

Photosynthetic acclimation of rice to global climate change: Will a same-cell C4 system help?

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Photosynthesis, water-use efficiency, and growth of rice (*Oryza sativa*) should increase as atmospheric CO₂ doubles, but higher temperatures could reduce yields. Also, ribulose biphosphate carboxylase-oxygenase (Rubisco) in rice is down-regulated by CO₂ enrichment, so photosynthesis and yield level off at 500 μ bar CO₂. Yield increases from climate change are thus unlikely to keep pace with population growth. Molecular engineering of a C4 system could enhance the C3 photosynthesis of rice, but adverse temperature effects on reproduction must still be countered. Engineering C4 biochemistry and Kranz anatomy into rice is a daunting task. However, the facultative C4 monocot *Hydrilla verticillata* serves as a model because it lacks Kranz anatomy. Instead, the C4 and Calvin cycles coexist and operate in series in the same cell, rather than in separate cells. Cytosolic phosphoenolpyruvate carboxylase (PEPC) is segregated from Rubisco and the decarboxylase (NADP malic enzyme) in the chloroplasts, where CO₂ is concentrated. A specific PEPC isoform (*Hvpepc4*) is induced, along with other C4 cycle enzymes, and functions in photosynthesis of the C4 leaf.

Rising atmospheric CO₂, like the C4 cycle, is a CO₂ concentrating mechanism (CCM) for Rubisco. Consequently, we review the responses of rice grown at elevated CO₂ and temperatures, as might be experienced in future climate scenarios. We also describe the *Hydrilla* CCM and discuss whether, in a CO₂-enriched atmosphere, its "minimalist" C4 system could enhance yields of a transgenic rice cultivar.

According to FAO records, over the past 40 years, grain yields for Asian paddy rice (*Oryza sativa*) have doubled from around 2 to 4 t ha⁻¹, although the rate of increase has now declined (Dawe 2000, Doberman 2000). Over this time frame, the Keeling curve shows that atmospheric CO₂ has risen from 316 μ bar in 1960 to 370 μ bar in 2000 (Keeling and Whorf 2001). Because rice has C3 photosynthesis, which is thus not saturated by current atmospheric CO₂, part of the yield increase is likely attributable to the additional CO₂. However, to meet the demand for food imposed by population growth, it is estimated that further increases of 40% to 50% are needed before the middle of this century. There is anticipation that the production of transgenic C4 rice varieties could enhance future yields (Sheehy 2000) given that C4 photosynthesis is generally more effective than C3 at current CO₂. This hope is bolstered by the report that transgenic rice lines with one or two overexpressed C4 enzymes, including *phosphoenolpyruvate* carboxylase (PEPC), the initial fixation enzyme of C4 plants, have increased photosynthesis, growth, and yield (Murphy et al 2001). But not all growth and yield results for transgenic rice have been as positive (Miyao-Tokutomi et al 2001).

Engineering into rice the multigenic traits for a full C4 system like that of maize (*Zea mays*), including all C4 cycle enzymes and their regulation and cell-specific expression along with the genes for C4 (Kranz) leaf anatomy, is a daunting task. In this regard, over two decades ago, we made the first discovery of a plant that performs C4 photosynthesis without Kranz anatomy—*Hydrilla verticillata* (Bowes et al 1978, Holaday and Bowes 1980, Salvucci and Bowes 1981, 1983a,b). In *Hydrilla*, the C4 and Calvin cycles coexist and operate in series in the same cell, rather than in separate cell types as in other C4 species. The PEPC in the cytosol is segregated from ribulose biphosphate carboxylase-oxygenase (Rubisco) and the decarboxylase (NADP malic enzyme, NADP-ME) that are localized in the chloroplasts. In this manner, futile cycling of CO₂ is minimized and the chloroplast is the specific site where CO₂ is concentrated for assimilation by Rubisco (Reiskind et al 1997). Because it does not require a specialized leaf anatomy, *Hydrilla* has been proposed as a model for engineering a C4 cycle-based CO₂ concentrating mechanism (CCM) in rice (Edwards 1999).

An important factor when considering molecular engineering of rice is the inexorable rise in atmospheric CO₂, along with the attendant, but less predictable, increases in temperature and changes in precipitation patterns. Anthropogenic CO₂ production is concentrating CO₂ in the atmosphere and at the Rubisco fixation site of C3 plants. This resembles the CCM of C4 species and could mitigate the need for a C4 system in rice. Consequently, in this paper, we review the responses of rice when grown season-long at elevated CO₂ and temperatures, as might be experienced in future global climate change scenarios. We also describe the components of the *Hydrilla* CCM and discuss whether a similar "minimalist" C4 system in rice could achieve higher-yielding cultivars.

Rice responses to growth under elevated CO₂ and temperature

Several studies have examined how rice responds when it is grown at elevated CO₂ and/or temperature (reviewed by Horie et al 2000, Sage 2000, Gesch et al 2001). As with other C3 plants, it is well established that rice responds positively to CO₂ in terms of photosynthesis, growth, and yield. This is mainly attributable to the competition between CO₂ and O₂ for the active site of Rubisco (Bowes and Ogren 1972). Elevated CO₂ reduces O₂ inhibition and the oxygenase activity of Rubisco, which then reduces the loss of photorespiratory CO₂ and thereby enhances net CO₂ assimilation. Baker et al (1990a,b) conducted a rather rare dose-response study, in which rice (cv. IR30) was grown

season-long under subambient, ambient, and superambient CO₂, from 160 to 900 flbar, in outside sunlit chambers that mimicked agricultural paddy-culture conditions. Canopy photosynthesis rates rose as CO₂ increased, but leveled off at about 500 flbar, while water-use efficiency (WUE) continued to increase up to 900 flbar (Baker et al 1990a). Concomitantly, shoot and root biomass, tillering, and grain yield increased, but again only up to about 500 flbar CO₂, even though NPK fertilizer was applied regularly to minimize photosynthetic acclimation (Baker et al 1990b). These results for rice differ from those of soybean (*Glycine max*), which continued to respond positively up to 990 flbar CO₂ (Gesch et al 2001). Ziska et al (1996) have shown in pot experiments that rice cultivars differ in the degree to which CO₂ and temperature influence growth and yield. Even so, by extrapolating from several data sets, Horie et al (2000) concluded that an overall 30% increase in rice grain yield is a reasonable estimate with a doubling in CO₂. However, the gains caused by such a rise still fall short of what are required to keep pace with projected population increases in the next half century.

The major reason why IR30 rice has minimal increases above 500 flbar CO₂ is because Rubisco amount and activity are down-regulated in a linear fashion with increasing growth in CO₂ (Rowland-Bamford et al 1991). Thus, gains from more substrate CO₂ are negated by the loss of Rubisco capacity. However, in an optimization process, the ability to handle the extra leaf carbohydrate may be aided by the upregulation of enzymes such as sucrose phosphate synthase (Hussain et al 1999). In contrast to rice, soybean typically exhibits little acclimation in photosynthesis and Rubisco activity, possibly because it is able to create sinks for the additional photosynthate (Campbell et al 1988, Gesch et al 2001).

Elevated temperatures can increase the CO₂ enhancement of C₃ photosynthesis and growth because Rubisco's oxygenase activity and photorespiration increase markedly with temperature (Long 1991). In the case of soybean, doubling the CO₂ enhanced photosynthesis linearly from 32% to 95% over a growth temperature range of 28 to 40 °C (Vu et al 1997). In contrast, rice (cv. IR72) did not follow the theoretical pattern; the CO₂ enhancement was relatively constant at about 60% from 32 to 38 °C, but clearly CO₂ enrichment can offset to some extent negative effects of high temperatures on rice photosynthesis (Vu et al 1997). However, this benefit does not extend to reproduction. Even though rice photosynthesis has a high temperature optimum for a C₃ plant (35 °C), high temperatures are deleterious to its reproductive processes and cause reduced grain yields even under elevated CO₂. Yields of an indica rice declined by 10% for every 1 °C over 26 °C, reaching zero at 36 °C, and similar outcomes occur with other cultivars, including japonica types (Baker and Allen 1993, Ziska et al 1996, Horie et al 2000). If temperatures rise in rice-growing regions, especially during the flowering period, sterility, abortion, or poor grain-fill will become serious problems that cannot be solved by engineering a C₄ photosynthetic system into the plant.

Elevated CO₂ and temperatures have opposing effects on water use. The WUE of a rice crop is enhanced as CO₂ increases, mainly because assimilation is greater, but evapotranspiration is also lower because of decreased stomatal conductance (Rorie et al 2000). The difference in WUE because of a doubling of CO₂ can be as much as 26% to 50% (Nakagawa et al 1997, Vu et al 1998). Furthermore, CO₂ enrichment delays the effects of severe drought on rice plants and minimizes a drought-induced down-regulation of Rubisco amount and activity that would otherwise impair CO₂ assimilation (Vu et al 1998). However, air temperatures above 26 °C diminish these enhancement effects of CO₂ on rice WUE (Horie et al 2000). The positive effects of CO₂ enrichment on water use by rice would be most obvious in upland regions, less so for paddy-grown crops.

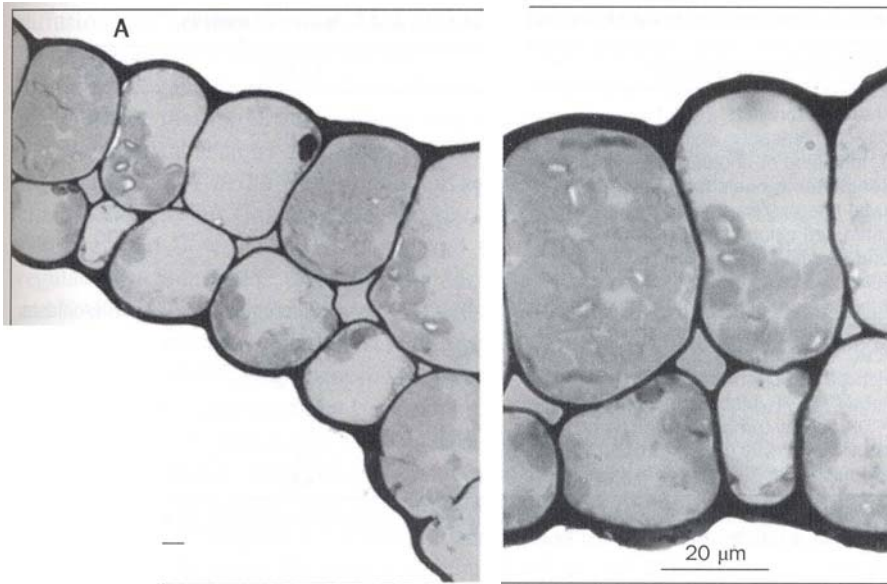
Same-cell (Kranz-less) C₄ systems

Until recently, it was considered dogma that C₄ photosynthesis functioned fully only when Kranz anatomy was present, with the carboxylases and decarboxylase segregated in mesophyll and bundle sheath cells to prevent futile CO₂ cycling. That view has now changed. Since our discovery of the *Hydrilla* C₄ system, other Kranzless C₄ systems have been reported. There is substantial evidence for a C₄-based CCM in the marine macroalga *Udotea flabellum*, derived from techniques using gas exchange, enzymes, and isotopic labeling, and inhibitor studies (Reiskind and Bowes 1991). The marine diatom *Thalassiosira weissflogii* may perform C₄ photosynthesis in a single cell (Reinfelder et al 2001), but it is not established if it concentrates CO₂ for Rubisco. The presence of C₄ photosynthesis in organisms other than angiosperms indicates that its origins predate them.

There are reports of C₄ photosynthesis in freshwater monocots that lack Kranz anatomy, in addition to *Hydrilla*, including *Egeria densa* (Casati et al 2000) and *Sagittaria subulata* (Bowes et al 2002). The grasses (Poaceae) contain more than 60% of C₄ species (Sage 2001) and in almost all the C₄ cycle is associated with Kranz anatomy. Aquatic *Orcuttia* species are an exception; they are C₄ NADP-ME species, as evidenced by ¹⁴C-pulse-chase labeling and enzyme studies, but the submersed leaves lack Kranz anatomy (Keeley 1998). The sedges (Cyperaceae) also contain a substantial number of C₄ Kranz species (Sage 2001), but one, *Eleocharis acicularis*, has Kranz-less submersed leaves that produce C₄ acids. Whether this constitutes a C₄-based CCM remains to be seen.

The ability to perform C₄ photosynthesis in the absence of Kranz anatomy is not confined to submersed leaves. The

terrestrial halophyte *Borszczowia aralocaspi* (Chenopodiaceae) lacks Kranz anatomy but is a C₄ NAD-ME species, on the basis of $\delta^{13}C$, gas exchange, enzyme activity, and localization studies (Voznesenskaya et al 2001). Its carboxylases and decarboxylase are spatially separated in a cell containing dimorphic chloroplasts. Similarly, the terrestrial plant *Bienertia cycloptera* is described as a facultative C₄ system that operates with the Calvin cycle in the same chlorenchyma cells (Freitag and Stichler 2002).



60 μm

Fig. 1. Light micrograph of a cross section of part of a *Hydrilla verticillata* C₄ leaf. (A) Lamina (which is the major photosynthetic region) and part of the midrib (which lacks Kranz anatomy and contains few chloroplasts). (B) An enlargement of part of the lamina to show the many small chloroplasts in both the adaxial and lower abaxial cells.

The facultative C₄ system of *Hydrilla*

Hydrilla verticillata is a tropical submersed monocot that was introduced as an aquarium plant into Florida around 1960, but it escaped, and its rapid distribution and growth soon made it a major weed problem. *Hydrilla* has a small leaf, about 14 mm long X 3 mm wide, with a single vein (midrib), and there is no differentiation into mesophyll and bundle sheath cells (Kranz anatomy) as normally occurs in a C₄ plant. The anatomy of part of a C₄ *Hydrilla* leaf is shown as a cross section in Figure 1. The C₃ leaf (not shown) is essentially the same. The lamina, where photosynthesis occurs, is composed of just two cell layers (Fig. 1A). The upper adaxial layer has larger cells, with more prominent vacuoles, than the lower, abaxial layer. There are no stomata. The midrib is only 5-6 cells thick and part of it is shown at the right of Figure 1A. It contains relatively few chloroplasts compared with the lamina that extend on either side of it. The lamina adaxial and abaxial cells contain many small chloroplasts (Fig. 1B), like terrestrial leaf cells, and not one or a few large chloroplasts as found in many algae with CCMs. The chloroplasts in both C₃ and C₄ leaves are granal and presumably have a competent PSII that produces NADPH. Immunocytochemical gold labeling shows that the adaxial and abaxial cells contain Rubisco in their chloroplasts and PEPC in the cytosol (Reiskind et al 1989). Thus, the C₄ and Calvin cycles are not segregated between the adaxial and abaxial cells; instead, the

Table 1. Photosynthesis-related characteristics of C₃ and C₄ leaves of *Hydrilla*.

Parameter and (reference) ^a	C ₃ leaf	C ₄ leaf
Kranz anatomy (1)	None	None
CO ₂ compensation point (μbar) (2)	>40	<10
O ₂ inhibition of photosynthesis (%) (2)	>28	<5
Net photosynthesis rate (μmol mg ⁻¹ Chl h ⁻¹) (2)	5	15
Ratio internal/external inorganic carbon (3)	0.8	4.2
Estimated chloroplast CO ₂ (μM) (3)	7	400
Leaf abaxial surface (pH) (3)	3.6	4.7
Rubisco activity (μmol g ⁻¹ fresh wt h ⁻¹) (4)	45	36
Rubisco location: adaxial and abaxial cells (1,4)	Chloroplasts	Chloroplasts
PEPC activity (μmol g ⁻¹ fresh wt h ⁻¹) (4)	<10	>150
PEPC location: adaxial and abaxial cells (1,4)	Cytosol	Cytosol
Induced PEPC isoform (5)	None	<i>Hvpepc4</i>
Ratio PEPC/Rubisco activity (4)	0.2	>4.2
PPDK activity (μmol g ⁻¹ fresh wt h ⁻¹) (2)	3	35
NADP-ME activity (μmol g ⁻¹ fresh wt h ⁻¹) (2)	16	44
¹⁴ C pulse-chase: 50% mal + asp turnover(s) (6)	None	<180

^a(1) Reiskind et al (1989), (2) Salvucci and Bowes (1981), (3) Reiskind et al (1997), (4) Magnin et al (1997), (5) Rao et al (2002), (6) Salvucci and Bowes (1983b).

compartmentation is between the chloroplasts and the cytosol (Magnin et al 1997, Reiskind et al 1997). *Hydrilla* represents a "minimalist" C₄ system and, even though the vast majority of C₄ plants exhibit Kranz anatomy, this feature is not a sine qua non for C₄ photosynthesis; neither does it differentiate terrestrial from submersed C₄ plants (Bowes et al 2002).

Terrestrial C₄ species are obligate, whereas *Hydrilla* has a facultative C₄ system. From ecological, physiological, and biochemical perspectives, *Hydrilla's* C₄ photosynthesis is one of the most thoroughly documented and is clearly in the C₄ NADPME subgroup, not in the C₃-C₄ intermediate or CAM categories. Evidence that it is a facultative C₄ plant, despite its lack of Kranz anatomy, is shown in Table 1, which compares some characteristics of C₃ and C₄ *Hydrilla* shoots. C₄ photosynthesis is induced by low CO₂, which in nature usually occurs in dense vegetation in summer (Holaday et al 1983, Spencer et al 1994), but is also inducible in the laboratory. Thus, shoots with previously high, C₃-like CO₂ compensation points (an indicator of substantial photorespiration) exhibit low, C₄-like values (Table 1). Concomitantly, the % inhibition of photosynthesis declines and net photosynthesis at limiting CO₂ increases. The photosynthetic temperature optimum is high (35 DC), as in other C₄ species (Bowes and Salvucci 1989). Perhaps the best evidence that the system is an effective CCM comes from measurements of internal inorganic-C in the leaves (Table 1). With an external solution of 0.5 mM, the inorganic-C in C₃ leaves was 0.4 mM, but it was fivefold higher (2.1 mM) in C₄ leaves (Reiskind et al 1997), akin to the situation in terrestrial C₄ leaves (Furbank and Hatch 1987). The high CO₂ is mainly confined to the C₄ leaf chloroplasts and is far greater than in the surrounding medium. The CCM is able to operate in a low pH medium, where HCO₃⁻ is negligible, showing that it is independent of the uptake of HCO₃⁻. In fact, the active secretion of H⁺ causes the abaxial leaf surface to become acidified (Table 1), which facilitates the diffusion of CO₂ into the leaf. Thus, *Hydrilla's* CCM is not located at the plasma membrane nor is it dependent on HCO₃⁻ use (Reiskind et al 1997). Consequently, dissolved CO₂ enters *Hydrilla* leaf cells, which is the same situation as in terrestrial plants whose mesophyll cell walls are wet with an acidic solution.

Biochemical changes accompany physiological changes. C₄ cycle enzyme activities increase in *Hydrilla* leaves concomitant with the induction of C₄ gas exchange characteristics, but Calvin cycle and photorespiratory enzymes do not increase (Holaday and Bowes 1980, Salvucci and Bowes 1981). C₄ enzymes that are upregulated include FEPC, NADP-malic enzyme (NADP-ME) and NAD-ME, NAD and NADP malate dehydrogenases (MDH), aspartate and alanine aminotransferases, pyrophosphatase, adenylate kinase, and pyruvate orthophosphate dikinase (PPDK). An activity of PPDK equivalent to the photosynthetic rate is significant as it plays a major role in the light-dependent conversion of pyruvate to PEP in C₄ photosynthesis. In terms of a decarboxylase, NADP-ME increases tenfold during C₄ induction (Salvucci and Bowes 1981, Magnin et al 1997). Western analysis time courses for PEPC, NADP-ME, and PPDK show that these enzyme proteins increase as the C₄ cycle is induced (Magnin et al 1997). There is a 10- to 15-fold increase in FEPC activity, which becomes the predominant carboxylase, while Rubisco is essentially unchanged and the PEPC:Rubisco activity ratio increases 20-fold (Table 1).

We have recently identified a specific FEPC isoform (*Hvpepc4*) whose transcript is induced as the leaf becomes C₄, and which is responsible for the initial photosynthetic carboxylation reaction (Rao et al 2002). The FEPC activities in C₄ and C₃ *Hydrilla* leaf extracts exhibit very different properties. In C₄ extracts, FEPC is light-activated *in vivo*, with daytime values 53% higher than at night, and it thus resembles a terrestrial C₄-PEPC isoform (Bowes et al 2002). Sensitivity to malate inhibition is also less during the day. In contrast, FEPC in C₃ leaf extracts shows no light activation and little malate inhibition day or night. Unlike *Hvpepc4*, another PEPC isoform (*Hvpepc3*) may have an anapleurotic

function in *Hydrilla* leaves or recycle respiratory CO₂ at night.

For C₄ *Hydrilla* leaves, malate and aspartate are major products initially labeled with ¹⁴C in the light, and, as Table I indicates, the label is rapidly turned over into Calvin cycle compounds and carbohydrates (Holaday and Bowes 1980, Salvucci and Bowes 1983b). These kinetic characteristics, like those of a terrestrial C₄ plant, demonstrate that C₄ acids are photosynthetic intermediates. In contrast, C₃ *Hydrilla* leaves do not turn over ¹⁴C-malate during a 2-h chase period. Carbon flow through photorespiratory compounds is reduced in C₄ leaves, which is consistent with an effective CCM (Salvucci and Bowes 1983b).

Under inorganic carbon-limited conditions, the C₄ shoots have ¹⁴C-insensitive relative growth rates that are up to 60% greater than those of their C₃ counterparts, and higher photosynthetic nitrogen-use efficiency (Spencer et al 1994). These features are like those of terrestrial C₄ plants. However, the C₄ cycle has an energy cost, perhaps more than a terrestrial C₄ plant. Field studies suggest that the quantum yield may be half that of the C₃ shoots (Spencer et al 1994).

Will a C₄ system be effective in rice?

Most researchers agree that a well-designed and optimized C₄ system in rice could increase CO₂ assimilation, biomass production, and grain yield. It should also improve crop water, and possibly nitrogen, use efficiency. The same is true for rising atmospheric CO₂. However, extrapolating from the current increase of about 2 Ilbar CO₂ y⁻¹, atmospheric CO₂ would be around 470 Ilbar by 2050, which is insufficient to saturate the photosynthesis of C₃ rice and increase yields to satisfy the projected needs. Moreover, if temperatures rise, an engineered C₄ system may also show positive responses to [CO₂], especially in dry upland areas where stomatal conductance is reduced (Sage 2000). Adverse temperature effects on reproduction need to be examined, but this important subject is separate from attempts to engineer a more effective CO₂ assimilation system.

The evidence from *Hydrilla* and *Borszczowia*, that C₄ systems can function without Kranz anatomy, increases the likelihood that a minimalist system could be realized in rice. However, several subjects need to be examined when creating such a system, some of which are described as follows.

It has been suggested that the *Hydrilla* C₄ CCM may not work in a terrestrial plant if the diffusion resistance of water around the submersed leaf retards leakage of the high internal CO₂. But this ignores the observation that the high CO₂ is in the chloroplasts, not in the cytosol or at the cell surface where the aqueous resistance would be operative (Reiskind et al 1997). The "same-cell" C₄ systems in terrestrial plants also undermine the "aqueous resistance" argument. However, a valid criticism is that we do not know how *Hydrilla* chloroplasts minimize CO₂ leakage, especially given that, as in terrestrial plants, they are small and mobile, with a greater surface area than a single, large chloroplast. Until this is resolved, it is difficult to predict whether rice mesophyll chloroplasts could retain a high CO₂ and prevent excessive leakage and futile cycling.

The FEPC reaction produces OAA, but malate and aspartate are the stable products observed in studies with *Hydrilla*. It is important to identify the major C₄ acid transported into the chloroplast. This is because *Hydrilla* chloroplasts, unlike the bundle-sheath chloroplasts of most NADP-ME C₄ plants, have grana like those of rice, and presumably produce NADPH. The direct import of malate, as in other C₄ NADP-ME species, could decrease the NADP⁺/NADPH ratio and exacerbate photoinhibition. In contrast, if OAA is imported, the coupling of the NADPH-MDH and NADP-ME reactions in the chloroplast could recycle NADPH. This would reduce the potential for photoinhibition and eliminate the need to down-regulate PSII activity. *Hydrilla* is not especially prone to photoinhibition, which lends weight to the argument that OAA and/or aspartate, rather than malate, are the major imported acids. We are searching for chloroplast transporters that may be induced along with *Hydrilla's* C₄ system. An up-regulated chloroplast transporter may be crucial to direct C₄ acids produced in the cytosol to the chloroplasts, rather than to the mitochondria where they would fuel a futile cycle.

Carbonic anhydrase (CA) catalyzes the interconversion of HCO₃⁻ and CO₂ in the stroma of C₃ chloroplasts. However, preliminary data suggest that it may not be present in *Hydrilla* chloroplasts, just in the cytosol. At a stromal pH of 8, the CA-catalyzed conversion of CO₂ to HCO₃⁻ should compete with Rubisco for CO₂ and be counterproductive for the *Hydrilla* CCM. This concept needs to be verified, but, if true, it may require that the stromal CA be down-regulated in rice by antisense technology to maximize the effectiveness of a C₄ CCM.

Attention must be given to Rubisco and its activase because they can be ratelimiting in a C₄ system (von Caemmerer et al 1997, Spreitzer and Salvucci 2002). In C₄ plants, Rubisco typically has a higher K_m(CO₂) and 20% to 50% greater turnover capacity (k_{cat}) than in C₃ species. The C₄ CCM enables Rubisco to operate closer to CO₂ saturation and places a premium on maximum rate, rather than on CO₂ affinity. A high k_{cat} enables rapid photosynthesis rates to be maintained with less Rubisco protein, which enhances the nitrogen-use efficiency. The downside is that the C₄ leaf may have less nitrogen available for remobilization and this might affect seed yield (Sage 2000). Rice Rubisco, with its lower k_{cat} may be limited in its ability to respond to a C₄-based CCM. In addition, if Rubisco protein and activity are down-regulated when a CCM is inserted, as they are when IR30 rice is grown at elevated atmospheric

CO₂, then this would further reduce the effectiveness of a C₄ system. It is worth noting that *Hydrilla* Rubisco shows minimal down-regulation when the C₄ cycle is induced (Magnin et al 1997). A reduction in leaf Rubisco protein might also compromise the subsequent protein content of the rice grain, which is already lower than that of other grains. The acclimation response may be linked to sink limitations, in which case the best candidates for a transgenic C₄ system would be cultivars that have the capacity to handle the additional carbohydrate loading. They could be selected from plants that exhibit the least acclimation of Rubisco when grown at twice-ambient atmospheric CO₂ with natural sunlight and adequate nitrogen.

Various isoforms of PEPC are found in C₃, CAM, and C₄ species, which differ in function and in regulatory and kinetic characteristics (Chollet et al 1996). We have found several isoforms in *Hydrilla*. Curiously, phylogenetic sequence analyses indicate that all the *Hydrilla* isoforms resemble "C₃" rather than other monocot "C₄" forms (Rao et al 2002). However, one of them, *Hvpepc4*, is inducible and functions as the photosynthetic isoform (Rao et al 2002). A C₄ signature serine residue (Ser770 or -772) that is ubiquitous in other C₄ isoforms, and influences the kinetics of PEPC, is absent from *Hvpepc4* and is replaced by alanine, which is typical of C₃ sequences (Rao et al 2002). We are investigating the regulation and kinetics of PEPC to determine how a "C₃" sequence can function in C₄ photosynthesis. Similarly, the decarboxylase in *Hydrilla* and *Egeria*, NADP-ME, resembles kinetically and in molecular mass a nonphotosynthetic isoform (Casati et al 2000, Bowes et al 2002). A detailed understanding of how specific isoforms of key enzymes are tailored to function in *Hydrilla*'s C₄ photosynthesis should enable us to better predict the properties needed for these components to operate optimally in a C₄ rice system.

In summary, the CO₂ component of climate change will be positive for rice yields, whether rice is upland- or paddy-grown. In contrast, if the predicted increases in air

References

temperatures occur, their detrimental effects on reproduction must be countered. However, even if they can be offset, rising atmospheric CO₂ over the next 25 to 50 years will not increase rice yields enough to meet the predicted needs, especially given the substantial Rubisco acclimation that rice displays; better-yielding cultivars will still be required. Improved cultivars may be engineered to have less downregulation of Rubisco, a Rubisco that has a greater specificity for CO₂ relative to O₂ (Evans and von Caemmerer 2000), or a C₄ system similar to that of *Hydrilla* (Bowes et al 2002).

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Notes

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