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C_2 photosynthesis: a promising route towards crop improvement?

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Summary

C_2 photosynthesis is a carbon concentrating mechanism that can increase net CO_2 assimilation by capturing, concentrating, and re-assimilating CO_2 released by photorespiration. Empirical and modelling studies indicate that C_2 plants assimilate more carbon than C_3 plants under high temperature, bright light, and low CO_2 conditions. I argue that engineering C_2 photosynthesis into C_3 crops is a promising approach to improve photosynthetic performance under these, and temporally heterogeneous, environments and review the modifications that may re-create a C_2 phenotype in C_3 plants. While a C_2 engineering program would encounter many of the same challenges faced by C_4 engineering programs, the simpler leaf anatomical requirements make C_2 engineering a feasible approach to improve crops in the medium term.

Key words: bundle sheath cells, C_2 photosynthesis, C_3-C_4 intermediate, carbon concentrating mechanism, crop improvement, food security, glycine shuttle, photorespiratory CO_2 pump

I. Introduction

Plants have evolved an extraordinary diversity of approaches to perform the carbon fixation pathways of photosynthesis that facilitate their expansion broadly across all of the Earth's biomes. Most plants use only C_3 photosynthesis, in which Rubisco binds CO_2 to initiate the Calvin-Benson cycle within the chloroplasts of mesophyll cells (Fig. S1). However, more than a quarter of this assimilated CO_2 can be later lost to photorespiration, the metabolic pathway initiated when Rubisco binds O_2 instead of CO_2. While photorespiration has been co-opted for a variety of metabolic functions, it releases previously assimilated carbon and nitrogen and is energetically costly (Eisenhut *et al.,* 2019). Photorespiration is exacerbated in hot, arid, bright, and saline conditions, where the concentration of CO_2 compared to O_2 decreases around Rubisco and is further compounded by Rubisco kinetics favouring oxygenation at high temperatures. In response, some plant lineages have evolved carbon concentrating mechanisms (CCMs) to improve net carbon assimilation in these high photorespiration environments.

C_2 photosynthesis, also called the glycine shuttle and photorespiratory CO_2 pump, is a simple CCM that captures, concentrates, and re-assimilates CO_2 released by photorespiration (Fig. S1). The peculiar phenotype of C_2 plants was first identified 45 years ago (Kennedy & Laetsch, 1974); however, the biochemistry behind these plants was not proposed (Monson *et al.* 1984) nor fully understood (Rawsthorne *et al.,* 1988) for a decade or more. It is now understood that glycine decarboxylase (GDC) is functional only in the bundle sheath cells of C_2 plants, such that this CCM works by shuttling photorespired glycine from the mesophyll peroxisomes into the bundle sheath mitochondria for decarboxylation (Fig. S1). This shuttle releases CO_2 in the bundle sheath compartment, approximately tripling the CO_2 concentration (Keerberg *et al.,* 2014) and facilitating its re-assimilation via the Calvin-Benson cycle within bundle sheath chloroplasts. Therefore, the C_2 CCM supplements C_3 photosynthesis to improve the re-assimilation rate of photorespired CO_2 and boost net CO_2 assimilation in warm, bright, and low CO_2 conditions (Bellasio & Farquhar 2019). Throughout the paper, the term 'C_2 photosynthesis' specifically refers to the use of this glycine shuttle, however, it should be noted that some C_2 species also engage a weak C_4 photosynthesis system (Sage *at al.*, 2014).

II. Diversity and distribution of C_2 photosynthesis

C_2 photosynthesis has been identified in over 50 species from 4 monocot and 16 eudicot lineages representing 11 plant families, including the agriculturally important Poaceae, Brassicaceae,

Asteraceae, and Amaranthaceae (Table 1). However, only one crop species, the salad green arugula (*i.e.,* rocket; *Diplotaxis tenuifolia*, Brassicaceae) has been identified to use C_2 photosynthesis. However, C_2 photosynthesis is likely used by more species than current records indicate. This is partially because the phenotype is difficult to identify, as a clear confirmation of C_2 photosynthesis requires multiple lines of evidence, including assessments of immunohistochemistry, leaf ultrastructure, and CO_2 compensation point (*e.g.,* Khoshravesh *et al.* 2016). Moreover, high intraspecific and intraplant photosynthetic diversity and plasticity exists in C_2 lineages (*e.g.,* Sayre and Kennedy 1977*;* Lundgren *et al.,* 2016), likely hindering confirmation of C_2 species further.

As a group, C_2 species are broadly distributed in geographical and ecological space, having been recorded across all major plant biomes and in every continent except Antarctica (Lundgren & Christin, 2017). However, while some C_2 lineages are remarkably widespread (*e.g.,* Diplotaxis, Mollugo), others are confined to small geographical and ecological niches (*e.g.*, Alloteropsis, Euphorbia, Portulaca). C_2 species live broadly across precipitation, seasonality, and soil quality spectra (Christin *et al.,* 2011; Lundgren *et al.,* 2016; Sage *et al.,* 2018), although their most consistent ecological feature is a tendency to shift into warmer habitats than their close C_3 relatives (Lundgren & Christin, 2017).

III. C_2 photosynthesis is a stable evolutionary state

C_2 photosynthesis is often associated with its role as an intermediate physiological state during the evolution of another, stronger CCM, C_4 photosynthesis, as C_2 physiology underlies most C_3-C_4 intermediate species (Schlüter & Weber, 2016; Sage *et al.,* 2018). Both the phenotype and ecology of C_2 plants may facilitate the evolution of C_4 photosynthesis. Firstly, the C_2 glycine shuttle also releases ammonium into bundle sheath cells (Fig. S1), which may create a nitrogen imbalance that could be efficiently remedied via the introduction of a C_4 cycle (Mallmann *et al.,* 2014), potentially causing some C_2 lineages to transition quickly to a C_4 state (Bräutigam & Gowik, 2016). Second, because C_2 photosynthesis increases net carbon assimilation under high temperatures (Monson 1989; Bellasio & Farquhar, 2019), evolution of C_2 physiology also shifts lineages into warmer environments than their C_3 relatives (Lundgren & Christin, 2017). Like the nitrogen imbalance hypothesis, warm environments, where photorespiration rates are high, create strong selection for C_2 lineages to rapidly transition to C_4 photosynthesis.

Despite biochemical and environmental selection pressures, many C_2 lineages entirely lack C_4 species (Table 1). This may indicate the existence of factors that limit or slow C_4 emergence in some plant lineages, such as anatomical limitations to efficient metabolite exchange or whether they inhabit cooler climates with low selection pressure for C_4 evolution (*e.g.,* Schlüter *et al.,* 2017). Alternatively, or additionally, C_2 physiology is likely sufficient in some circumstances, reducing selection pressure for further C_4 evolution. Indeed, C_2 lineages that lack close C_4 relatives occupy areas with higher precipitation and higher quality soils compared to their C_3 relatives (Lundgren & Christin, 2017), perhaps indicating weak selection for further C_4 evolution under these environmental conditions. Furthermore, lineages such as Mollugo have remained in a C_2 state for over 10 million years (Christin *et al.,* 2011), implying that C_2 photosynthesis is a stable evolutionary state and not inherently a step along an inevitable C_4 trajectory (Blätke & Bräutigam 2019; Edwards, 2019).

IV. C_2 photosynthesis is a tractable route to improve food security

Recent findings from both theoretical analyses and field experiments suggest that alternations to photosynthesis can deliver large increases in productivity (Kromdijk *et al.,* 2016; South *et al.,* 2019). The urgent need to achieve large improvements in crop photosynthetic efficiency has consequently catalyzed rapid recent progress in the use of synthetic biology as an approach to overcome the limitations of C_3 photosynthesis. The most important direct sources of global human calories (*i.e.,* rice and wheat) use C_3 photosynthesis, so any improvement to this system would have far reaching benefits in feeding a growing human population. Scientists therefore aim to engineer C_4 photosynthesis into C_3 crops, as C_4 plants requires less nitrogen and water and are consequently more efficient and ultimately faster growing and higher yielding than C_3 plants under certain environmental conditions (Christin & Osborne, 2014; Atkinson *et al.,* 2016). This large improvement in efficiency, however, requires major reconfigurations of leaf anatomy, ultrastructure, and biochemistry, the genetics behind which are still not fully understood (Sedelnikova *et al.,* 2018), making engineering C_4 photosynthesis into C_3 crops a long-term challenge. By contrast, C_2 photosynthesis offers some of the benefits of C_4 photosynthesis but with fewer required anatomical modifications (Fig. 1), suggesting that C_2 conversions may be more tractable than C_4 conversions (Leegood, 2002; Gowik & Westhoff, 2011). Moreover, for some lineages, such as the agriculturally and nutritionally important Brassicaceae, which

repeatedly evolved C_2 but never C_4 photosynthesis, C_3 species may be readily improved with C_2 but intractable to C_4 engineering efforts.

From an engineering perspective, all of the genes required for C_2 biochemistry are present in C_3 species, such that only changes to regulation and expression would be needed to recreate the glycine shuttle. Figure 2 describes the modifications suggested to engineer C_2 photosynthesis into C_3 plants. Briefly, C_2 plants require abundant chloroplasts with active Rubisco in both mesophyll and bundle sheath cell types, and GDC activity must be exclusive to the bundle sheath. Anatomical modifications, other than those to functionalize the bundle sheath, may not be required. Lundgren *et al.* (2019) compared closely related C_3, C_2 and C_4 phenotypes of the grass *Alloteropsis semialata* to find that recently diverged C_3 and C_2 populations only differed in the number of mesophyll cells separating veins, with C_2 plants having on average fewer mesophyll cells (3-6) than C_3 plants (5-11). Importantly, vein density did not differ between C_3 and C_2 *A. semialata* but did increase via the development of minor veins in *A. semialata* plants engaging predominately C_4 photosynthesis. Thus, the ongoing challenge faced by C_4 engineering programs to increase leaf vein density may be unnecessary in C_2 engineering programs, making it one substantial step easier to implement.

Additional modifications, such as increases to bundle sheath cell size, movement of chloroplast and mitochondria positioning within the bundle sheath, shifts in Rubisco proportioning between bundle sheath and mesophyll cells, or changes to minimize the ratio of CO_2 leakage out and metabolite fluxes into the bundle sheath, may help to optimize the C_2 CCM, however the degree to which these components are required are likely lineage specific. Further modifications to ameliorate the nitrogen imbalance between mesophyll and bundle sheath cells may be required, however, no obvious overarching requirements have been identified yet and, as such, may not be required for a successful C_2 engineering effort (Schlüter *et al.*, 2017).

Recent studies have already made important strides in understanding the genes that underlie the requirements to engineer C_2 photosynthesis, thanks in large part to the progress made via the C_4 Rice Project (reviewed in Sedelnikova *et al.*, 2018; Ermakova *et al.,* 2019). For example, Wang *et al.* (2017) showed that, compared to wild type, constitutive expression of the GLK transcription factor in rice conveyed (1) larger bundle sheath chloroplasts with two to three times more Rubisco and Rubisco activase enzymes; (2) larger bundle sheath mitochondria with GDC; and (3) more plasmodesmata junctions to functionally increase the connectivity between mesophyll and bundle sheath cells. However, constitutive GLK expression did not convey overall chloroplast and

mitochondria areas in bundle sheath tissue comparable to other C_2 species, suggesting that additional modifications may be required to achieve the large bundle sheath organelle areas potentially required for C_2 photosynthesis. Furthermore, Adwy *et al.* (2015) found that deleting the M-box, a 59 bp region in the promoter region upstream of the *AtGLDP1* and *AtGLDP2* genes in the C_3 model species *Arabidopsis thaliana*, established a bundle sheath specific expression pattern. More recently, Adwy *et al.* (2019) confirmed the presence of the M-box promoter region in a C_3 *Moricanida* species and lack of this region in three C_2 *Moricandia* species. These findings have promising applications for C_2 engineering, as deletion of the M-box region may also restrict GDC expression to the bundle sheath mitochondria in other C_3 species. In theory, the modifications described by Wang *et al.* (2017) and Adwy *et al.* (2015, 2019) could, in combination, functionalize the bundle sheath and consequently facilitate a glycine shuttle. While additional modifications will very likely be needed to optimize this engineered glycine shuttle, large anatomical changes such as increased vein density or bundle sheath cell sizes are unlikely to be required to successfully recreate C_2 photosynthesis. Thus, despite facing similar challenges as C_4 engineering efforts, successful C_2 engineering programs seem a tangible prospect.

V. Engineering C_2 photosynthesis should convey benefits to C_3 crops

Photorespiratory CO_2 loss is a major factor limiting productivity in C_3 plants, cutting crop yields by more than 20%, such that reducing photorespiratory losses by as little as 5% may translate to over $500 million annually from the additional production in just soy and wheat alone (Walker *et al.,* 2016). Because C_2 plants suffer less net carbon loss from photorespiration, engineering C_2 photosynthesis into C_3 crops could therefore have a large impact on crop production. The physiological benefits that engineering C_2 photosynthesis could convey are not entirely clear though, and seemingly depend to some degree on temperature, light level, and ambient CO_2 concentration (Table 2; *e.g.,* see Schuster & Monson 1990). Some of the physiological diversity characterised across C_2 plants undoubtably arises from mixed photosynthetic systems, as some C_2 plants also engage a weak C_4 cycle, such that distinguishing the physiological effects of C_2 photosynthesis alone is difficult. To clearly ascertain the potential physiological benefits of engineering C_2 photosynthesis into C_3 crops, a comprehensive comparative survey of C_2 vs C_3 physiology across diverse plant lineages under consistent growth and measurement conditions is needed.

A short review of the literature suggests that C_2 plants generally have higher rates of photosynthesis and water- and nitrogen-use efficiencies compared to C_3 plants (Table 2). Indeed, Bellasio & Farquhar (2019) used a modelling approach to quantify the effects on net carbon assimilation from engineering C_2 photosynthesis into the globally important C_3 crop rice. They found that, compared to traditional C_3 rice, C_2 rice assimilated more carbon under ambient CO_2 conditions, but also across a broad environmental space including warm temperatures (> ~35 ºC), high light (> ~700 µmol m^{-2} s^{-1}), and low CO_2 concentrations (< ~400 µmol mol^{-1}). Moreover, C_2 photosynthesis conveys such strong benefits under warm temperatures that even under the high CO_2 concentrations predicted under climate change scenarios, C_2 rice will outpace C_3 rice in terms of carbon assimilation above ~ 35 ºC (Bellasio & Farquhar 2019).

Engineering C_2 photosynthesis into C_3 crops may also convey physiological flexibility to tolerate a broader range of environmental conditions. C_2 plants are inherently flexible, as the C_2 glycine shuttle initiates only under photorespiratory conditions, meaning that C_2 plants act like C_3 plants in the absence of photorespiration. Plants using C_2 photosynthesis should therefore perform well under environmental conditions that alternatively favour either typical C_3 or C_4 physiologies (*i.e.,* low or high photorespiration environments, respectively), and could be particularly successful in temporally heterogeneous environments. One disadvantage of this flexibility, however, may be sub-optimal partitioning of Rubisco and other photosynthetic enzymes to the bundle sheath when rates of photorespiration are not high. Despite this, the physiological flexibility of C_2 plants may be particularly beneficial for plants experiencing unpredictable weather events, such as those anticipated as a consequence of climate change.

VI. Conclusions

The prospect of engineering C_2 photosynthesis into C_3 crops to improve photosynthetic efficiency is receiving increasing attention (Leegood, 2002; Gowik & Westhoff, 2011; Bellasio & Farquar, 2019; Blätke & Bräutigam 2019). Engineering C_2 photosynthesis should convey improved net carbon assimilation to C_3 crops, especially in high temperature and light environments, and seems to avoid the high vein density requirements of C_4 photosynthesis, making it easier to implement than C_4 engineering programs. However, to accurately assess the potential for C_2 photosynthesis to improve C_3 crop performance, several important questions remain to be answered. (1) To what extent, and in which environments, does C_2 physiology translate into larger, faster growing, and higher yielding plants? (2) Does C_2 photosynthesis convey costs? If so, under which environments

are these costs most strongly realized? (3) How would engineering C_2 photosynthesis interact with other metabolic pathways (*e.g.,* nitrogen metabolism)? (4) Which crops would benefit most from a C_2 engineering effort? Answering these questions will not only clarify a potentially lucrative crop improvement strategy, but also reveal fascinating insights into the evolution of complex traits and diverse photosynthetic systems.

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Figure legends

Fig. 1. Biochemical (left) and anatomical (right) modifications that occur in the transitions between C_3, C_2, and C_4 photosynthetic types. Major steps along this transition are noted as enabling phenotypes within C_3 individuals (blue), establishment of a C_2 cycle (green), establishment of a C_4 cycle (light pink), and optimization of a C_4 cycle (dark pink). Each minor modification that facilitates these major steps is listed along the side of the transition landscape in the respective color. A dotted line distinguishes anticipated modifications to recreate C_2 or C_4 photosynthesis from a typical C_3 phenotype. Note that more steps are needed to establish a C_4 phenotype than a C_2 one. M, mesophyll; BS, bundle sheath; GDC, glycine decarboxylase; PEPC, phosphoenolpyruvate carboxylase. Asterisks denote modifications that are likely to be lineage specific.

Fig. 2. Proposed modifications required to engineer C_2 photosynthesis into C_3 plants. (a) Basic C_3 phenotype, highlighting the abundance of chloroplasts (green) with active Rubisco (R), mitochondria (red) with active glycine decarboxylase (GDC), and peroxisomes (blue) in mesophyll cells, while the bundle sheath cells have fewer organelles. (b) Step 1: prepare the bundle sheath by enhancing chloroplasts, mitochondria, and photosynthetic enzymes in bundle sheath cells and improving connectivity between mesophyll and bundle sheath cells via more plasmodesmata or pit fields (*e.g.,* via constitutive GLK expression). Organelle repositioning along the inner centripetal wall may be required in some lineages. (c) Step 2: functionalize the bundle sheath via restricting GDC activity to bundle sheath cells (*e.g.,* via M-box deletion). (d) Step 3: additional modifications to optimize the C_2 shuttle, for example, by enlarging bundle sheath cells may be required in some lineages.

Tables

Table 1. List of C_2 species by family and lineage [1].

Family	Lineage [2]	C_2 Species
Eudicots		
Acanthaceae	Blepharis	*Blepharis acuminate, B. diversispina, B. espinosa, B. gigantea, B. natalensis, B. nolimetangere, B. pruinose, B. sinuate, B. subvolubilis*
Amaranthaceae	Alternanthera	*Alternanthera crucis, A. ficoidea, A. tenella*
	Salsola	*Salsola arbusculiformis, S. divaricate, S. laricifolia*
	Sedobassia	*Sedobassia sedoides*
Asteraceae	Flaveria	*Flaveria angustifolia, F. anomala, F. chloraefolia, F. floridana, F. linearis, F. oppositifolia, F. pubescens, F. ramosissima, F. sonorensis*
	Parthenium	*Parthenium hysterophorus*
Boraginaceae	Heliotropium	*Heliotropium convolvulaceum, H. greggii, H. racemosum*
Brassicaceae	**Brassica**	*Brassica gravinae*
	Diplotaxis	*Diplotaxis erucoides, D. muralis* [3]*, D. tenuifolia*
	Moricandia	*Moricandia arvensis, M. nitens, M. sinaica, M. spinosa, M. suffruticosa*
Cleomaceae	Cleome	*Cleome paradoxa*
Euphorbiaceae	Euphorbia	*Euphorbia acuta, E. johnstonii, E. lata*
Molluginaceae	Hypertelis	*Hypertelis spergulacea, Paramollugo nudicaulis*
	Mollugo	*Mollugo verticillata*
Portulaceae	Portulaca	*Portulaca cryptopetala* [4]*, P. hirsutissima*, *P. mucronata*
Scrophulariaceae	Anticharis	*Anticharis ebracteate, A. juncea*
Monocots		
Poaceae	Alloteropsis	*Alloteropsis semialata zambezian*
	Homolepis	*Homolepis aturensis*
	Neurachne	*Neurachne minor*
	Steinchisma	*Steinchisma cuprea, S. decipiens, S. hians, S. spathellosum, S. stenophyllum*

[1] Table modified from Lundgren & Christin, 2017; Voznesenskaya *et al.,* 2017. [2] Lineages in bold lack close C_4 relatives. [3] *Diplotaxis muralis* is hybrid between *D. tenuifolia* (C_2) and *D. viminea* (C_3) (Ueno *et al.,* 2006). [4]*Portulaca cryptopetala* contains facultative CAM, and this lineage lacks close C_3 relatives.

Table 2. Published comparisons of C_2 and C_3 physiology.[1]

Species[2]	Key Findings
Empirical Studies (eudicot)	
Alternanthera (Rajendrudu *et al.,* 1986)	
Tridax procumbens (C_3), *Achyranthes aspera* (C_3), *Alternanthera ficoides* (C_2), *A. tenella* (C_2)	Compared to the C_3 species, both C_2 species had higher A_{net}. Measurements collected at 29°C and 340 µl/L [CO_2].
Diplotaxis (Ueno *et al.,* 2006)	
Diplotaxis viminea (C_3), *D. muralis* (C_3 x C_2 hybrid), *D. tenuifolia* (C_2)	Both *D. muralis* and *D. tenuifolia* had higher A_{net} (on per area and per chlorophyll basis) than *D. viminea*. Measurements collected at 25°C and 350 µl/L [CO_2].
Heliotropium (Vogan *et al.,* 2007)	
Heliotropium europaeum (C_3), *H. karwinskyi* (C_3), *H. tenellum* (C_3), *H. convolvulaceum* (C_2), *H. greggii* (C_2), *H. racemosum* (C_2)	Compared to the C_3 species, the C_2 species had - higher WUE at 370 [CO_2] µmol mol^{-1} - similar carboxylation efficiency - higher A_{net} at 200, 300, and 370 [CO_2] µmol mol^{-1} - similar stomatal conductance - higher C_i/C_a at 370 [CO_2] µmol mol^{-1} Measurements were collected at 30°C.
Cleome (Voznesenskaya *et al.,* 2007)	
Cleome monophylla (C_3), *C. paradoxa* (C_2)	The C_2 species had higher WUE than the C_3 species at 27°C and 370 [CO_2] µmol mol^{-1}.
Moricandia (Schlüter *et al.,* 2017)	
Moricandia moricandioides (C_3), *M. suffruticosa* (C_2), *M. arvensis* (C_2)	Compared to the C_3 species, both C_2 species had - lower carboxylation efficiency - lower A_{net} at 400 ppm [CO_2] - lower WUE at 400 ppm [CO_2] Measurements were collected at 25°C.
Flaveria (Monson, 1989)	

Flaveria cronquistii (C_3), *F. pubescens* (C_2 + weak C_4), *F. floridana* (C_2 + weak C_4), *F. ramosissima* (C_2 + weak C_4)	Compared to the C_3 species, the three C_2 species had: - higher A_{net} at all [CO_2] at 35°C - higher A_{net} at sub-ambient [CO_2] at 30°C - lower A_{net} over 200 µbar [CO_2] at 30°C - similar WUE under well-watered or water-stressed conditions - higher pi/pa when well-watered, well fertilized, ambient [CO_2] - lower stomatal limitation to photosynthetic rate Compared to the C_3 species, *F. ramosissima* had higher NUE while the other two C_2 species had similar NUE (defined as initial slope of A_{net} vs leaf N curve).
Mollugo (Kennedy *et al.,* 1980)	
Mollugo pentaphylla (C_3), *M. nudicaulis* (C_2), *M. verticillata* (C_2 + weak C_4)	Compared to the C_3 species, *M. nudicaulis* had similar A_{net} (at 300 ppm CO_2) and carboxylation efficiency, but higher transpiration. Compared to the C_3 species, *M. verticillata* had higher A_{net} and transpiration. Measurements were collected at 30°C.
Empirical Studies (monocot)	
Steinchisma / Homolepis / Neurachne (Khoshravesh *et al.,* 2016)	
Dicanthelium oligosanthes (C_3), *Panicum bisulacatum* (C_3), *Steinchisma hians* (C_2), *Homolepis aturensis* (C_2), *Neurachne minor* (C_2 + weak C_4)	Compared to the C_3 species, the C_2 species had - similar A_{net} and WUE at 400 µmol mol^{-1} [CO_2] - similar carboxylation efficiency Measurements were collected at 31°C.
Alloteropsis (Lundgren *et al.,* 2016)	

Alloteropsis semialata (C_3, C_2 + weak C_4, C_4 populations)	C_2+weak C_4 *A. semialata* populations had similar A_{net}, g_s, WUE, C_i/C_a, and carboxylation efficiency to C_3 populations.
Modelling study (Bellasio & Farquhar, 2019)	
rice (C_3), hypothetical C_2 rice	Under best case scenarios, the hypothetical C_2 rice had higher A_{net} compared to C_3 rice broadly across temperatures (15°C - 45°C) when light levels were above ~700 µmol m^{-2} s^{-1}. When light levels were below ~700 µmol m^{-2} s^{-1}, the hypothetical C_2 rice had higher Anet compared to C_3 rice only at higher temperatures (~ >35°C). The hypothetical C_2 rice maintained a CO_2 assimilation advantage over C_3 rice when $[CO_2]$ < 400 µmol mol^{-1} along a broad range of temperatures (15°C – 45°C). When $[CO_2]$ was greater than 400 µmol mol^{-1}, the C_2 assimilation advantage over C_3 rice only occurred at high temperatures (~ >35°C).
Modelling study (Way *et al*., 2014)	
Modelled C_3 and C_2 photosynthesis using published *Flaveria* data	Using a stomatal optimisation approach with measured biochemical parameters corrected to 30°C, C_2 plants have higher A_{net} than C_3 plants at 280, but not 400 µmol mol^{-1} $[CO_2]$. At 280 µmol mol^{-1} $[CO_2]$, C_2 species have similar WUE as C_3 species.

[1] A_{net}, net rate of photosynthesis; gs, stomatal conductance; WUE, water use efficiency; NUE, nitrogen use efficiency; C_i/C_a, ratio of intercellular to ambient $[CO_2]$.

[2] Species reported to use a weak C_4 cycles are labelled as C_2 + weak C_4.

Supporting Information

Fig. S1. Simplified diagram of photosynthesis and photorespiration in C_3 and C_2 plants.

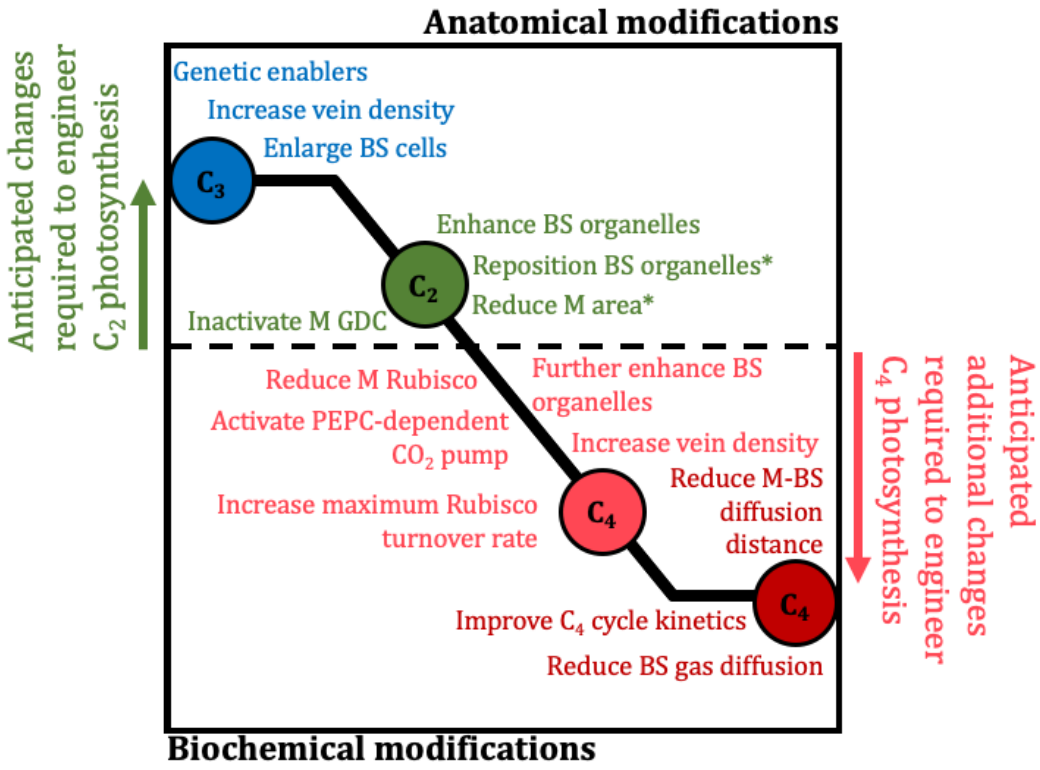

Figure 1.

nph_16494_f1.tiff

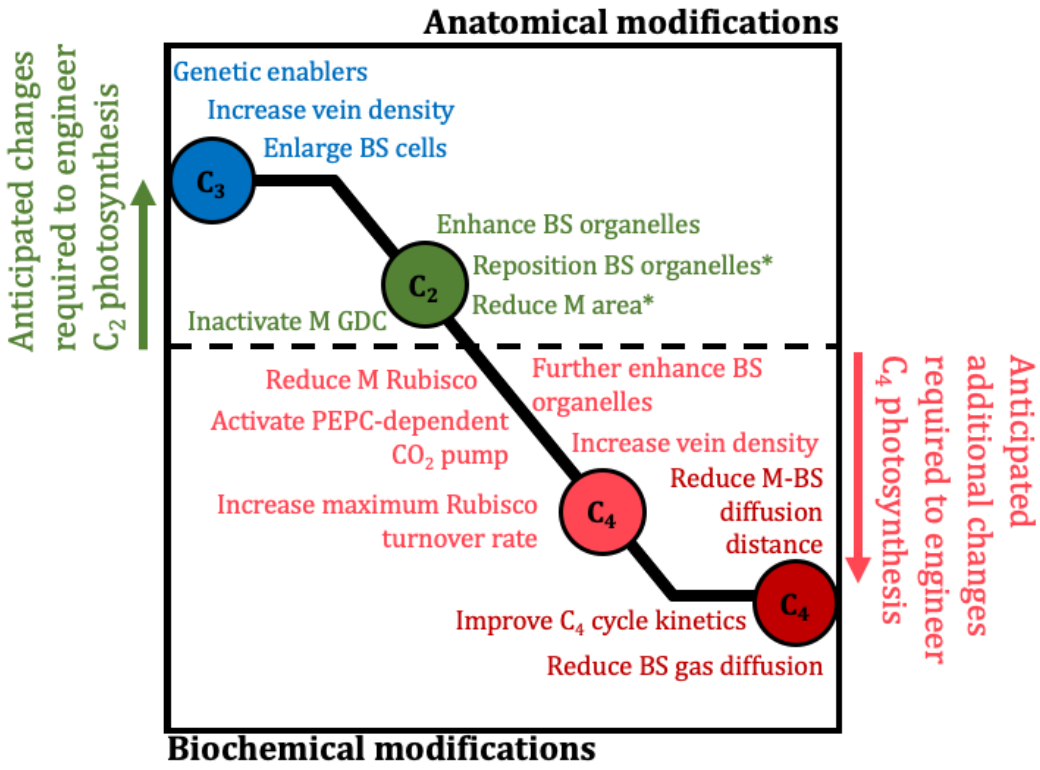

Figure 1.

nph_16494_f1.tiff

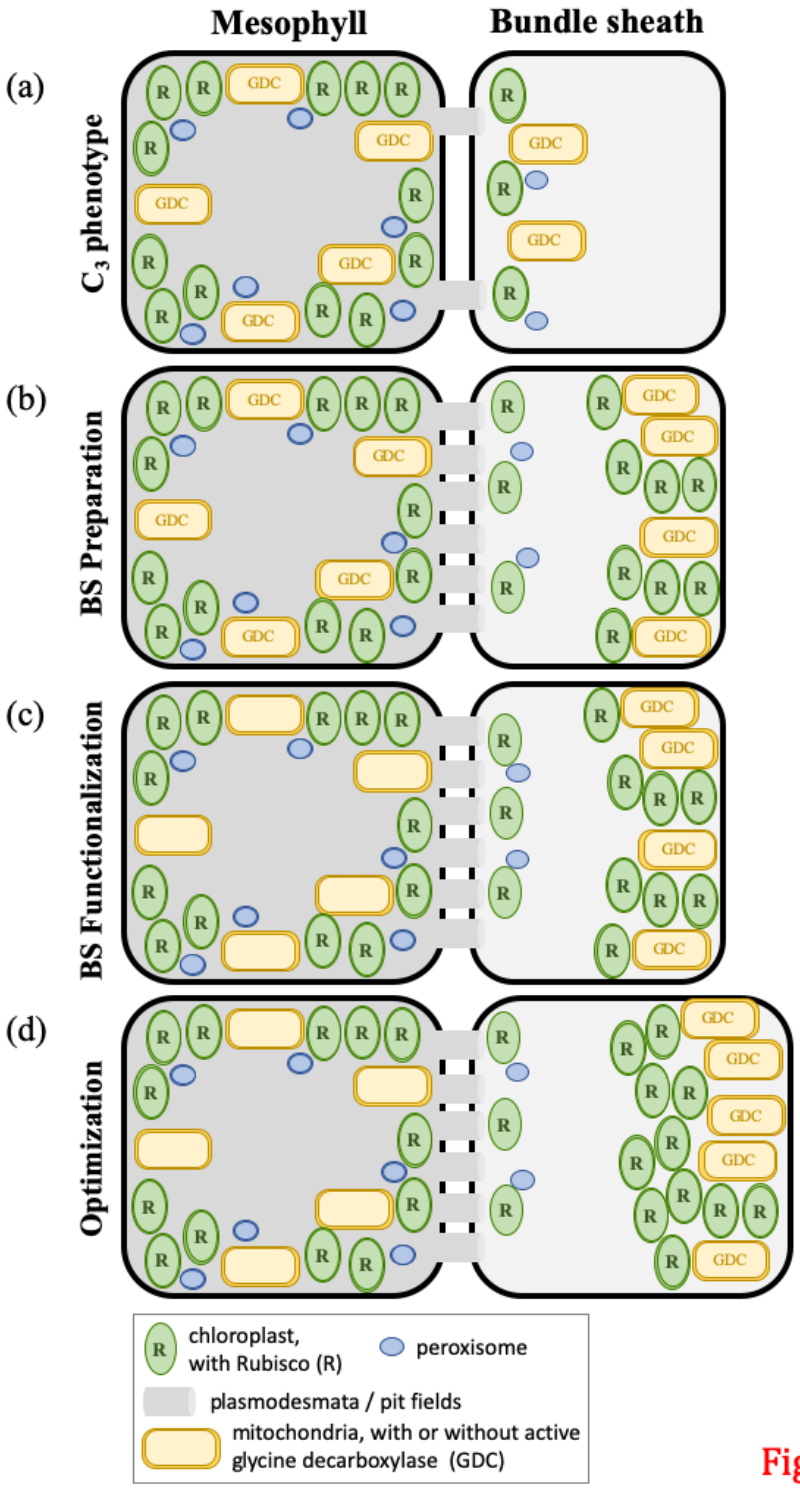

Figure 2

nph_16494_f2.tiff

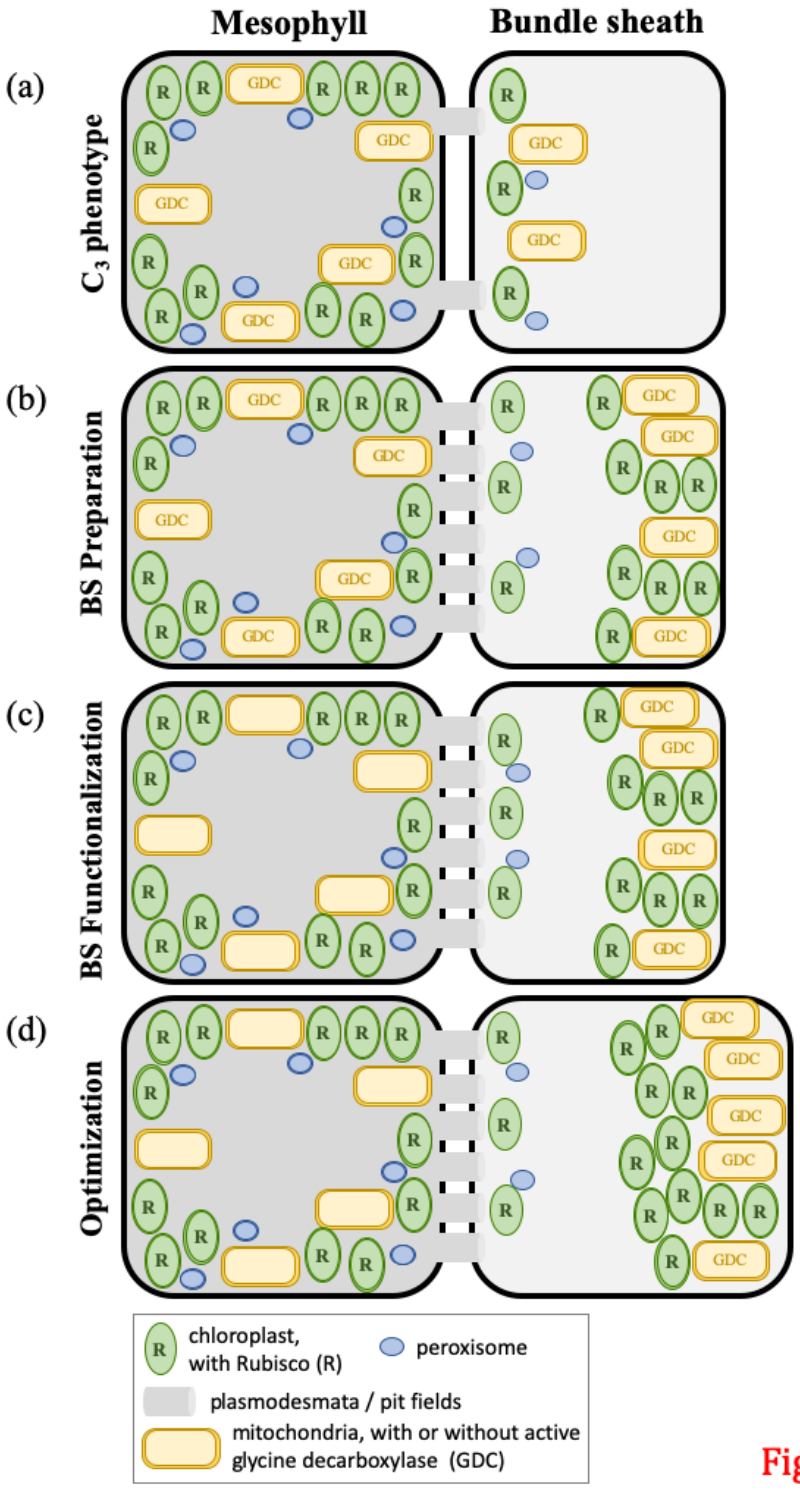

Figure 2

nph_16494_f2.tiff