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REVIEW PAPER

Raising yield potential of wheat. II. Increasing photosynthetic capacity and efficiency

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Abstract

Past increases in yield potential of wheat have largely resulted from improvements in harvest index rather than increased biomass. Further large increases in harvest index are unlikely, but an opportunity exists for increasing productive biomass and harvestable grain. Photosynthetic capacity and efficiency are bottlenecks to raising productivity and there is strong evidence that increasing photosynthesis will increase crop yields provided that other constraints do not become limiting. Even small increases in the rate of net photosynthesis can translate into large increases in biomass and hence yield, since carbon assimilation is integrated over the entire growing season and crop canopy. This review discusses the strategies to increase photosynthesis that are being proposed by the wheat yield consortium in order to increase wheat yields. These include: selection for photosynthetic capacity and efficiency, increasing ear photosynthesis, optimizing canopy photosynthesis, introducing chloroplast CO_2 pumps, increasing RuBP regeneration, improving the thermal stability of Rubisco activase, and replacing wheat Rubisco with that from other species with different kinetic properties.

Key words: Activase, photorespiration, Rubisco, RuBP, CO_2.

Introduction

The primary determinant of crop biomass is the cumulative rate of photosynthesis over the growing season. Although yields of wheat have increased over time, comparatively little of this increase can be attributed to increased biomass. Instead, improvements in agronomic practice and harvest index (the proportion of biomass that is grain) are largely responsible for the increased yield (Austin *et al.*, 1989; Fischer *et al.*, 1998). The harvest index of two major food crops, rice and wheat, is now approaching a plateau and further increases in yield will necessitate an increase in productive biomass and, therefore, an increase in photosynthesis. Provided that other constraints do not become limiting, increasing photosynthesis will increase crop yields, as demonstrated by the effects on yield of CO_2-enrichment

Abbreviations: RuBP, ribulose 1,5 *bis*phosphate; SBP, sedoheptulose 1,7 *bis*phosphate; SBPase, sedoheptulose 1,7 *bis*phosphatase; FBPaldolase, fructose 1,6 *bis*phosphate aldolase; HI, harvest index; CA, carbonic anhydrase; PGA, 3-phosphoglycerate.

experiments (Ainsworth and Long, 2005). Also, experiments on historic genotypes of wheat suggest that improvements in photosynthesis per unit leaf area may already have occurred, in concert with improvements in harvest index and grain number, although not apparently in greater biomass (Fischer *et al.*, 1998).

Total crop photosynthesis is dependent on (i) the ability of the canopy to intercept and capture light; (ii) the duration of light capture; and (iii) the photosynthetic capacity and efficiency of the canopy. All three can be considered as targets for crop improvement. For wheat and rice grown under conventional high-input systems, canopy architecture has been effectively optimized for light capture and there are few obvious opportunities for further improvements (Horton, 2000). There may still be opportunities to extend the duration of light capture by improving the rate of early leaf area growth or introducing 'staygreen' phenotypes to increase total photosynthesis by extending the growing season (Dohleman *et al.*, 2009; Dohleman and Long, 2009). Also, while the gap between yield potential and yield realisation in the field is still large, particularly in third world agriculture, this can be due both to genetic and agronomic reasons (Leegood *et al.*, 2010). However, improvements in agronomic practice alone are unlikely to allow us to meet projected world food demands and genetic gains will be required in addition to agronomic improvements (Leegood *et al.*, 2010). It is also likely that improvements in yield potential will translate directly to increased yield in many water-limited and stressful environments, as demonstrated by the successes of the CIMMYT wheat breeding programmes, which makes selections under optimal conditions that still perform well under less than optimal conditions (Fischer *et al.*, 1998).

Once canopy architecture, light interception, and photosynthetic duration have been optimized, total photosynthesis can only be increased by increasing the photosynthetic rate per unit leaf area (Long *et al.*, 2006; Raines, 2006; Parry *et al.*, 2007). The theoretical photosynthetic energy conversion efficiency of C_3 plants is about 4.6% (Zhu *et al.*, 2008), while the recorded energy conversion efficiency in the field is usually less than one-third of this value. This suggests that there is substantial room to increase photosynthetic energy conversion efficiency (Reynolds *et al.*, 2000; Zhu *et al.*, 2008).

The biochemistry involved in C_4 photosynthesis is segregated into specialized cell types: in the mesophyll cells, which are in contact with the intercellular air spaces, gaseous CO_2 is initially fixed by PEPC into C_4 acids. The C_4 acids are then transported to deeper, gas-tight, bundle sheath cells where decarboxylation occurs releasing CO_2 which is then recaptured by Rubisco. This complexity is advantageous because Rubisco has the potential to catalyse a competing and wasteful reaction with oxygen, initiating photorespiration and the release of CO_2 through glycine decarboxylation. Since C_4 photosynthesis elevates the CO_2 concentration within the bundle sheath cells to levels approximately 10-fold higher than would be possible with normal atmospheric concentrations of CO_2, the wasteful 'oxygenase' activity of Rubisco is effectively suppressed (Furbank *et al.*, 2000; Carmo-Silva *et al.*, 2008). Another advantage of C_4 photosynthesis is that the PEPC enzyme is a more efficient carboxylase than Rubisco; having a much faster turnover and much higher affinity for inorganic carbon (bicarbonate, HCO_3^-, being the substrate) Thus, C_4 photosynthesis is particularly valuable in warm temperatures and/or water-limited environments when the differential solubility of CO_2 and O_2 would otherwise favour photorespiration and stomatal closure often reduces the CO_2/O_2 in the intercellular spaces to levels that also strongly favour photorespiration (Long *et al.*, 2006). The suppression of photorespiration in C_4 plants relative to C_3 is illustrated in Fig. 1A, and B, where the ambient atmospheric oxygen concentration has little effect on CO_2 assimilation by sorghum (C_4), but a significant effect on wheat (C_3).

Supplementing C_3 plants with PEPC and the other C_4 pathway enzymes has long been viewed as a daunting task because of the complexities of inserting a multi-step and highly regulated pathway in an anatomically correct configuration (Bjorkman *et al.*, 1971; Edwards *et al.*, 2001). Optimizing the properties of Rubisco represents an alternative and more direct approach for increasing biomass in C_3 plants.

Not only does Rubisco initiate photorespiration, but it is also a slow catalyst, which is why comparatively large amounts of this enzyme are required to sustain high photosynthetic rates. Indeed, the enzyme can often exceed 50% of the soluble protein in a C_3 leaf. Despite this great abundance, Rubisco activity is often limiting to assimilation under field conditions (Parry *et al.*, 2003, 2007).

Theoretical models that describe the limitations to photosynthesis have been used to identify the major

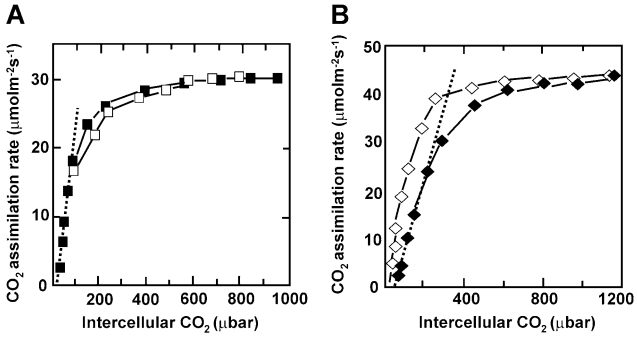

Fig. 1. The response of CO_2 assimilation to CO_2 concentration for sorghum, a C_4 plant (A) and wheat, a C_3 plant (based on data from Furbank *et al.*, 2009). Open symbols show gas exchange carried out under 2% O_2 and closed symbols at 21% O_2. Note that for sorghum, the initial slope of this curve and the intercept with the *x*-axis (CO_2 compensation point) is defined by the kinetics of PEP carboxylase and hence is insensitive to O_2 due to a lack of photorespiratory influences (von Caemmerer, 2000). In the case of wheat, this slope is defined by Rubisco kinetics and is not only steeper under non-photorespiratory conditions but the CO_2 compensation point is reduced considerably.

biochemical 'bottlenecks' to photosynthesis. The predictions of these models have been confirmed by analysis of targeted transgenic plants (von Caemmerer, 2000). From an idealized A/C_i curve, which illustrates the relationship between photosynthetic CO_2 assimilation (A) and the intercellular CO_2 concentration (C_i) under saturating light, three potential constraints can be identified (Fig. 1A). At the lower C_i values, such as those that occur as stomata close, net assimilation is limited by Rubisco abundance and kinetics, while at higher values of C_i, such as those that occur when the stomata are fully open, the limitation shifts to the regeneration of the Rubisco substrate, RuBP. At the highest C_i values, rarely achieved in nature but achieved experimentally by raising the external CO_2 concentration to artificially high levels, net assimilation becomes limited by phosphate regeneration or sugar synthesis and export. At any instant in a crop—whether the limitation is caused by Rubisco or RuBP regeneration—carbon assimilation is determined by the plant species in question, the ambient CO_2 concentration, the stomatal conductance (which is affected by water availability), the light intensity and the temperature (see, for example, Yamori *et al.*, 2010). It is generally considered that under ideal conditions most crops sit at a point where both Rubisco activity and RuBP regeneration co-limit photosynthesis but that any stomatal closure (e.g. caused by very moderate stress) will cause C_3 photosynthesis to become Rubisco limited. This is illustrated by the modelled curves (dotted lines) for wheat shown in Fig. 2.

Previous reviews have explored in detail a range of approaches that can potentially increase photosynthesis (Raines, 2003; Long *et al.*, 2006; Zhu *et al.*, 2010). In this paper we focus on targets that are of particular relevance for enhancing photosynthesis in wheat.

Strategies to overcome limitations of Rubisco

More Rubisco

Theoretically there are several strategies that could be employed individually or together to overcome the limitations of Rubisco (Box 1). One obvious approach would be to increase the activity of Rubisco by increasing the amount of Rubisco protein in the chloroplast. This would be particularly beneficial in conditions of high irradiance and high temperatures when internal CO_2 concentrations are low. To be effective, this strategy would require a major supplement of 'added' Rubisco protein resulting in a higher leaf nitrogen concentration. This would be technically feasible as, although Rubsico already exceeds 50% of soluble protein, recent evidence with transgenic tobacco plants grown under moderate light suggests that the protein concentration in the chloroplast can be increased significantly without a detrimental effect on growth (Yubuta *et al.*, 2008). However, this strategy might be more problematic under high light if the higher protein concentrations interfere with starch granule formation. Furthermore, this approach has limited appeal as it would increase the requirement for nitrogen, which is already a major limitation and an expensive nutrient in global agricultural systems.

'Better' Rubisco

An alternative strategy to increase Rubisco activity would be to identify or create a Rubisco with a higher catalytic rate (Fig. 2A), a higher affinity for CO_2 (Fig. 2C), and/or a lower affinity for O_2 (Fig. 2D). The ability of Rubisco to discriminate between the two gaseous substrates is described by the specificity factor, τ ($\tau = V_c K_o / V_o K_c$). The specificity factor is high when the carboxylase activity is favoured and low when the oxygenase activity is favoured. Investigation of critical amino acid residues governing the catalytic properties of Rubisco from wheat and other crop plants by *in vitro* mutagenesis has been hampered by an inability to obtain active enzyme by expressing the associated genes in *E. coli*. Even so, progress has been made with genes for Rubisco from cyanobacteria whose expression in *E. coli* does result in active enzyme (Gatenby *et al.*, 1985; Gatenby and Ellis, 1990). The identification of amino acid residues that confer key catalytic properties (Parry *et al.*, 1987), and their modification by site-directed mutagenesis has provided great insight into the structure/function relationships of Rubisco as well as highlighting the challenges of this site-directed approach.

Interspecies comparison reveals that there is considerable variation in the kinetic properties of Rubisco isolated from diverse sources that could be exploited in crop improvement (Table 1). Despite an apparent negative relationship between the rate constant for carboxylation, K^c_{cat} and the Rubisco specificity factor, first reported by Bainbridge *et al.* (1995) and more recently by Tcherkez *et al.* (2006) and Savir *et al.* (2010), there is sufficient variation between species and sufficient deviation from this apparently negative correlation to suggest a means to improve photosynthesis. For example, replacing wheat Rubisco with that from *Limonium gibertii* (Fig. 2E) would give significant increases in assimilation at concentrations of CO_2 up to the current ambient concentration (significant for photosynthesis under water-limited conditions) and small increases at higher CO_2 concentrations where RuBP regeneration is limiting. In combination with enhanced RuBP regeneration promoted by increased expression of sedoheptulose *bis*phosphatase (Miyagaura *et al.*, 2001; Lefebvre *et al.*, 2005) the improvement at ambient CO_2 could also be significant (Fig. 2F). The impact on assimilation of simple changes to Rubisco kinetic constants, achieved by replacing wheat Rubisco with that from other species, together with modest enhancements of RuBP regeneration capacity, is shown in Fig. 2A–H.

Over the course of a day, total crop canopy CO_2 uptake is the result of both light-limited and light-saturated photosynthesis. Increased specificity factor (τ) would increase light-limited photosynthesis, while the associated

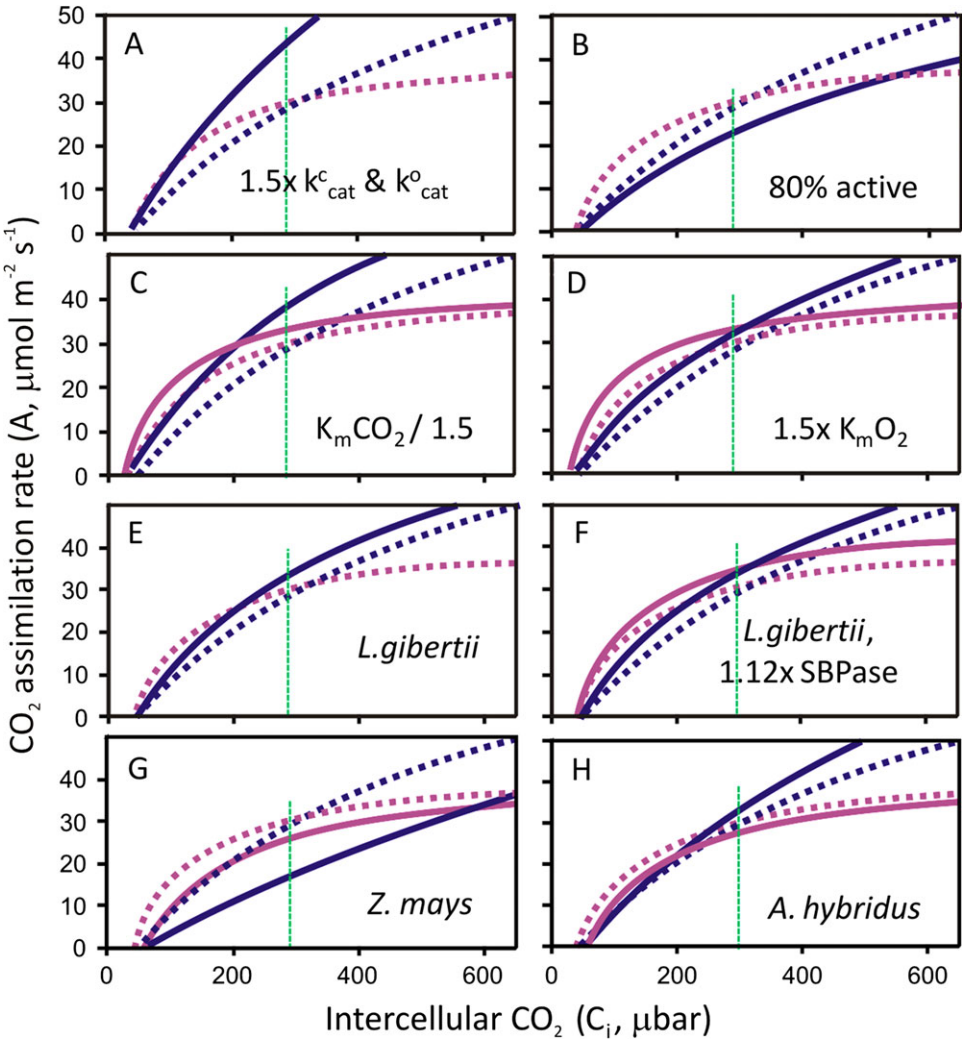

Fig. 2. Modelled photosynthetic responses to changes in activity, kinetics or species of Rubisco and of enhancing RuBP regeneration. The Rubisco-limited (A_c) and electron-transport limited (A_j) rates of CO_2 assimilation for wheat are represented as blue and red dotted lines, respectively, derived from the kinetic constants of Carmo-Silva *et al.* (2010) (Table 1) and the biochemical model of Farquhar *et al.* (1980). The maximal electron transport rate (178 μmol m^{-2} s^{-1}) was chosen so that the wheat curves bisected (i.e. shared control) at an intercellular CO_2 (C_i) of approximately 300 μbar. Other assumptions were: Rubisco content (35 μmol m^{-2}), dark respiration rate (1.2 μmol m^{-2} s^{-1}), saturating light, 210 mbar O_2, and intracellular CO_2 conductance non-limiting. The actual rate of assimilation is the lower of the two values (A_c or A_j) at any C_i. Solid blue line: effect of stated change on A_c. Solid red line: effect of stated change on A_j (not shown when change relative to control was marginal). (A) 1.5-fold increase in rate constants for carboxylase (k^c_{cat}) and oxygenase (k^o_{cat}) activity. (B) Rubisco with 80% of full activity. (C) Affinity for CO_2 increased by lowering K_mO_2 by one-third. (D) Affinity for O_2 decreased by increasing K_mO_2 by 50%. (E) Replacing wheat Rubisco with that from *Limonium gibertii* (Table 1). (F) As in (E) in combination with a 12% increase in A_j resulting from overexpression of SBPase. (G, H) Replacing wheat Rubisco with that from *Zea mays* and *Amaranthus hybridus*, respectively (Table 1). A typical C_i corresponding to ambient CO_2 (390 μbar) is indicated by the broken green line.

decrease in k^c_{cat} would lower the light-saturated rate of photosynthesis.

Zhu *et al.* (2004*a*) examined the consequence of the inverse relationship between k^c_{cat} and τ on canopy photosynthesis. The simulated daily integral canopy photosynthesis suggested that the present average specificity found in C_3 terrestrial plants is supra-optimal for the present atmospheric CO_2 concentration of 387 ppm, but would be optimal for around 220 ppm, a value remarkably close to the average of the last 400 000 years. The possibility that increased [CO_2] favours the selection of forms of Rubisco with increased k^c_{cat} and decreased τ is consistent with the observation that Rubisco from C_4 plants, where the enzyme functions in a high CO_2 environment, typically have a higher k^c_{cat} and lower specificity factor than in C_3 land plants (Sage, 2002; Seemann *et al.*, 1984). In spite of this argument, Zhu *et al.* (2004*a*) showed that if Rubisco from the red algae *Griffithsia monilis*, with a massively high specificity factor of 167 and a respectable k^c_{cat} of 2.6 s^{-1}, could be expressed in place of the present 'typical' C_3 crop Rubisco, then canopy carbon gain could be increased by 27%. They further proposed that, if the increase in specificity can only be

Box 1. WYC approaches to increase photosynthesis

- Phenotypic selection for photosynthetic capacity and efficiency
- Phenotypic selection for ear photosynthesis
- Optimizing and modelling canopy photosynthesis and photosynthetic duration
- Chloroplast CO_2 pumps
- Increasing RuBP regeneration
- Improving thermal stability of Rubisco activase
- Replacement of LS Rubisco

achieved at the expense of k^c_{cat} (which the *G. monilis* example mitigates against), a crop should ideally express a high k^c_{cat} Rubisco in the upper canopy leaves exposed to full sunlight and a high τ Rubisco in the shaded lower canopy leaves. This design has the potential to increase canopy photosynthesis by up to 30%.

To undertake the replacement of wheat Rubisco with that of another species is technically challenging, requiring context-specific expression, assembly, post-translational modification, and activity regulation (Sharwood *et al.*, 2008; Whitney and Sharwood, 2008; Whitney *et al.*, 2009). Considerable progress has been made but there are still technical hurdles that need to be overcome (Liu *et al.*, 2010). For example, the large subunit of Rubisco, which is thought to determine most of the kinetic properties, is chloroplast encoded (Criddle *et al.*, 1970). Modifying the large subunit of Rubisco by transgenesis requires chloroplast transformation which has only been developed for a few crop plants, excluding monocots (Bock, 2007), and must, therefore, become a priority for future research.

An alternative to transgenesis for the improvement of Rubisco kinetic properties is to use a forward genetic/phenomic screen to mine existing genetic variation in Rubisco kinetic properties in wheat and related species. The contribution of photosynthesis to plant yield is integrated in space, over the entire leaf mass, and in time, over the entire growing season. Thus, very small increases in the rate of net photosynthesis can translate into large increases in net carbon gain. A biochemical screen could be employed where diverse sets of germplasm could be examined for diversity in Rubisco kinetic properties, such as k^c_{cat}, K_m, catalytic misfire, and τ. While there is evidence for considerable interspecific variation in these kinetic parameters (see Table 1, and references above) there have been no major studies undertaken thus far to examine intraspecific variation in wheat. Measurement of Rubisco kinetic properties is technically challenging and not amenable to high-throughput phenotyping, however, the modelled response of leaf gas exchange to CO_2 concentration can be used to predict Rubisco kinetic properties non-destructively if certain parameters are known (Fig. 1; von Caemmerer *et al.*, 1994, 2000). As discussed above, the initial slope of the response of CO_2 assimilation to CO_2 concentration in a C_3 leaf is determined by the amount of Rubisco present and its kinetic properties (von Caemmerer *et al.*, 2000). For a given leaf nitrogen/Rubisco content, this slope can thus be used to infer k^c_{cat} and τ. If an attempt is made to normalize such data to total amounts of Rubisco, a rapid determination of leaf CO_2 assimilation at three or four low CO_2 concentrations, particularly if done at high and low O_2 concentration, would provide a screen for genetic variation in Rubisco kinetics. This approach has previously been used successfully to predict Rubisco kinetic constants *in vivo* for tobacco (von Caemmerer *et al.*, 1994). While a comprehensive survey has not been carried out, preliminary evidence exists in the literature for such variation in wheat (Condon *et al.*, 1990), where the slope of the A/C_i curve was seen to vary by as much as 30% between genotypes of wheat, although these data were not normalized to Rubisco content.

Table 1. Variation in some key kinetic constants for Rubisco between various species of land plants

Unless otherwise specified, the data apply to 25 °C. Source of the original data is given by the letters where: (a) Carmo Silva *et al.*, 2010; (b) Parry *et al.*, 1989; (c) Makino *et al.*, 1988, (d) Zhu *et al.*, 1998, (e) Cousins *et al.*, 2009; (f) Jordan *et al.*, 1981; (g) Sage and Seeman, 1993; (h) Whitney *et al.*, 2001, (j) Kubien *et al.*, 2008; (k) Jordan and Ogren, 1983; (l), Jordan and Ogren, 1983; (m) PJ Andralojc and J Galmes, personal communication; (n) Galmes *et al.*, 2005; (o) Makino *et al.*, 1985; (p) Kane *et al.*, 1994.

Species	K_mCO_2 (μM)	K_mO_2 (μM)	SF	k^c_{cat} (s^{-1})
Triticum aestivum	10.9 a	341 a	107 b	2.91 a
	11.2 c	383 c	120 c	2.5 d
	9.7 e	nd[a]	114 e	3.8 e
Spinacea oleracea	14 f	480 f	80 f	3.7 g
Nicotiana tabacum	11 h	295	82 h	3.4 h
Oryza sativa	8.1 o	370 o	85 p	1.85 o
Zea mays	16 e	183 e	108 e	4.7 e
	21 j	157 j	75 j	4.1 j
	34 f	810 f	78 f	4.4 g
Sorghum bicolor	30 l	nd	70 k	5.4 l
Limonium gibertii	8.9 m	594 m	113 n	2.62 m
Amaranthus hybridus	16 f	640 f	82 f	3.8 l *
P. dilatatum	20 a	415 a	88 a	3.55 a
C. dactylon	21 a	402 a	89 a	3.90 a
Z. japonica	18 a	403 a	84 a	4.35 a

[a] Data marked nd were not available.
* Data for the closely related species *A. retroflexus*.

Maintaining Rubisco activity

Photosynthesis is particularly sensitive to inhibition by moderate heat stress and this inhibition generally translates into a decrease in yield (Lobell and Field, 2007). Modern wheat cultivars have been developed for current climatic conditions and display symptoms of heat stress above a relatively low critical temperature. Climate models predict that average global temperatures will increase by 0.6–2.5 °C

over the next 50 years, which will be accompanied by more-frequent episodes of extreme heat. The inhibition of photosynthesis by moderate heat stress correlates with a decrease in the activation state of Rubisco (reviewed in Salvucci and Crafts-Brandner, 2004). As temperatures increase, Rubisco active sites progressively become inactive either through decarbamylation or catalytic inactivation. The impact of this on carbon assimilation is shown in Fig. 2B. Restoration of activity requires a specific chaperone, Rubisco activase. Rubisco activase has a relatively low temperature optimum for reactivating Rubisco, above which it is very heat sensitive. Hence, as the temperature increases beyond this optimum (e.g. above 30 °C) Rubisco activity declines precipitously.

Research with the model plant, *Arabidopsis*, has already demonstrated that the thermotolerance of photosynthesis can be improved by increasing the thermal stability of Rubisco activase (Kumar *et al.*, 2009). This same strategy could be modified to improve the performance of wheat and other crops at elevated temperature. An alternative strategy that has yet to be investigated is to eliminate Rubisco's dependence on activase by minimizing the formation of misfire inhibitors during catalysis, either by decreasing the affinity of Rubisco for oxygen or by altering the active-site chemistry of Rubisco to avoid catalytic inactivation (Pearce, 2006; Parry *et al.*, 2008) or even to make the enzyme permanently carbamylated. These strategies would achieve the same result: improved photosynthetic performance at elevated temperature and probably much higher catalytic rates. Since proof-of-concept has already been established for the activase-based strategy, this approach should be amongst the first to be pursued by means of nuclear transformation of wheat.

Faster RuBP regeneration

As illustrated in Figs 1 and 2, photosynthesis in well-watered crop plants at high light in air is limited both by the flux through Rubisco and by the rate at which RuBP can be regenerated. There is good experimental evidence that increasing the RuBP supply to Rubisco by increasing the activity of the Calvin cycle enzyme sedoheptulose-1,7-*bis*phosphatase (SBPase) can ameliorate the limitation to assimilation caused by RuBP regeneration when stomata are fully open and increase both photosynthetic rate and biomass accumulation (Harrison *et al.*, 1998; Lefebvre *et al.*, 2005; Tamoi *et al.*, 2006). Over-expression of SBPase in rice suggested that yields were also improved under drought and heat stress by protecting the Rubisco chaperone, Rubisco activase (Feng *et al.*, 2007). Experimental evidence has also shown that some of the other enzymes involved in the regeneration of RuBP can limit regeneration (e.g. fructose 1,6-*bis*phosphate aldolase, Haake *et al.*, 1999; plastid transketolase, Henkes *et al.*, 2001). These experimental results have been supported by a numerical simulation using an evolutionary algorithm to optimize the distribution of resources between enzymes of carbon metabolism. This approach has suggested that, in a situation where leaf nitrogen levels are kept constant, increasing fructose 1,6-*bis*phosphate aldolase and SBPase activities and decreasing some photorespiratory enzymes would dramatically increase photosynthetic rate (Zhu *et al.*, 2007; Fig. 2E, F).

Increased CO_2 at the Rubisco catalytic site

The inefficiency of Rubisco has been overcome during evolution by the appearance of a variety of CO_2-concentrating mechanisms in cyanobacteria, algae, and higher plants. Higher plants have evolved a CO_2-concentrating mechanism in the form of C_4 photosynthesis which requires both biochemical and anatomical specialization. An attractive approach to improving carbon assimilation would be to introduce CO_2-concentrating mechanisms to C_3 crop plants thereby favouring the carboxylation reaction and reducing photorespiratory losses. Given that C_4 photosynthesis has evolved independently many times and is found in a number of different plant families (Sage, 2004) it may be possible to introduce a C_4-like pathway into C_3 plants which would not only increase photosynthesis and yield but would also improve water use efficiency (Sheehy *et al.*, 2007). Although C_4 photosynthesis has independently evolved in a number of different plant families, it appears to have repeatedly recruited the same key genes. Initially, the complexity of the anatomical and biochemical changes needed for the operation of C_4 photosynthesis has limited the level of interest in introducing this system into C_3 plants. The discovery in several plant species of a single cell C_4-like mechanism has raised hopes that it will be possible to introduce components of the C_4 system into C_3 crops without fundamentally altering leaf anatomy (reviewed in Edwards *et al.*, 2004). However, on closer inspection of these C_4 single cell systems, it is clear that some level of separation occurs between atmospheric CO_2 uptake and assimilation, allowing some concentration of CO_2 at the Rubisco catalytic site. Without this separation the potential benefits of C_4 photosynthesis are lost. In keeping with this, all attempts thus far to install a single cell C_4 mechanism in rice have been unsuccessful in producing significant effects on photosynthetic performance (Taniguchi *et al.*, 2008; Hibberd and Covshoff, 2010).

With the increasing demand for food and the plateau in annual yield increases in wheat and rice (Long and Ort, 2010), the possibility of achieving large increases in yield through introducing a C_4-like mechanism in such crops has received more attention. A major new project has been initiated (funded by the Bill and Melinda Gates Foundation) to transfer C_4 characteristics into rice, including the anatomical specialization required for a 2 cell-type C_4 mechanism (Kranz anatomy). This is a highly ambitious project utilizing a wide array of approaches to reach this goal including a phenotypic screen for 'C_4-ness' applied to rice and sorghum mutants combined with an approach to install the necessary genes into rice using genetic transformation (Hibberd *et al.*, 2008; Furbank *et al.*, 2009; http://beta.irri.org/projects15/c4rice). Over and above the

technical issues that would need to be overcome to create such plants there will be a cost in terms of increased nitrogen demand, to support the additional enzymes and an increased demand for ATP and NADPH, to provide the energy to synthesize the C_4 pathway intermediates required for the biochemical CO_2 pump. The negative impact of the nitrogen requirement for the C_4 proteins may be mitigated by a requirement for less Rubisco, when operating at higher CO_2 concentrations. A reduced nitrogen requirement would be more likely, however, if such plants also contained a catalytically superior Rubisco. This type of approach could also be applied to wheat but it is important to realize that C_4 photosynthesis would not be as advantageous in cool environments (where photorespiration is lower) or in light-limited environments because of the need to divert light energy away from the Calvin cycle to operate the C_4 carbon concentrating mechanism (Sage, 2004).

While the installation of a 'Kranz' C_4 cycle (involving anatomic as well as metabolic changes) into wheat could require the transfer of many genes, an alternative strategy based on one or two transgenes may be possible by mimicking the inorganic CO_2 concentrating mechanism (CCM) present in cyanobacteria and algae (Lieman-Hurwitz *et al.*, 2003; Price *et al.*, 2008). By requiring a lower number of transgenes and no major anatomical modification, this approach might be less technically challenging and the energy costs of a CCM may be inherently lower than that of the C_4 pathway. Although a CCM localized at the plasma membrane or the chloroplast envelope has never been observed in a terrestrial plant, there are opportunities to introduce well-characterized cyanobacterial bicarbonate pumps into terrestrial mesophyll cells, or more specifically into the chloroplast envelope. Single-subunit HCO_3^- transporters such as BicA and SbtA which have been well characterized as bicarbonate transporters would be obvious choices, although multi-subunit transporters such as the BCT1 HCO_3^- transporter and NDH1-based CO_2 uptake systems could also be considered (see Fig 3; Price *et al.*, 2008). Likewise, HCO_3^- transporters from micro-algae such as *Chlamydomonas* could also be considered as candidates for installation (Spalding, 2008). The first objective, however, would be to place a cyanobacterial HCO_3^- transporter, BicA or SbtA, on the chloroplast inner envelope membrane to target the estimated 20–50 ppm CO_2 drawdown between the leaf intracellular space and the chloroplast stroma (Evans and von Caemmerer, 1996). This initial approach, not aimed at accumulating CO_2 in the chloroplast above cytoplasmic levels, would target a 5–15% improvement in photosynthetic CO_2 fixation efficiency. Introduction of a more effective CCM, allowing significant accumulation of HCO_3^-, would require more modifications (Price *et al.*, 2008).

Whether a modified C_4 cycle or bicarbonate-pump approach is to be used to elevate the concentration of CO_2 around Rubisco, a major gap in our knowledge of the diffusion properties of the chloroplast envelope and plasma membrane/cell wall prevents us from predicting the outcome of such a transformation (Evans *et al.*, 2009). If CO_2 could freely pass across the compartment where it is being concentrated and back into the atmosphere, the energetic costs of the CCM could be too high to provide a benefit translatable to yield. It has been suggested that aquaporins may be involved in modulating membrane permeability to CO_2 and that permeability could be manipulated by altering levels of these proteins (Uehlein *et al.*, 2003, 2008). It should also be noted that leaf morphology in C_3 plants appears to have evolved to minimize the diffusion path for CO_2 to reach Rubisco, a characteristic which may be undesirable for plants concentrating CO_2 in the chloroplast compartment (Evans *et al.*, 2009). Repackaging Rubisco in some type of carboxysome or pyrenoid structure, as found in certain algae, might be necessary to achieve effective operation of a CCM (Price *et al.*, 2008) as illustrated in Fig. 3. Interestingly, pyrenoids with CCMs are present in the earliest land plants, *Anthocerotophyta*, which are assumed to be the ancestors (or close ancestors) of modern vascular plants (Meyer *et al.*, 2008).

Decreasing photorespiratory losses

The preceeding subsections have considered the options for increasing the CO_2/O_2 ratio at the Rubisco catalytic site. A different approach would be to alter part of the higher plant photorespiratory pathway, thereby reducing the energetic cost of photorespiration or increasing the probability of recapturing CO_2 released in the process. This could be achieved using metabolic engineering to introduce genes encoding proteins sourced from non-photosynthetic organisms that short-circuit the normal photorespiratory cycle (Kebeish *et al.*, 2007; Parry *et al.*, 2007; Maurino and Peterhansel, 2010). Recently, this approach has shown promise in *Arabidopsis* where a positive effect on both photosynthesis and growth was detected. However, these approaches not only alter the subcellular compartmentation of the photorespiratory cycle but also affect the energy balance within these compartments. Care would have to be taken to prevent the accumulation of toxic intermediates which could occur if there was a high flux through this bypass pathway.

Strategies to improve efficiency of light capture

Relaxing the photoprotected state more rapidly to normal state

Light is required for photosynthesis. However, when the photosynthetic photon flux density (PPFD) exceeds the photosynthetic capacity of leaves, the extra energy can potentially cause photooxidative damage to the photosynthetic apparatus, especially PSII reaction centres. This is largely avoided by an induced increase in the thermal dissipation of energy within the photosystem II (PSII) antenna system via the formation of epoxidated xanthophylls (Baroli and Niyogi, 2000; Havaux and Niyogi, 1999; Long *et al.*, 1994). This reversible increase in thermal

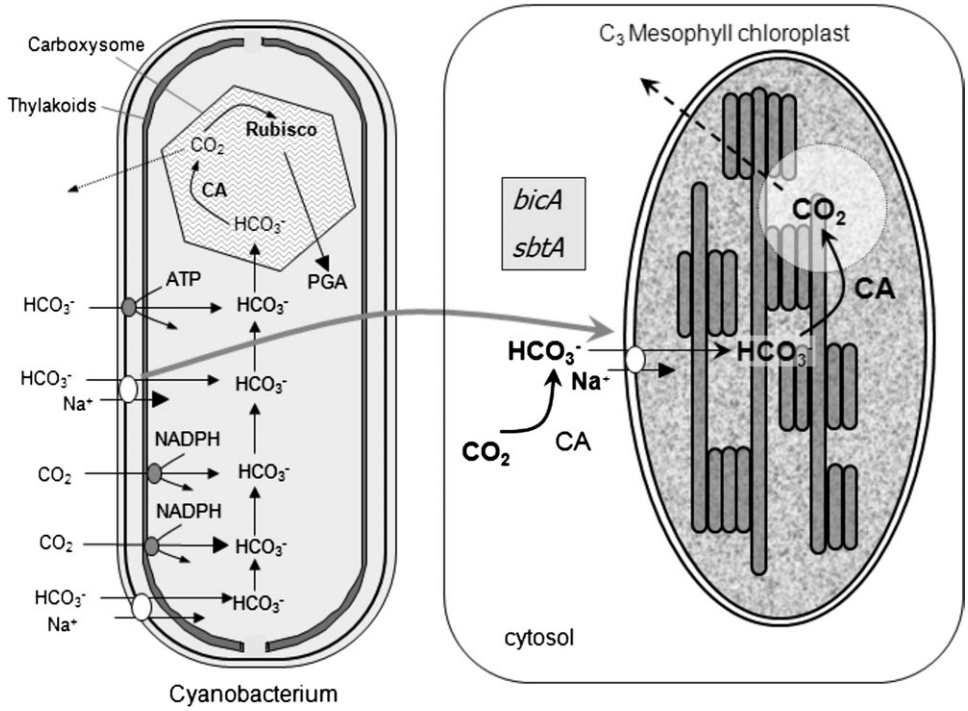

Fig. 3. Model of how the HCO3- transporter might be installed in mesophyll cells of C3 plants.

quenching of excitation is termed photoprotection. Dissipating more energy as heat instead of driving primary charge separation and, thereby, energy capture decreases the quantum yield of PSII (Niyogi, 1999), lowering the efficiency of CO_2 fixation by the photosystems. This lower efficiency can be reflected by a decrease in the initial slope (the quantum yield, Φ_{CO_2}) and convexity (θ) of the PPFD dependence of CO_2 assimilation. At high light, a decrease in Φ_{CO_2} and θ has minimal impact on carbon gain, while the increased thermal energy dissipation protects PSII against oxidative damage. However, the decrease in Φ_{CO_2} and θ reduce carbon gain at low light, for example, for leaves at a lower layer within the canopy or for all leaves at dawn or dusk. A finite period of time is required for the recovery of Φ_{CO_2} and θ when solar radiation drops from saturating PPFD as, for example when a cloud obscures the sun or change in sun-angle places one leaf in the shade of another. Given that sunlight upon and within leaf canopies in the field is continually fluctuating, photoprotection can cause substantial decreases in total canopy CO_2 uptake. Using a ray tracing algorithm Zhu *et al.* (2004b) analysed the impact of fluctuating light levels in the field on total canopy CO_2 uptake, predicting that the delay in recovery from photoprotection could decrease CO_2 assimilation by 6.5–17% at 30 °C and 12.5–32% at 10 °C for chilling-tolerant and -susceptible species, respectively. This modelling suggested that plants with an increased capacity for photoprotection and repair will gain a competitive advantage in high-light stress conditions. How realistic is it to manipulate photoprotection in wheat? Algae associated with the coral *Stylophora pistillata* can withstand 1.5× full sunlight without evidence of loss of maximum photosynthetic efficiency or photoinhibition, showing that the loss of efficiency is not an intrinsic requirement of the photosynthetic apparatus (Falkowski and Dubindky, 1981). Much recent evidence suggests that processes or components related to photoprotection can be manipulated to change the heat-dissipation process. For example, over-expressing β-carotene hydroxylase in *Arabidopsis thaliana*, which controls the biosynthesis of carotenoids of the xanthophyll cycle, changed the rate of formation and relaxation of non-photochemical quenching (*NPQ*), a parameter describing the heat-dissipation process (Johnson *et al.*, 2008). Besides the possibility of genetically engineering properties of photoprotection, genetic variation in susceptibility to photoinhibition within a single species or among species is evident from the diversity in the extent to which Φ_{CO_2} decreases and/or recovers after photoinhibition (Long *et al.*, 1994; Pimentel *et al.*, 2005; Wang *et al.*, 2008). This indicates a valuable approach to enhancing photosynthesis under fluctuating irradiance, namely, identifying and engineering optimal non-photochemical quenching kinetics.

Canopy light capture

There is some potential to improve cumulative radiation interception over the crop cycle by promoting fast early-leaf area growth to more rapidly reach maximum radiation interception, and by extending the duration of green leaf area as the crop matures. It is important that such approaches are not considered in isolation (on a leaf area or whole plant basis), but in the context of the whole crop and the resources available to it (Reynolds *et al.*, 2009). Rapid leaf area growth is likely to be most beneficial in favourable

environments where the time at full radiation interception is restricted by short crop duration, such as in warm spring-wheat cropping regions, and in many water-limited environments that suffer terminal drought (Condon *et al.*, 2004), whereas the same strategy could be disadvantageous in other water-limited environments (Parry *et al.*, 2005). Rapid leaf area growth may also be counterproductive in longer-season favourable environments if it results in greater within-canopy shading extending over a large proportion of the crop cycle. This will penalize radiation use efficiency (E) because of the increased proportion of fixed carbon which is consumed by respiration, required to maintain this greater leaf area (Murchie *et al.*, 2009). Radiation interception reaches about 70% at a leaf area index of 3, but a leaf area index of 6 may be required to achieve 85% radiation interception, in other words increasing (in this case doubling) the leaf area beyond a certain point will not greatly improve light interception. Also, while much of the radiation incident on the crop may be absorbed by the upper canopy, it may not be used effectively to drive assimilation because, as elaborated above, at high light intensities an increasing proportion of the energy is diverted to non-photochemical processes and, at the same time, leaves lower in the canopy may be deprived of light. The detrimental effects of within-canopy shading on E may be minimized through changes to canopy architecture during crop development towards more-erect leaf angles and/or smaller leaves that allow greater light penetration deeper into the canopy, so that intercepted radiation is distributed over a higher proportion of the deployed leaf area (Horton, 2000). More-erect leaf angles are also likely to reduce the proportion of leaves that become light-saturated at high light intensities, thereby further contributing to greater E (Murchie *et al.*, 2009). Dense, high-input wheat crops under conventional cropping systems in many regions of the world already display erect-leaf characteristics, so it seems that this aspect of canopy architecture is already very close to optimal (Horton, 2000). It is likely that canopy architecture may need to be 're-optimized' to match some recent developments in cropping practice aimed at increasing the sustainability of wheat production. In many regions, wider row-spacing is needed to enable efficient trash clearance by seeding machinery. Also, the bed-furrow cropping systems being widely adopted in South and East Asia (Wang *et al.*, 2004) are effectively 'skip-row' configurations that encourage more-open canopies than conventional planting configurations.

Extended leaf area duration, via 'functional stay green' or persistent green leaf area late in development, should be a beneficial trait in many environments. An extended canopy duration has been implicated as an important component of yield potential gains under high-input conditions in the UK (Shearman *et al.*, 2005). Genetic improvement in this trait would need to be coupled with superior nitrogen remobilization to ensure that both C and N are adequately supplied to the developing grain. Unless all of the N is ultimately reassimilated to the grain, protein content of the grain may be adversely affected. There is considerable genetic variation in the phenomenon of delayed senescence and providing there is enough water during late grain-filling, this could be a valuable trait for increasing photosynthesis over the whole life cycle of wheat (Spano *et al.*, 2003).

Canopy CO_2-exchange

The height of the wheat crop canopy may also have a substantial impact on canopy E. Modern semi-dwarf canopies are substantially shorter than the canopies of wheat crops grown 50 years ago. If the substantially higher grain yields required 50 years from now are to be supported by the stems of future wheat canopies, then canopy height may need to be reduced even further, to improve lodging resistance. In reviewing options to achieve greater lodging resistance, Berry *et al.* (2007) concluded that future, higher-yielding wheat canopies will need to be shorter still, ideally closer to 70 cm in height rather than current canopy heights of *c.* 80–100 cm. But any gains in lodging resistance from height reductions of this magnitude will need to be assessed carefully against the strong likelihood that they will restrict canopy E and biomass accumulation. Miralles and Slafer (1997) found no difference in E, before anthesis, between near-isogenic lines of wheat differing in height due to the presence/absence of either one of the widely-deployed semi-dwarfing genes *Rht-B1b* and *Rht-D1b*. The lack of difference in E is an interesting observation because the expectation would have been for the semi-dwarf isolines to have higher photosynthetic capacity due to a higher Rubisco concentration on a leaf area basis (Morgan *et al.*, 1990). For dwarf near-isogenic lines, with both dwarfing genes (and probably even greater Rubisco concentration), E before anthesis was actually substantially less than that of tall lines (Miralles and Slafer, 1997). The lack of difference in E between semi-dwarf and tall lines and the lower E of dwarf lines indicates that the difference in plant height resulted in a disconnection between leaf-level photosynthetic characteristics and canopy C gain. There are several possible reasons for this disconnection, perhaps contributing additively to the observed outcome. One may be a better distribution of direct and diffuse light, and therefore enhanced C-capture, by taller canopies because of greater physical separation between successive leaf layers (Miralles and Slafer, 1997). Lack of sink strength may be another reason for E being lower than expected for canopies of semi-dwarf and dwarf wheats (see above): stem growth may have been insufficiently rapid, and hence sink strength lowered, due to the presence of dwarfing genes. It may also be that the ambient CO_2 concentration around the upper, most photosynthetically-active leaves of tall canopies is greater than around the upper leaves of shorter canopies, due to the closer coupling of tall canopies to the atmosphere. Closer coupling of tall canopies is the most likely explanation for a previously unpublished observation made by AG Condon and RA Fischer (1995) during data collection contributing to Fischer *et al.* (1998). While collecting data on photosynthetic characteristics of an

historic series of wheats, an adjacent breeding population segregating for height was also studied. In this population it was found that canopies of tall lines were cooler than canopies of semi-dwarf lines, despite the fact that the flag-leaves of semi-dwarf lines had greater stomatal conductance and, all else being equal, the canopies of these lines should have been cooler. From energy balance considerations it can be concluded that the canopies of the taller lines were better coupled to the atmosphere, allowing much more effective exchange of latent heat and better canopy cooling. There are other canopy characteristics, not just canopy height, which will influence atmospheric coupling. The dense canopies of modern high-input crops present very smooth upper surfaces to the canopy boundary layers above them. Breeding options, but also management options, that 'roughen' the canopy surface will be important to improve coupling to the atmosphere and better facilitate CO_2 exchange, thereby improving canopy E and translating future gains in leaf photosynthetic characteristics into biomass gains.

There is a great need to understand better and exploit the interactions between CO_2 exchange of source leaves and the sinks in the plant where the sugars generated by photosynthesis are destined. The uppermost flag-leaves are the major source of sugars for expanding grain in well-watered wheat crops. It has long been known that surgical alteration to the balance between source flag-leaf (trimming leaves) and sink spike (removing grain) results in changes in the rate of photosynthesis for the subtending flag leaf during grain-filling (Gifford and Evans, 1981). Recent experiments (Reynolds *et al.*, 2005) demonstrate that increasing the strength of the sink spike of field-grown wheat, by artificially boosting grain number per spike, can stimulate flag-leaf photosynthesis during grain-filling. An important implication taken from this observation is that the photosynthetic capacity present in current wheats may be under-utilized already, because of insufficient sink strength (either too few grains being set, or grains that have a size limitation). It follows that effective exploitation of any future gains in leaf photosynthetic capacity must be accompanied by a coincident boost in sink strength. Options for boosting sink strength, as part of the wheat yield consortium, are further explored in Foulkes *et al.* (2011). To accompany these efforts at boosting sink strength, it would be very useful to have rapid diagnostic measures that allow screening of field-grown germplasm for evidence of sink limitation to photosynthesis. Development of such diagnostics will be another target of work under the canopy photosynthesis banner of the consortium. It is anticipated that newly-developed diagnostics of sink limitation would be employed prior to anthesis, when the crop's yield potential is being established, and during grain-filling, when that potential is being realized.

Photosynthesis by the spike

Reproductive structures in grasses, as well as many other species, are photosynthetic, an adaptation to the fact that they intercept a significant amount of radiation and, probably, to safeguard seed-filling when leaf area is reduced by pests or other stresses. The spikes are displayed above the leaf canopy for up to half the crop duration and once they have emerged in a dense wheat crop, almost half of the incident radiation may be intercepted by spikes. That given, any strategy to improve E of wheat should also consider genetic modification of spike photosynthesis (SP). Furthermore, it has been shown that SP can contribute substantially to grain-filling (Tambussi *et al.*, 2007). However, relatively little is known about the trait, and as far as the authors are aware no cereal breeding programme has ever made a systematic attempt to improve it. In fact a number of basic questions need to be answered before genetic improvement becomes feasible.

The first priority will be to establish the range of genetic variation for SP. This has a number of components which may interact. Discrete organs of the spike (glumes, awns, etc) show different photosynthetic capacity determined by their morphology, development, and metabolic capacity (Tambussi *et al.*, 2007) and are likely to be under independent genetic control. However, just as for leaves, a more important question than whether individual spikes show measurable differences in photosynthetic rate is whether the 'canopy' of spikes shows genetic differences in assimilation capacity (i.e. SP m^{-2}). Finally, given that spikes and leaves essentially compete in terms of resource capture (e.g. for light and N) it may be salient to consider genetic variation for 'SP as a proportion of the total carbon fixed' in terms of achieving an efficient balance between SP and that of the rest of the canopy. The ability accurately to measure these effects is a perequisite to being able to combine their favourable expression through breeding, as well as allowing potentially unfavourable genetic linkages to be broken.

As mentioned, one of the challenges in designing efficient wheat canopies will be to optimize the apparent trade-off in light interception between leaves and spikes. The lower limit for light interception by spikes will be determined by the minimum tissue required to permit a progeny load conducive to expression of high yield (Austin *et al.*, 1980). The upper limit is less obvious, however, the use of gigas spike types to boost potential grain number (Gaju *et al.*, 2010) could have application in increasing the photosynthetic capacity of the 'spike-canopy'. Another factor in terms of optimizing the morphology of the spike canopy will be the trade-off between spike size and spike density. This is especially important given the fact that high yield potential is realized in cultivars which express a diverse range of spike densities. While empirical approaches are tested using available genetic diversity, modelling approaches (e.g. Zhu *et al.*, 2008) that consider the response of both leaf and spike photosynthesis to light intensity can help to establish thresholds of efficient light distribution within the whole canopy including spikes. An example of a simple question that could be answered by modelling is whether awns make an efficient contribution to SP, especially as these are easily removed or enhanced through breeding. Other approaches will need to consider nitrogen use, including the distribution

and composition of pigments and enzymes (especially Rubisco) between leaves and spikes to define theoretical targets and establish search parameters for the screening of genetic resources. In this context, it is necessary to consider developmental and environmental effects too; for example, spikes may express delayed chlorosis relative to leaves especially under stress. This phenomenon suggests that SP is better adapted to the harsh conditions, which tend to occur in the latter part of grain-filling in a number of wheat agro-ecosystems, and genetic variation for 'stay-green spike' has been reported in wheat (Abbad *et al.*, 2004). Part of the explanation may come from the fact that SP partially uses respiratory CO_2 made available during grain-filling, thereby increasing the transpiration efficiency of the organ compared with leaves (Araus *et al.*, 1993) as well as potentially increasing E as a whole.

The main bottleneck to improving SP in crops is that it is especially difficult to phenotype. For example, gas exchange measurements to establish CO_2 fixation rate can be confounded by the spike's ability to recycle respiratory carbon and, due to spike architecture, these measurements are technically difficult and slow. It is difficult to standardize the units of C fixation for SP; an area basis is normal for leaves which are expressed as two dimensional structures, spikes on the other hand have a complex three-dimensional geometry. The other problem, which is not unique to SP, relates to the feasibility of recording integrated values of photosynthesis over representative periods of the crop cycle, while at the same time encompassing more than a single organ. A recent attempt to overcome these problems used shading treatments (of either spikes or leaves and stems) throughout grain-filling to estimate the relative contribution of SP to grain weight in field-grown wheat plots. Results have indicated highly significant genetic variation among cultivars (M Reynolds *et al.*, unpublished data). Another alternative to gas analysis that permits instantaneous measurement of photosynthetic parameters is modulated chlorophyll fluorescence, which has the advantage of being very rapid and, as such, can be adopted into high-throughput phenotyping platforms and estimates electron transport rate, a parameter which will integrate over both net gas exchange and recycling of respiratory carbon (Munns *et al.*, 2010), providing care is taken to control [CO_2] and [O_2] around the screened individuals carefully. Such approaches have value in both identifying genetic variation and in gene discovery. Using the treatments described above, promising mapping populations have already been developed through screening parents for relative contribution of SP to grain weight. Once reliable molecular markers have been identified–via precision phenotyping to genetically map SP traits—they can be applied in breeding, facilitating the combination of SP with other E-related traits.

Conclusions

The wheat yield consortium has identified several strategies that have the potential either individually or in combination to increase photosynthesis and, therefore, the yield potential of wheat. The benefit of each of the approaches proposed will depend on environmental conditions and thus their impact will vary over time; nevertheless Table 2 indicates the expected impact and possible time for adoption into breeding programmes. The approaches proposed will deliver improved germplasm both through the exploitation of natural variation and biotechnology (Box 2) and new technological tools. There is clearly an urgent need to develop crop plants that give higher outputs per unit area of land, without having to increase inputs of fertilizer or water. It is for these reasons that all of the most promising avenues to achieve this goal are considered. Of these, the

Table 2. Possible increases in net photosynthesis that may be achieved by selected modifications to wheat, and speculated time for availability of wheat lines for first crosses in breeding programmes

The predictions assume that water and nutrients would not be limiting although some modifications are likely to have a greater effect if accompanied by increased sink capacity.

Modification	Predicted increase (%)	Time scale (years)
Mine existing germplasm	5–20	<5
Short-circuiting photorespiration	0–5	5
Increased mesophyll conductance	5–10	5
Increased RuBP regeneration	0–10	5
Exploiting existing species variation in Rubisco	0–20	12
Exploiting existing species variation in Rubisco and increased RuBP regeneration	10–35	15
Optimized Rubisco regulation	5–20	10
CO_2 pump	0–30	10
CO_2 pump with Kranz anatomy	50	20
Rubisco without oxygenase and high K_{cat}	100	20

Box 2. WYC key deliverables

- High throughput screens for photosynthetic characteristics in CE and field
- Germplasm with varying photosynthetic capacity and efficiency
- Determine the physiological, structural, and biochemical basis for high P_{max}, P_{eff}/P_{leff} and heat-stable Rubisco activase
- New molecular markers for component traits
- Transgenic wheat plants with increased RuBP regeneration
- Transgenic wheat plants with thermally tolerant activase
- Transgenic wheat plants with decreased compensation point
- Plastid transformation protocols for wheat
- Data for impact of transgenes on yield in field-grown plants for possible enhanced growth and photosynthetic performance

improvement of photosynthetic carbon fixation offers a realistic, timely and overlooked target for the production of crops with improved yields in the near future. Whilst individually the manipulation of photosynthetic capacity and efficiency, increasing ear photosynthesis, optimizing canopy photosynthesis, introducing chloroplast CO_2 pumps, increasing RuBP regeneration, improving the thermal stability of Rubiosco activase and replacing wheat Rubisco with that from other species with different kinetic properties can increase photosynthetic productivity, many can also be pyramided to even greater advantage. A concerted integrated international approach is essential to make progress in delivering food security to a hungry world.

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