Using C₄ photosynthesis to increase the yield of rice—rationale and feasibility

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90% of the world's rice is grown and consumed in Asia, with each hectare of rice-producing land providing food for 27 people. By 2050, because of population growth and increasing urbanisation, each remaining hectare will have to feed at least 43 people. This means that yields must be increased by at least 50% over the next 40 years to prevent mass malnutrition for the 700 million Asians that currently rely on rice for more than 60% of their daily calorific intake. Since predictive models suggest that yield increases of this magnitude can only be achieved by improving photosynthesis, and because evolution has increased photosynthetic efficiency by 50% in the form of the C₄ pathway, one solution is to generate C₄ rice. However, this is an ambitious goal that requires proof of concept before any major investment of time and money. Here, we discuss approaches that should allow proof of concept to be tested.

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Introduction

Rice—supply and demand in the 21st century

About halfway through the past century, plant breeding programmes generated cereals with high yield potential and led to large improvements in grain production. This became known as the 'Green Revolution'. The short-stature rice cultivars that came to dominate production in Asia have, however, reached a yield barrier [1], and the gains associated with the Green Revolution have virtually ceased. This comes at a time when about 548 million Asians are undernourished [2••]. Over the next 50 years, the population of Asia will increase by about 1.5 billion. At the same time, climate change will probably result in more extreme variations in weather and have adverse effects on rice production [3], water scarcity will become more common possibly leading to a loss of irrigated rice land [4], and the increasing demand for biofuels will generate competition between grain for fuel and food resulting in price increases [5].

Currently, 75% of total rice production (600 Mt) comes from irrigated land and 22% from land receiving only rain; the balance comes from deepwater systems. Given the absence of additional agricultural land, the demands generated by population increases alone will require rice yields to rise by 50% in all ecosystems over the next half century.

Theoretical models suggest that the yield increases required to match the projected population growth can only be achieved by increasing the efficiency with which photosynthesis uses solar energy [6,7]. The radiation use efficiencies of C₃ and C₄ plants differ by about 50% [8,9], and such differences are attributable to differences in canopy photosynthesis. This has led to the radical suggestion that the C₄ photosynthesis pathway should be placed into rice [10]. Introducing the C₄ pathway into cultivars grown in irrigated, rainfed and deepwater ecosystems could be the solution to the problem of raising rice yields in all rice ecosystems [3]. In comparison to C₃ crops such as rice, C₄ crops have higher yields, reduced water loss and increased nitrogen use efficiency, particularly when grown in hot and dry environments [11••].

C₄ versus C₃

Since photosynthetic organisms became terrestrial around 450 million years ago, land plant morphology and photosynthetic mechanisms have diversified [12]. Morphologically, 'leaves' have become more structured. For example, in extant mosses, leaf-like structures comprise relatively undifferentiated photosynthetic cells and gas exchange occurs through pores in the epidermis. In lycophytes, leaves are more complex, with stomata in the epidermis and chloroplasts in both epidermal and mesophyll cells. Within the angiosperms, there has been a further elaboration of morphology such that leaves develop multiple cell types, two or three of which are highly evolved to carry out photosynthesis. This morphological complexity is associated with higher photosynthetic capacity per unit area.

There are three photosynthetic pathways used by plants [11••]. In most species, CO_2 is fixed by Ribulose Bisphosphate Carboxylase/Oxygenase (RuBisCO) in the Calvin-

Benson cycle to generate a three-carbon compound. These plants are referred to as C_3. Species that use the C_4 and Crassulacean Acid Metabolism (CAM) pathways evolved from C_3 plants, and in both cases, a four-carbon compound is initially formed from fixation of HCO_3^-. C_4 and CAM plants increase the efficiency with which CO_2 is supplied to RuBisCO through the addition of this C_4 carbon shuttle. Thus, C_3 is the ancestral pathway, with C_4 and CAM representing recently diverged forms.

The evolution of C_4 from C_3 occurred independently at least 45 times in angiosperms [13], yet the differences between the two photosynthetic types are complex. Most C_4 plants, and certainly all known C_4 grasses, compartmentalise photosynthetic reactions between two morphologically distinct cell types that are arranged in concentric circles around veins [14]. This so-called Kranz anatomy comprises enlarged bundle sheath (BS) cells immediately adjacent to the veins that are surrounded by mesophyll (M) cells. This arrangement generates a consistent interveinal distance of four cells (vein-BS-M-M-BS-vein). Metabolic reactions are divided between BS and M such that C_4 acids are generated in M cells and then diffuse to the BS where the Calvin-Benson cycle operates. This separation of metabolism is achieved by restricting the expression of a small number of genes to either the BS or M cells. Carbonic anhydrase (CA), phospho*enol*pyruvate carboxylase (PEPC), NADP-malate dehydrogenease (MDH), pyruvate orthophosphate dikinase (PPDK) and the proteins involved in their post-translational regulation accumulate in the M cells, whereas NADP-malic enzyme (ME) and RuBisCO are restricted to the BS [15]. Genes encoding all of these enzymes are present in C_3 plants, but expression levels are much lower than in C_4 species.

The polyphyletic evolution of the C_4 pathway suggests that the transition from C_3 to C_4 is relatively simple. This suggestion is supported by the observation that both C_3 and C_4 plants possess inherent plasticity in their photosynthetic characteristics. Biochemical characteristics of C_4 photosynthesis are present in some cell types of C_3 species [16], and C_3 patterns of cell-type differentiation can be found in some tissues of C_4 plants [17]. These observations suggest that C_3 and C_4 characteristics can develop in a single plant and thus that mechanisms to induce high levels of C_4 gene expression are present in C_3 plants. Indeed some plants are able to switch between C_3 and C_4 photosynthesis [18,19], indicating that the processes governing the function of these apparently complex systems must be flexible.

In addition to C_3 and C_4, there are a number of genera that contain C_3–C_4 intermediate species. The characteristics of these intermediates have been used to hypothesise the sequence of events that occurred as C_4 evolved [11••]. However, there is no evidence that C_3–C_4 intermediates are in the process of evolving the C_4 pathway, and it is equally possible that they represent evolutionary dead ends.

The way forward?

Although generating C_4 rice is extremely ambitious, the polyphyletic evolution of C_4 photosynthesis provides cause for optimism. The challenge is how to repeat the process in a reasonable timeframe.

Some species use a form of the C_4 pathway that operates in single cells [20,21]. While this may at a first glance seem a simpler system to attempt to install into rice, in all domesticated, highly productive C_4 crops, 'Kranz' anatomy and the compartmentalisation of photosynthetic metabolism are inextricably linked. Therefore, in our opinion, the generation of C_4 rice will require a mechanistic understanding of this apparently necessary interface between leaf morphology and metabolism in classical 'Kranz' C_4 species. Such a goal demands an examination of photosynthetic characteristics in C_3 plants with altered leaf morphology and of leaf morphology in C_3 plants with altered photosynthetic metabolism.

To determine the extent to which rice leaf morphology can vary, screens are being carried out on mutant *O. sativa* populations and on accessions of the 22 wild-rice relatives. Preliminary screens have shown variations in both interveinal distance and the degree of chloroplast development in BS cells (unpublished). For example, the domesticated *O. sativa* inbred line IR64 has approximately twelve M cells between adjacent veins, and chloroplasts occupy approximately 20% of the BS cell area. By contrast, accessions of *O. barthii* and *O. australiensis* have an average of six M cells between veins. These observations suggest that genetic variation within existing *Oryza* germplasm resources may be sufficient to develop morphological features that would support an efficient two-cell C_4 pathway. The challenge is to find accessions of rice with decreased interveinal distance, and optimal chloroplast volume within BS cells. Notably, *O. barthii* and *O. australiensis* have also been reported to accumulate elevated levels of PEPC [22] implying that in species with altered leaf anatomy, there are also changes in leaf metabolism.

There is also evidence that altering primary metabolism of C_3 leaves has profound effects on leaf development. For example, constitutive manipulation of components of the Calvin-Benson cycle causes alterations to leaf morphology [23,24] and specific metabolites can act as signalling molecules to alter leaf development [25•,26]. In the case of C_4 metabolism, induction of high levels of NADP-ME in rice leads to a reduced stacking of thylakoids in chloroplasts [27] and specific manipulation of translocators that have been co-opted into key roles in C_4 plants alter both leaf and cell development [28•,29]. Surprisingly, overexpression of a *PEPC* gene from *Corynebacteium* in *Vicia narbonensis* leads to accumulation of

transcripts encoding enzymes involved in the methylation cycle [30$^{\bullet\bullet}$]. As DNA methylation is known to be important in controlling gene expression, the link between high activities of PEPC in a specific cell type, flux through the methylation cycle, and its influence on gene expression should be investigated. Overall, these observations indicate that an altered metabolite flux generated by the C_4 pathway could lead to altered leaf morphology.

Conclusions

To avoid a humanitarian disaster, rice production needs to keep pace with the rate of population growth. Because plants that use the C_4 pathway can maintain faster rates of photosynthesis and higher yields than those that use the C_3 pathway, it has been proposed that rice should be engineered to use C_4 photosynthesis. This is extremely ambitious, and so we argue that proof of concept is needed. Furthermore, because there is increasing evidence that leaf morphology and metabolism are co-ordinated, we argue that initial studies should be designed to investigate the mechanistic interface between leaf morphology and metabolism in C_3 and C_4 leaves.

Acknowledgements

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