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Integrating C_4 photosynthesis into C_3 crops to increase yield potential

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The growth rate of the human population is faster than improvements in crop yields. To feed people in the future, multiple strategies are required. One proposed approach is to raise the yield potential of C_3 crops by modifying photosynthesis to the more efficient C_4 pathway. Owing to complex changes associated with C_4 photosynthesis, it is no understatement to define this conversion as one of the Grand Challenges for Biology in the 21st Century. Here we outline the challenges of installing a C_4 system and assess how new approaches and knowledge may help achieve this goal.

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Background to crop yields

The Green Revolution led to large improvements in grain production. However, in recent years, plant breeders have failed to systematically increase yields in line with population [1,2]. It is estimated that world cereal production must increase by 50% by 2030 to meet the projected demand for food [3]. Owing to increases in climate uncertainty, it would be most beneficial if genetic improvements increased yields across a range of environments. Increasing the maximum attainable yield of existing food crops could be part of the solution. It is theoretically possible to increase yield potential by 50% in some species by raising their photosynthetic capacity [2,4–6]. If this proved possible in practice, then it would greatly contribute to food security.

Increasing photosynthetic capacity raises yield potential

Dramatically increasing yield potential is not trivial because the outcome results from complex interactions between contributing components. Yield potential is the product of four factors: (1) total incident solar radiation accrued over the growing season, (2) efficiency of the plant to intercept photosynthetically active radiation (PAR), (3) efficiency with which intercepted PAR is converted into dry matter (radiation use efficiency, RUE) and (4) amount of resources partitioned to the grain (harvest index). During the Green Revolution, light interception and harvest index were maximised. Extending the growing season is undesirable because management practices are tied to cyclical weather patterns that allow production within specific time frames, and canopy production and architecture are thought to be optimised [2,4]. This leaves RUE as a potential source for significant new genetic improvement. Theoretical models predict RUE of C_3 crops would be improved by approximately 50% by using C_4 photosynthesis [2,4]. This led to the suggestion that converting crops from C_3 to C_4 could mitigate the global food crisis [4,7].

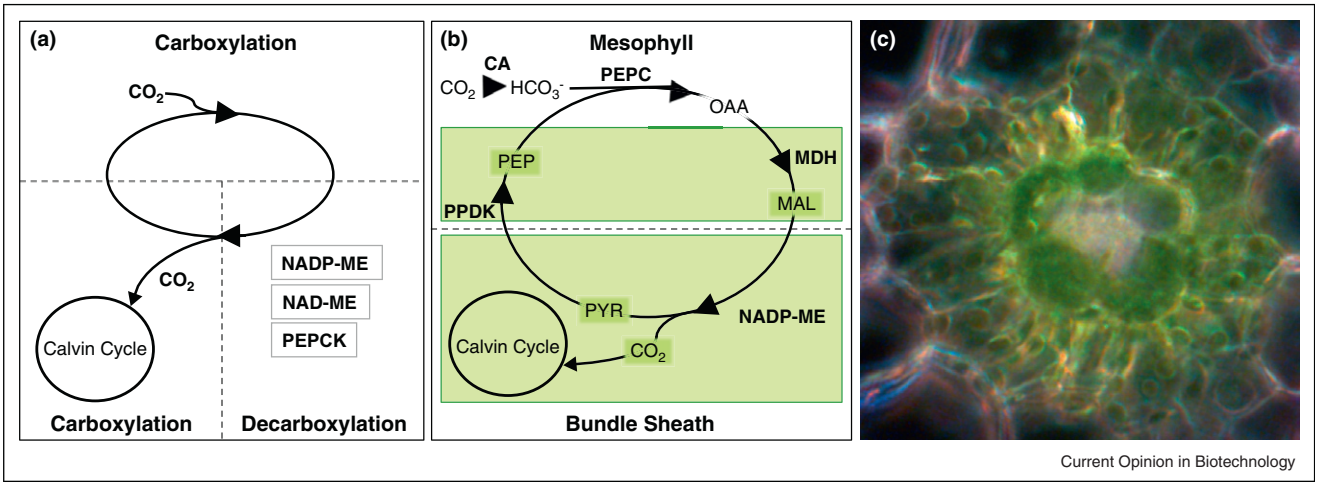
Flavours of C_4 photosynthesis

There are multiple forms of C_4 photosynthesis, but all involve specialised anatomy and biochemistry of leaves. Three major subtypes of biochemistry [8] are superimposed onto at least twenty-five types of leaf anatomy [9] (Figure 1a), and evidence is mounting that these biochemical subtypes are an oversimplification [10,11••, 12•,13•,14]. This diversity leads to the important question of which C_4 flavour should be selected to engineer into C_3 crops. Two main approaches have been undertaken, both of which use NADP-malic enzyme (NADP-ME) biochemistry (Figure 1b) as a basis for converting rice from C_3 to C_4. These are the development of a single-celled C_4 system [15,16] and a two-celled system [17] that would require the development of mesophyll (M) and bundle sheath (BS) cells arranged in classical Kranz anatomy (Figure 1b,c). The latter effort, which is the subject of this review, has been selected by the C_4 Rice Project [18] because it is the type utilised by many of the most productive C_4 crops and is relatively simple. However, it will still be difficult to engineer.

Challenges associated with placing C_4 photosynthesis into C_3 leaves

The complexity of C_4 photosynthesis indicates that its integration into C_3 leaves will be an enormous challenge. Indeed, many domesticated C_3 crops, including rice, belong to genera that are deeply embedded in clades consisting only of C_3 species [19••] and so it can be argued that there is some inherent incompatibility between the

Figure 1



C_4 photosynthesis requires specialised leaf biochemistry and anatomy.
(a) There are three major subtypes of C_4 biochemistry. In each, CO_2 is initially fixed by the cytosolic enzyme phospho*enol*pyruvate carboxylase (PEPC) to form a four carbon molecule that is subsequently decarboxylated by at least one of three enzymes: NADP-malic enzyme (NADP-ME), NAD-malic enzyme (NAD-ME), and/or phospho*enol*pyruvate carboxykinase (PEPCK). While NADP-ME operates in the plastid, NAD-ME and PEPCK function in the mitochondria and cytosol, respectively, requiring diffusion of released CO_2 to the chloroplasts. In all subtypes, a high concentration of CO_2 builds in the vicinity of Ribulose-1,5-Bisphosphate Carboxylase Oxygenase (RuBisCO), favouring its use as a substrate to initiate the Calvin–Benson cycle and dramatically reducing photorespiration. These biochemical reactions may be superimposed onto many different types of leaf cellular anatomy.
(b) The C_4 Rice Project aims to convert rice to a two-celled NADP-ME C_4 photosynthetic system with classical Kranz anatomy. In this system, two distinct photosynthetic cell types, mesophyll (M) and bundle sheath (BS), differentiate to form an interdependent biological unit with a defined spatial arrangement. M and BS cells form concentric circles around the veins, generating a consistent pattern of vein-BS-M-M-BS-vein across the leaf. The C_4 cycle starts in the M cells, where CO_2 is converted to bicarbonate in the cytosol by carbonic anhydrase (CA) and is fixed to phospho*enol*pyruvate (PEP) by PEPC to form oxaloacetate (OAA). OAA moves into the chloroplast where it is converted to malate (MAL) by malate dehydrogenase (MDH). MAL moves from the M cell chloroplast to the BS cell chloroplast where it is decarboxylated by NADP-ME to form pyruvate (PYR) and CO_2. The PYR moves from the BS cell chloroplast to the M cell chloroplast where it is converted to PEP by pyruvate, orthophosphate dikinase (PPDK), thereby completing the C_4 carbon cycle. The CO_2 released in the BS chloroplast is used in the Calvin–Benson cycle.
(c) *Sorghum bicolor* performs two-celled NADP-ME C_4 photosynthesis with classical Kranz anatomy. Shown here is a representative cross section of a *S. bicolor* leaf with the vein (centre) surrounded by a layer of BS and M cells, respectively. The C_4 Rice Project aims to duplicate this anatomical and physiological arrangement in rice.

current genomes of these species and operation of C_4 photosynthesis. Additionally, major gaps in our knowledge of the C_4 leaf must be addressed. No master regulator(s) has been isolated and loci for many of the transporters associated with metabolite fluxes, modifications to cell biology as well as the specialised anatomy of C_4 leaves remain to be identified.

On a more pragmatic note, the number of genes essential to a functional C_4 pathway is large. Existing methods of genetic engineering are probably insufficient for its installation, and the engineering challenge will probably increase as we identify more genes essential to C_4. In the next sections, we propose opportunities that may allow some of these challenges to be overcome.

Opportunities to introduce Kranz(-like) anatomy into C_3 leaves

For a two-celled NADP-ME C_4 leaf to be engineered, a key modification will be the introduction of Kranz(-like) anatomy into C_3 leaves. Classical Kranz anatomy (Figure 1c) is proposed as a target because the most productive C_4 crops have this cellular pattern. Reduction in interveinal distance, larger and/or increased number of chloroplasts within BS cells, specialisation of M and BS chloroplast proteomes, and sufficient plasmodesmata for transport between M and BS cells will be necessary modifications to the C_3 leaf. Although no genes controlling development of Kranz anatomy are known, it is possible to disrupt cell specific functions and patterning in C_4 species. Large-scale screens of *Zea mays* [20–23] yielded mutants in BS and M specific pathways [23,24]. Mutants with large interveinal spaces or altered BS cell development have been identified in *Panicum maximum* [25]. A screen of sorghum [18] yielded lines with significantly increased vein-spacing [17]. Conversely, a screen of rice mutants identified lines with closer vein-spacing relative to wild type [17]. The presence of some flexibility in C_3 and C_4 leaf traits provides hope that Kranz anatomy can be introduced into C_3 leaves.