green Revolution. Unicellular green algae such as *Chlorella* and *Chlamydomonas*, as well as model plants with short life cycles, rapid transformation systems, and deep functional knowledge of their genomes such as *Arabidopsis*, remain crucial tools for tests of concept for a number of these potential

Table 1. This Table Lists the Manipulations That Could Be Undertaken to Improve Photosynthetic Efficiency in C3 Crops, the Type of Manipulation, and the Model Estimated Improvement in Efficiency of Conversion of Received Light Energy into Crop Biomass Relative to Today's Best Cultivars

	Manipulation	Type	Efficiency Gain	Timescale	Additional Benefits
1	extend usable spectrum of crop photosynthesis into NIR	CSyn	10%–30%[a]	L	could be used to power 3 or improve value of 10. C4
2	more rapid relaxation of heat dissipation at PSII	Syn	30%[i]	S	synergistic with all other changes. C4
3	convert C3 crops to C4	Syn	30%[c,e]	L	improved WUE and NUE
4	add cyanobacterial or microalgal CO2/HCO3 pumps	Syn	5%–10%[d]	M	improved WUE and NUE
5	add cyanobacterial carboxysome system	CSyn	60%[d]	L	improved WUE and NUE
6	add algal pyrenoid CO2 concentrating system	CSyn	60%[d]	L	improved WUE and NUE
7	substitute forms of Rubisco better adapted to today's CO_2	CSyn, B	15%–30%[h,j]	L	improved WUE and NUE
8	synthetic photorespiratory bypasses	Syn	15%[c,f]	S	improved WUE and NUE
9	optimize regeneration of RubP	Sys, B	60%[g]	S	synergistic with all; improved NUE. C4
10	transmit more light to lower canopy leaves	B, Syn	15%–60%[b,c]	S	synergistic with 1, and 3 thru 9. improved WUE and albedo. C4

CSyn indicates synthetic addition of foreign genes to the chloroplast or plastid genome; Syn indicates synthetic addition to the nuclear genome; Sys indicated up- or down-regulation of existing genes; and B indicates that the improvement may be tractable by breeding given adequate molecular markers for the specific genes. The efficiency gains are from modeled estimates and are largely untested; these vary greatly depending on different assumptions and can vary with environmental conditions. For example, the benefits of items three through eight will increase with temperature and so give the greatest increases in warm climates. Timescale is an estimated time to obtain material that could be used in a breeding program. S represents a 1–5 year timescale, since these have already been demonstrated in model plant species or actual crops as providing some clear improvement; M indicates a 5–10 year timescale; and L indicates a 10–30 year time scale. These involve manipulations that require as-yet-unachieved goals, such as plastid transformation, or a full understanding of what makes a plant C4. It should be noted that, with adequate resource, there is no reason to believe that these goals cannot be achieved. All timescales are estimates assuming adequate investment for intensive effort and the needed resources. Additional benefits indicate synergies, i.e., where 1+1 > 2, and simultaneous improvements in either the efficiency of water use (WUE) or of nitrogen use (NUE) per unit of biomass are improved. C4 indicates that this manipulation would also improve efficiency of carbon gain in C4 crops, such as maize and sorghum. Sources.

[a]Blankenship et al. (2011).
[b]Drewry et al. (2014).
[c]Long et al. (2006a).
[d]McGrath and Long (2014).
[e]von Caemmerer et al. (2012).
[f]Xin et al. (2015).
[g]Zhu et al. (2007).
[h]Zhu and Long (2009).
[i]Zhu et al. (2004a).
[j]Zhu et al. (2004b).

improvements (Table 1). However, these cannot predict or represent the complexities of a closed-crop canopy and its photosynthetic performance in the field. Transformation of our major crops, on the other hand, is generally slow and has been limited to a few public laboratories. Solanaceous crops have proved some of the easiest to transform. Tobacco is a valuable test bed, it is easily transformed, and it forms a closed canopy in the field with many of the same characteristics of food crops (Figure 3). Tobacco can serve as a key test bed to identify the most promising manipulations that might then be moved on to the more difficult tasks of transforming crops such as wheat, rice, soy, or cassava. However, conventional transformation of plants with constructs to up- or down-regulate specific genes or to introduce synthetic pathways through agro-bacterium or biolistic addition results in near-random insertions. This presents a challenge when quantifying and testing the improvement made by a specific addition to crop carbon gain in the field. Positional effects mean that no one event is the same, and some may be lethal in homozygotes of the second generation (T1) in which they have knocked out key genes. This necessitates the testing of many events as replicated stands of the transformants in the field, limiting the number of constructs that might be tested (Figure 2). Tools that would allow insertion of constructs at the same point in the genome, a point that does not interfere with expression of other genes, would decrease variability between events and increase comparability of transformations with different constructs. Recombination-mediated genetic engineering (recombineering), Zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and, in particular, clustered, regularly interspaced, short palindromic repeats (CRISPRs) widely used in engineering of microbial genomes provide a means to achieve directed insertions in crops (Jiang et al., 2013; Shan et al., 2013). Testing would be further facilitated by the development of haploid plants for transformation, so that homozygotes can be obtained rapidly (Lee et al., 2014; Suelter et al., 2014). Although, in the evolution of

Figure 3. The Pipeline of Transformation of Leaf Discs of Tobacco with Constructs for Improved Photosynthetic Efficiency through Regeneration on Selective Media, Growth of the Initial Transformants to Seed, and Then Testing of Transgenes in Replicated Field Plots

chloroplasts, most genetic information of the ancestral cyanobacteria has been transferred to the nucleus, the vestigial DNA codes for some key proteins of the photosynthetic apparatus, including the larger part of Rubisco. Achieving a number of the potential improvements suggested here requires or would benefit from successful transformation of the chloroplast DNA. As noted earlier, this has only been achieved in a few species and, as yet, in none of the major crops (Scharff and Bock, 2014). But there appears to be no fundamental barrier to achieving this, except adequate investment.

Sufficient knowledge is now available that modeling a whole crop plant in silico might be achievable (Chew et al., 2014), thus creating a tool that would be both a framework for testing hypotheses on improving net carbon gain and production and for applying optimization routines to improve efficiency (Figure 4). Mechanistic models of gene expression networks, proteins, metabolic pathways, shoot and root development, and canopy microclimate have all been developed. Although these models have been used in isolation to predict synthetic and systems means to improve photosynthetic efficiency, they ignore interactions with the rest of the plant system or crop ecosystem. Multi-scale modeling that integrates these different models can aid in system-wide predictions of photosynthetic efficiency across scales (Figure 4).

Why Now and Not 2050?

This article has focused on genetic yield potential improvement to increase yield per hectare as a means to protect against a potential future shortage of primary foodstuffs (Figure 1). There are of course other means of increasing food supply by using more land and by raising yields on all farms to those achieved by the best farmers. However, our major food crops require good soils and water supply to achieve high yields, and there is little land of this quality that is not already in production. Indeed urban sprawl, desertification, salination, and exhaustion of aquifers that have been used for irrigation suggest that less, not more, land may be available into the future (Alexandratos and Bruinsma, 2012). Crops could be raised on poorer land than that used today but with lower yields and greater risk of environmental and biodiversity degradation. Yields for a given crop can vary greatly between farms and countries. For instance, the average yield of maize in the USA in 2013 was 10.0 t/ha and in Zimbabwe 0.9 t/ha, even though both have similarly good climatic conditions for raising the crop (Food and Agriculture Organization of the United Nations, 2015). Raising yields to those achieved by the best farmers is an important aim but depends greatly on internal policies and farmer access to advice, seed, and fertilizers, none of which can have certainty into the future. So while in the best of worlds, seed with increased yield potential might not be necessary, we cannot and should not take that risk. Given the 20 to 30 year gap

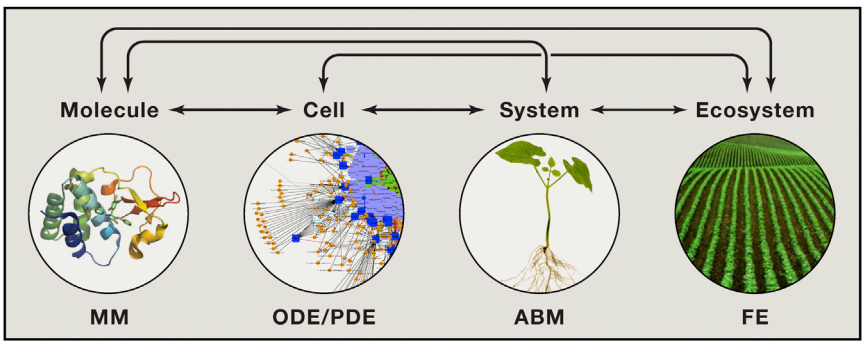

Figure 4. Multi-scale Modeling Concept
Models at different levels, generated with different mathematical strategies, inform one another and bridge the critical gaps in our knowledge of fundamental plant behavior. Examples of model types include MM, molecular modeling; ODE, ordinary differential equations; PDE, partial differential equations; ABM, agent based modeling; and FE, finite element modeling.

between demonstration of innovative solutions at the experimental level and provision of seed to farmers, the need to bridge and accelerate the gap between molecular engineering and practical crop breeding to achieve higher yields cannot be postponed, especially considering the forecast situation for 2050.

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