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PERSPECTIVE

The Development of C$_4$ Rice: Current Progress and Future Challenges

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Another "green revolution" is needed for crop yields to meet demands for food. The international C$_4$ Rice Consortium is working toward introducing a higher-capacity photosynthetic mechanism—the C$_4$ pathway—into rice to increase yield. The goal is to identify the genes necessary to install C$_4$ photosynthesis in rice through different approaches, including genomic and transcriptional sequence comparisons and mutant screening.

As the world population races toward 10 billion, agricultural scientists are realizing that another "green revolution" is needed for crop yields to meet demands for food. In rice, yield potential is limited by the photosynthetic capacity of leaves that, as carbohydrate factories, are unable to fill the larger number of florets of modern rice plants. One potential solution is to introduce a higher-capacity photosynthetic mechanism—the C$_4$ pathway—into rice. This is the goal of researchers in the international C$_4$ Rice Consortium: to identify and engineer the genes necessary to install C$_4$ photosynthesis in rice (1).

Rubisco, the primary CO$_2$-fixing enzyme in rice, is a poor catalyst of CO$_2$ at current atmospheric conditions. It has a tendency of confusing its substrate CO$_2$ with the more abundant O$_2$ as well as being a very slow catalyst of CO$_2$, turning over only once or twice per second. Rubisco's oxygenase activity requires the recycling of phosphoglycolate in the photorespiratory pathway, resulting in an energy cost and loss of previously fixed CO$_2$. Many photosynthetic organisms, including cyanobacteria, algae, and land plants, have developed active CO$_2$-concentrating mechanisms to overcome Rubisco's inefficiencies (2). Among land plants, this led to the development of C$_4$ photosynthesis, a biochemical CO$_2$-concentrating mechanism. C$_4$ pho-

in another type of specialized tissue, the bundle sheath cells. This process elevates the CO$_2$ concentration in the bundle sheath and inhibits Rubisco oxygenase activity, allowing Rubisco to operate close to its maximal rate (Fig. 1). In comparison with C$_3$ crops such as rice, C$_4$ crops (such as maize and sorghum) have higher yields and increased water- and nitrogen-use efficiency (1, 4).

Building the C$_4$ Machinery

In an evolutionary context, the transition from C$_3$ to C$_4$ photosynthesis has occurred independently in more than 60 different plant taxa (3). Genomic and transcriptional sequence comparisons of cell-
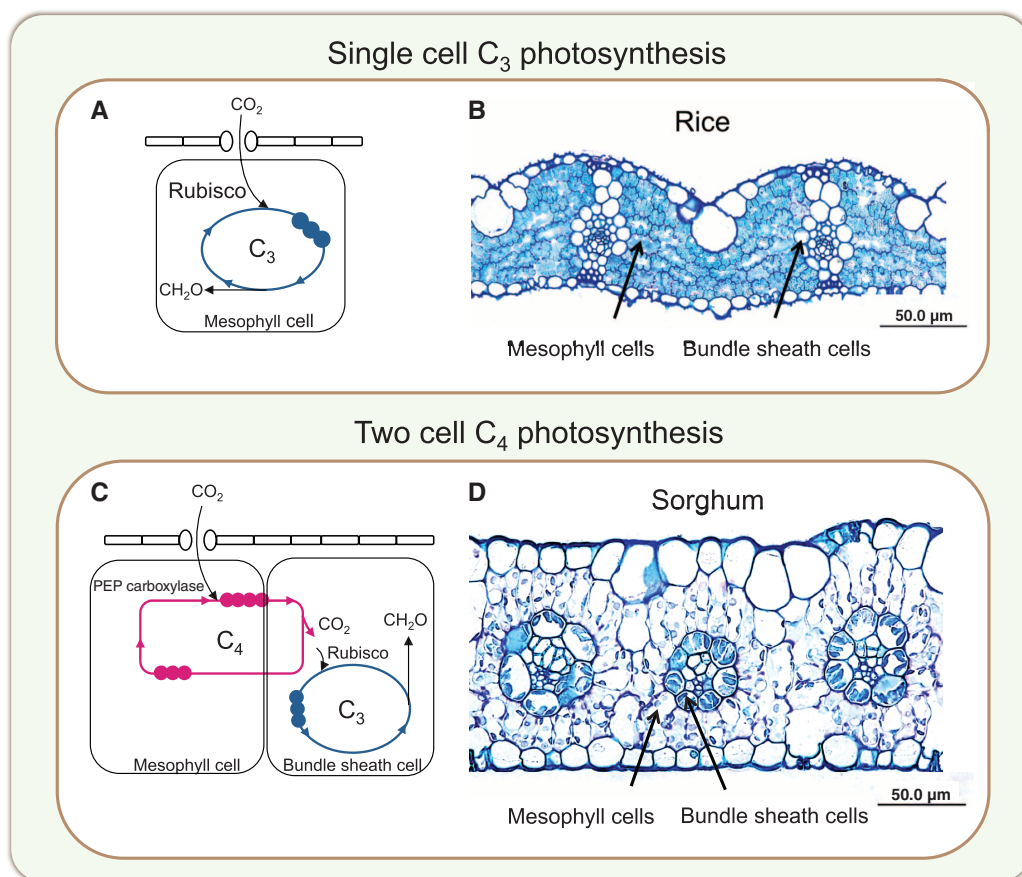

Fig. 1. (A) C$_3$ photosynthesis fixes atmospheric CO$_2$ into C$_3$ acids with Rubisco in single cells. **(C)** Two-cell C$_4$ photosynthesis requires spatial separation of fixation of atmospheric CO$_2$ into C$_4$ acids and the donation of CO$_2$ from these C$_4$ acids to Rubisco. Also shown are light microscopy images of transverse sections of leaves of **(B)** rice, a C$_3$ plant, and **(D)** sorghum, a C$_4$ plant. The rice section shows vascular bundles with few chloroplasts and large numbers of mesophyll cells between the vascular bundles typical for C$_3$ species. The sorghum leaf section shows chloroplasts in bundle sheath and only two or three mesophyll cells in between the vascular tissue typical of a C$_4$ species.

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tosynthesis arose multiple times in the past 60 million years in warm semi-arid regions, with early occurrences coinciding with low atmospheric CO$_2$ in the late Oligocene (3). During C$_4$ photosynthesis, CO$_2$ is fixed within specialized leaf tissues known as mesophyll cells to produce C$_4$ acids, which diffuse to and are decarboxylated

specific and leaf-developmental gradient transcription profiles between closely related C$_3$ and C$_4$ species are being used to identify C$_4$-specific regulatory genes (4). Combining this information in parallel with screens of mutagenized C$_4$ *Sorghum bicolor* and *Setaria viridis* along with activation-tagged rice populations hopefully will
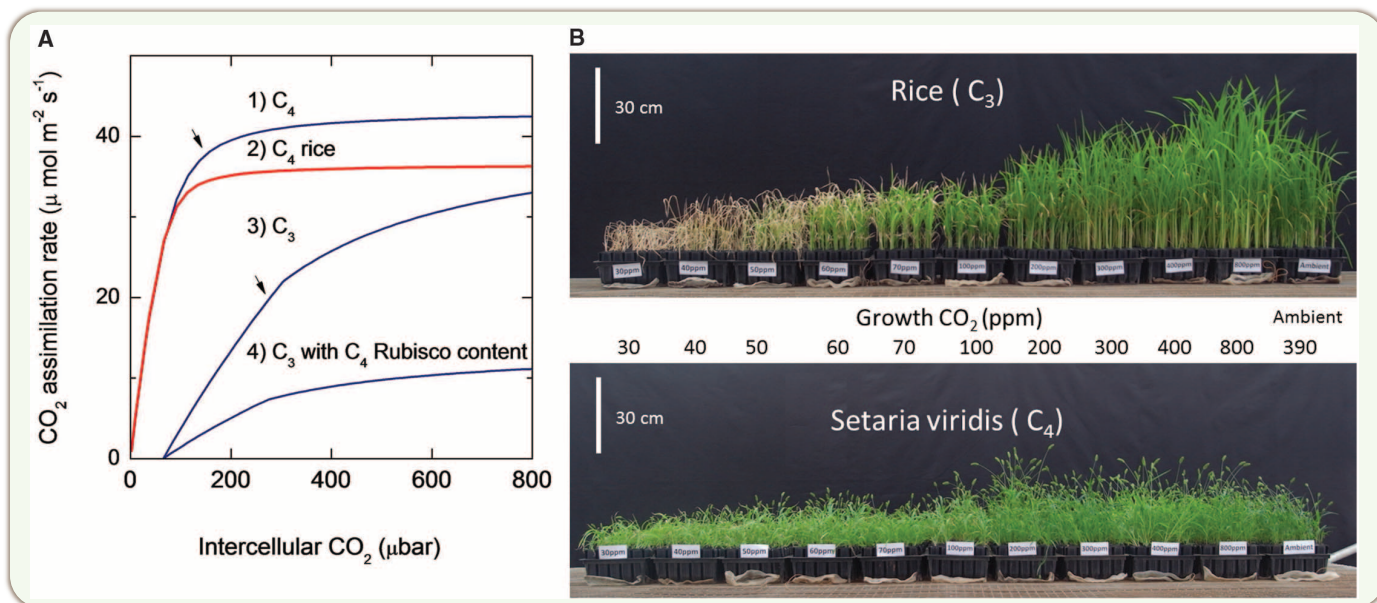
Plant Metabolism

Fig. 2. (A) Modeled changes in CO_2 assimilation rate in response to changes in leaf intercellular CO_2 partial pressure for C_3 and C_4 photosynthesis and for a hypothetical C_4 rice. Curves 1, 2, and 4 have Rubisco levels typically found in a C_4 leaf (10 μmol m^{-2} catalytic Rubisco sites). Curve 3 shows a typical response for C_3 leaves with three times the Rubisco level of C_4 leaves. Curve 1 shows the response of a C_4 leaf with C_4 Rubisco kinetic properties. Curve 2 models how a C_4 leaf with C_3 Rubisco kinetic properties would respond (a hypothetical C_4 rice with C_3 Rubisco kinetics). The comparison of these two curves shows the increase in CO_2 assimilation rate achieved with C_4 compared with C_3 Rubisco kinetic properties within a functional C_4 mechanism. Arrows to curves 1 and 3 show intercellular CO_2 partial pressures typical at current ambient CO_2 partial pressures for C_4 and C_3 photosynthesis. To generate the curves, model equations were taken from (*11*) and comparative Rubisco kinetic constants from (*12*). **(B)** Growth of 21-day-old rice and *S. viridis* seedlings at different ambient CO_2 concentrations ranging from 30 to 800 parts per million.

reveal candidate genes in the C_3-to-C_4 switch that can be tested in transgenic rice and *S. viridis* (*5*). Because C_4 plants can carry out net CO_2 assimilation at very low CO_2 levels whereas C_3 plants cannot (Fig. 2), we can use growth screens to identify gain of function in activation-tagged rice mutants and loss of function in *S. viridis* mutants (Fig. 2). We are also using the fact that C_4 photosynthesis imparts a distinct carbon isotope signature on dry matter (*6*) in a loss-of-function screen for C_4 mutants.

A subset of genes required for the major biochemical components and metabolite transporters involved in the C_4 pathway have been cloned and coupled to suitable promoters to give cell-specific expression in rice (*7*). Attempts to install C_4 photosynthesis in plants lacking the appropriate anatomy show that a biochemical approach alone will not be enough (*8*). Bundle sheath cells in rice are smaller than in C_4 plants and have less chloroplasts, and there are a large number of mesophyll cells between vascular bundles (Fig. 1) (*4*). Promising mutants have been identified in rice that show reduced vein spacing. Combined with studies of sorghum, we are optimistic that we will be able to identify the genes controlling this aspect of anatomy (*4*, *7*).

Lessons Learned and Future Challenges

Although C_4 leaves have close veins and high rates of photosynthesis, C_4 photosynthesis is also

naturally supported around widely spaced veins in maize husk tissue, albeit at lower rates (*6*). Thus, a prototype C_4 rice may be achievable with a subset of C_4 genes, but a "good" C_4 rice will require substantial fine tuning of biochemistry and anatomy. Particularly intriguing is the need for additional metabolite transport across membranes of organelles in C_4 photosynthesis (*4*). A functional C_4-concentrating mechanism in rice would allow for an approximately two-thirds reduction in Rubisco levels, relative to wild-type rice, but Rubisco would be sequestered in bundle sheath cells and ideally have a greater catalytic turnover rate (Fig. 2) (*2*). Antisense gene suppression of key photosynthetic enzymes has illuminated C_4 metabolism and engineering strategies, including the surprising find that phosphorylation of phosphoenolpyruvate (PEP) carboxylase by the regulatory enzyme PEP carboxylase phosphokinase is not needed for C_4 function (*9*). With the adoption of the C_4 model plant *S. viridis*—with its short life cycle, small stature, and genome size—along with advances in efficient transformation, we anticipate that much more will soon be learned (*5*). We expect to have a C_4 rice prototype within 3 years. However, we estimate that another 15 years of research are required for optimization of the phenotype and field testing for C_4 rice to become ready for cultivation in farmers' fields.

Norman Borlaug's green revolution was based on just a handful of genes (*10*). However, the

need for even greater food plant production looms. The promise of C_4 rice has resulted in one of the largest consortia of plant biologists pursuing a common goal. We optimistically take on this challenge, anticipating that advances in our understanding of plant metabolism, and C_3 and C_4 photosynthesis in particular, will better serve humanity in years to come.

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