Letters

Supercharging rice photosynthesis to increase yield

Rice (Oryza sativa and Oryza glaberrima are the cultivated species) is a plant with C_3 photosynthesis that is mostly grown in climates where photorespiration rates are high. The fact that rice is a C_3 crop is often a surprise, given the prevalence in the tropics and subtropics of C_4 photosynthesis in crops (maize, sorghum, sugar cane), fodder grasses (e.g. Cynodon dactylon), grass weeds (e.g. Imperata cylindrica) and sedges (e.g. Cyperus papyrus). Although C_4 photosynthesis has evolved independently at least 45 times in 19 families of angiosperms (Sage, 2004), it is not known in the genus Oryza or in any close relatives or anywhere in the Bambusoideae subfamily of grasses, many species of which are tropical.

Rice is the most important crop in the world for human food. Similar amounts of rice, wheat and maize are produced annually but a large proportion of wheat and maize goes for livestock feed or industrial uses (Rice Almanac, 2002). Asia accounts for 90% of the rice grown and consumed and the poorest people spend up to half their wages on rice (Dawe, 2000). Over the last 40 yr the production of rice in Asia has kept pace with the increase in population (Fig. 1) as more land has been brought into cultivation and the Green Revolution (better cultivars, use of irrigation, fertilizers and pesticides) has increased yields per hectare. As populations continue to grow, rice consumption must increase (currently half the population of south-east Asia has a calorie intake inadequate for an active life) and the area for cultivation will remain constant or decrease as land is taken for urban and industrial use. Ensuring food security and protecting the environment for the world is a continuing challenge (Evans, 1998) and requires a second Green Revolution.

It is therefore generally agreed that rice yields must increase but without proportionate increases in the use of water or fertilizer, and within the context of climate change (Evans, 1998; Hossain & Pingali, 1998; Dobermann, 2000; McCarthy et al., 2001; Tilman et al., 2001; Depledge, 2002). Improving rice productivity is the key to a better life for rice farmers and consumers, and contributes to economic development and the route out of poverty for less developed countries (Dawe, 2000). However, yields in some Asian countries may have reached a plateau (Cassman, 1999) and yield potentials in breeders’ trials at the International Rice Research Institute (IRRI; www.irri.org) have not increased for 30 yr (Sheehy, 2001a). Yield potential (Evans & Fischer, 1999) is the yield in an optimal physical environment (solar radiation, temperature, mineral nutrients) and with complete protection from weeds, pests and diseases. In general, farm yields can be pushed to c. 80% of yield potential; the gap is inevitable and allows for the physical environment being suboptimal on most farms, and for moderate use of fertilizer and crop protection measures in environmentally sensitive farming. A substantial increase in yield potential is required, along with better use of water and fertilizer, and we propose that only rice with supercharged, C_4 photosynthesis is likely to provide this.

How could a C_4 rice be constructed? Are there any traces of ‘C_4-ness’ in rice or its near relatives that could be used in a breeding programme? What are the features of productive C_4 plants that would need to be transferred to rice? These are questions for a workshop in July 2006 at IRRI in the Philippines. The aim is to form a consortium, led by IRRI, to co-ordinate and conduct the research necessary to construct a C_4 rice. Scientists with the relevant skills and experience from institutions all round the world will need to be included in this long-term project. Rice with C_4 photosynthesis could make a major contribution to the second Green Revolution, and may indeed ‘involve the most audacious feat of genetic engineering yet attempted’ (Surridge, 2002). In this paper we explain why we concentrate on photosynthesis to increase yield potential, why C_4 photosynthesis, and why now, and suggest how the endeavour could be started.

Fig. 1 Rice production and the population of Asia for the years 1961–2004. As an indication of the trend, the line is the regression of production on population (y = 191.1x – 98.3; P < 0.001, \(R^2 = 0.98\)), and the dotted line is an extrapolation to the population predicted for 2050 (square dot).
Why improve photosynthesis to increase yield potential?

Yield potential depends on the proportion (harvest index) of crop biomass that is directed towards the harvested part of the plant. Biomass is accumulated during the life of the crop as photosynthetically active radiation (PAR, 400–700 nm) is intercepted and used in photosynthesis; the effectiveness of the use and the production of biomass is quantified as the radiation use efficiency (RUE), the amount of dry matter per unit of PAR intercepted (Monteith, 1977). Summarizing these ideas in an equation (simplified from Mitchell & Sheehy, 2000) allows the components to be examined in turn.

\[ Y = H e \sum_{i=1}^{n} Q_i f_i \]  
Eqn 1

where \( Y \) is the grain yield as dry matter (g m\(^{-2}\)); \( H \) is the harvest index; \( e \) is the radiation conversion factor, so-called RUE (g MJ\(^{-1}\)); \( Q_i \) is the PAR incident on the crop on the \( i \)th day (MJ m\(^{-2}\)); and \( f_i \) is the fraction of incident PAR that is intercepted, averaged over the \( i \)th day.

Yield potential can be improved by increasing the value of any one or more of the terms in the equation. It is unlikely that harvest index can be increased much from its current value for modern cultivars in best conditions of c. 0.5, and it may have to decrease slightly to provide stronger stems that are more resistant to lodging. There is little interest in prolonging the growth duration (\( n \)) because the current durations coincide with suitable seasons or allow multiple cropping in a year. The incident PAR cannot be increased except by selecting sunnier locations or growing seasons. Intensively grown rice crops are probably close to intercepting the largest fraction of incident PAR that is possible, given that rice is an annual crop and so starts on bare ground. That leaves RUE as the only term for which a substantial increase might be available. The current general values are for rice 2.2 g MJ\(^{-1}\) (above-ground dry matter, intercepted PAR) and for maize 3.3 g MJ\(^{-1}\) (Kiniry et al., 1989); the higher value for maize is associated with \( C_4 \) photosynthesis, which is especially effective in hot, sunny environments. If a \( C_4 \) rice can be constructed by genetic engineering, with RUE increased by 50% to the value for maize, then yields should be increased by 50%. High values of RUE for rice (2.6 g MJ\(^{-1}\)) in well-fertilized experimental crops are associated with higher yields (Sheehy et al., 2000a).

Taking RUE as constant, on a given day it can be calculated from this equation (Mitchell et al., 1998):

\[ \varepsilon_{\text{total}} = \frac{P - m W - 0.25 P}{Q_{\text{int}}}, \]  
Eqn 2

where \( \varepsilon_{\text{total}} \) is the RUE (g MJ\(^{-1}\); for total dry matter including roots, and intercepted PAR); \( P \) is gross photosynthesis (g m\(^{-2}\); dry matter, ground) for the day; \( m \) is the coefficient for maintenance respiration (g \( 1^{-1}\); dry matter respiring, dry matter) for the day; \( W \) is the total dry matter (g m\(^{-2}\); dry matter, ground); and \( Q_{\text{int}} \) is the amount of intercepted PAR for the day (MJ m\(^{-2}\)).

The top line of the equation is the increase in total biomass as the balance between gross photosynthesis and respiration (neglecting shedding of plant parts during crop growth). Respiration is maintenance respiration (mW, proportional to total biomass) and synthetic respiration (0.25 P, a fraction of fixed carbon used to provide energy for synthesis). Large reductions in respiration are unlikely (Byrd et al., 1992) so increasing photosynthesis is probably the only option for raising the value of RUE. A 50% increase in RUE requires at least a 50% increase in the rate of gross photosynthesis for leaves (Sheehy, 2001b). Evans and von Caemmerer (2000) showed that maize leaves had substantially higher rates of photosynthesis than rice at the same nitrogen content. Rice leaves can double their maximum rate of photosynthesis when provided with an atmosphere of 900 ppm carbon dioxide (Murchie et al., 1999).

Harvest index is an empirical way of relating grain yield to biomass: grain yield (dry matter) is expressed as a proportion of the above-ground dry matter. Part of the actual mechanism is the formation of spikelets (florets), their pollination and then grain filling. It would be little use increasing photosynthesis if there were not the spikelets available for filling. In rice a much larger number of spikelets develop on the panicle when it is initiated than are harvested as filled grains, so there is unused sink capacity (Sheehy et al., 2001). If the output of the source, i.e. photosynthesis, is increased then a larger grain yield should be formed, a conclusion supported by results from experiments using elevated concentrations of carbon dioxide (Baker & Allen, 1993; Ziska et al., 1997; Kobayashi et al., 2005).

Why \( C_4 \) photosynthesis?

A 50% increase in canopy photosynthesis will require both higher leaf quantum yield (initial slope) and higher rates of leaf photosynthesis when saturated by PAR (plateau, on the curve of leaf photosynthesis vs. PAR). This allows all leaves in the canopy, in the range of conditions during the day and during the growing season, to contribute to increased photosynthesis overall. Higher quantum yield is important for leaves receiving low PAR, in canopies with PAR well distributed through the canopy or when incident PAR is low. Higher saturated rates of photosynthesis are required for leaves receiving high PAR, high in the canopy, at appropriate angles, when the incident PAR is high.

Ultimately, improved photosynthesis requires better performance from Rubisco (ribulose 1,5-bisphosphate
carboxylase–oxygenase) because there is no alternative to this enzyme (and the Calvin cycle) for continuous net fixation of carbon dioxide into carbohydrate (Sage, 2004). In modern atmospheres some oxygenase activity by Rubisco is inevitable, hence the occurrence of the pathways of photorespiration to minimize the loss of carbon skeletons from the Calvin cycle.

The options are more Rubisco, better Rubisco or make Rubisco work harder. Rubisco already accounts for up to 50% of soluble protein in leaves and increased amounts would require a proportionate increase in nitrogen; this is not an attractive choice. Moreover, Rubisco only exerts low control on rates of photosynthesis in many environments (Quick et al., 1991). Rubisco with a higher specificity for carbon dioxide is certainly of interest but is almost always accompanied by lower rates of catalysis. This option has been explored by Zhu et al. (2004) who concluded that worthwhile increases in canopy photosynthesis (up to 27%) could be obtained if Rubisco with the properties found in some nongreen algae could be incorporated into leaves. The success of the best versions of C₄ photosynthesis, supercharging the basic C₃ system, arises from an absence of photorespiration and more effective use of Rubisco in higher concentrations of carbon dioxide – Rubisco simply works harder, in conditions more like the primitive atmosphere in which it evolved. Several systems of C₄ photosynthesis in single cells are now known (Reiskind et al., 1997; Edwards et al., 2004; Sage, 2004) but none are associated with high productivity (von Caemmerer, 2003); instead they allow some photosynthetic gain at high energy cost in conditions conducive to high photorespiration. Consequently it is productive C₄ photosynthesis with Kranz anatomy, as occurs in maize, that will be required in rice.

What makes the C₄ system even more attractive is its economical use of water and nitrogen. The water-use efficiencies (transpiration) of crops in the field are notoriously variable, but the values for C₄ crops are about double those of C₃ crops (Brown, 1999; Mortlock, 2003). Data from various sources are given in Table 1 to compare the efficiencies of water use (WUE), photosynthetic nitrogen use (PNUE) and radiation use (RUE) in crops of rice and maize. The ratio of the maize to rice values suggests that changing from C₃ to C₄ rice would increase WUE by 89%, PNUE by 180% and RUE by 50%. In addition, Greenwood et al. (1990) showed that C₄ crops contained c. 60% of the nitrogen used by C₃ crops for maximum yield.

### Why attempt this project now?
The question of supercharging rice photosynthesis by constructing a C₄ rice was examined in a workshop at IRRI at the end of 1999 (Sheehy et al., 2000b). The conversion of C₃ plants to C₄ photosynthesis has been covered in several later reviews (Häusler et al., 2002; Leegood, 2002; Miyao, 2003; von Caemmerer, 2003; Raines, 2006). Nearly 7 yr after the workshop, a number of developments make the task appear feasible. There is an increased understanding of C₄ photosynthesis and of molecular biology, and techniques in genetic engineering continue to improve rapidly.

The C₄ pathway is an addition to the basic C₃ system of photosynthesis and plants are classified as C₃ or C₄ apart from a few intermediate species (and plants with crassulacean acid metabolism – CAM). It is now clear that the C₃ and C₄ syndromes are not as rigidly separated as was first thought. The enzymes that are prominent in the C₄ pathway also exist in C₃ leaves, although with very low activity (Matsuoka et al., 2001). More surprisingly, there is a well developed C₄ pathway in certain locations in C₃ plants: in the green tissue around vascular bundles (Hibberd & Quick, 2002), and probably in rice spikelets (Imaizumi et al., 1997). In the opposite direction, maize, an archetypal C₄ plant, has patches of C₃ tissue wherever a mesophyll cell is not adjacent to a bundle sheath cell, particularly in leaf sheaths and husk leaves (Langdale & Nelson, 1991). These observations help in understanding why the C₄ system has evolved repeatedly: apparently there is much preconditioning and whenever there are testing conditions of high photorespiration the C₄ system readily emerges (Sage, 2004).

In molecular biology, Brown et al. (2005) suggested that study of Cleome gynandra, the C₄ plant most closely related...
to *Arabidopsis*, would accelerate understanding of the C₄ syndrome. The advantages of this approach are that: (a) all the knowledge of *Arabidopsis* leaf development can be used to identify key genes in *Cleome*, (b) *Arabidopsis* can be used as a test system into which to transfer C₄ genes from *Cleome*, and (c) *Cleome* has a short life cycle. Hall and Langdale (1996) reviewed molecular aspects of leaf development and further work has elucidated the roles of the genes associated with differentiation of mesophyll and bundle sheath cells in C₄ leaves (Cribb et al., 2001; Rossini et al., 2001). Insertion of genes for C₄ enzymes into rice and successful expression is now routine (Miyao, 2003).

In genetic engineering there is a wider choice of promoters and of transit peptides to control the compartment in which the product of the transgene occurs. The transformation of plastids is possible so that genes can be expressed in the chloroplast (Maliga, 2002). A large number of enhancer trap lines have been made in rice (Wu et al., 2003; Johnson et al., 2005; Liang et al., 2006) and these are a promising resource.

**How do we start?**

The construction of C₄ rice must be planned from the top downwards, starting with the ultimate objective (food security in rice) and then working out the steps required to achieve it (higher yields, improved photosynthesis, etc.), as outlined in this paper. The opposite approach, pushed by genetic engineering from the bottom upwards, is frequently disappointing. Sinclair et al. (2004) gave examples of how improvements made at the level of molecular biology are dissipated when scaled up through biochemical and physiological levels to the response of crops in the field.

In the short term, we see five lines of research (and these will no doubt be refined by the July 2006 workshop at IRRI).

1. Establish benchmark values of yield potential, physiology and anatomy for rice against which to measure the effect of C₄ additions. These values will include the yield potential of current best cultivars in typical environments, characteristics of leaf and canopy photosynthesis, carbon dioxide compensation point and carbon isotope discrimination as measures as C₄-ness, distance between minor veins in the leaf, characteristics of bundle sheath cells, and so on.

2. Evaluate material in the IRRI genebank (100 000 lines of *Oryza sativa*, 6000 accessions of other species of *Oryza* for C₃-like phenotypes, from which a breeding programme could start. Yeo et al. (1994) reported carbon dioxide compensation points characteristic of C₃-C₄ intermediates in a few species of *Oryza*, and rice panicles show some aspects of C₄ photosynthesis (Imaizumi et al., 1997).

3. Produce and evaluate C₄ transgenics in the best current cultivars (high yield, pest and disease resistant) using maize genes involved in the C₄ biochemical pathway; to complement the initial work in other cultivars (Ku et al., 1999). Maize is the most productive C₄ grain crop, and many C₄ genes are available.

4. Use bioinformatics on the genomes of rice and maize to identify the key regulatory genes (e.g. transcription factors) that are involved in C₄ photosynthesis.

5. Use *Arabidopsis* and *Cleome* to find C₄ genes to understand how development of Kranz anatomy is controlled (Brown et al., 2005).

In the medium term, we would concentrate on producing model plants to enable a complete understanding of Kranz anatomy, and determine what regulates the expression of photosynthesis genes in the bundle sheaths of rice. Ultimately, it will be necessary to embed the full C₄ system in rice, to conduct field trials to determine its effectiveness, and to incorporate it in a range of cultivars for commercial use in various rice-growing systems.

**Conclusions**

Supercharging photosynthesis is the only way to improve yield potential substantially in rice whilst not increasing the demand for water and nitrogen. This means adding the C₄ biochemical pathway and modifying leaf anatomy so that the C₄ system works at its best. We are confident that now is a pivotal time for harnessing all current progress in understanding C₄ photosynthesis and in techniques of genetic engineering to try to construct a C₄ rice. To do this, partnerships will be required between institutions with the specialized expertise, and that is why IRRI is forming the C₄ Rice Consortium.

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**References**


