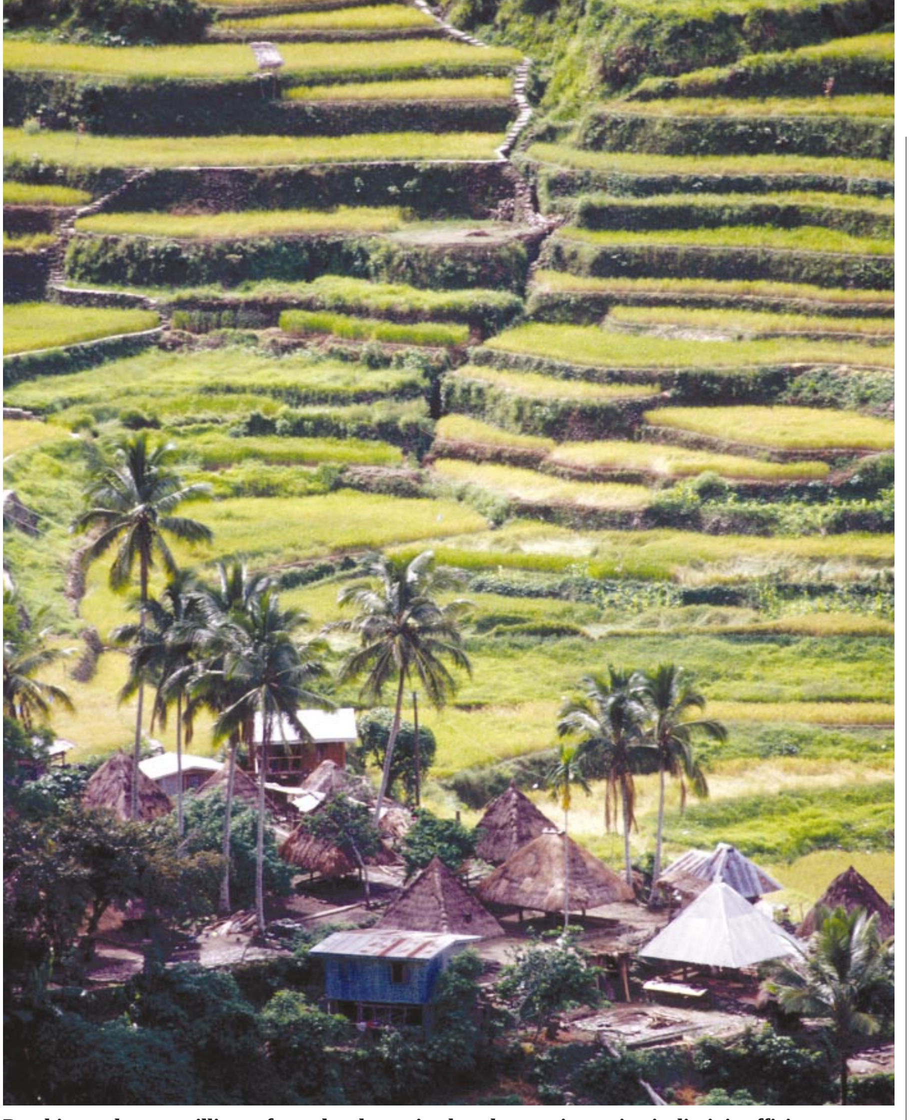
news feature

The rice squad

Feeding the world in the twenty-first century could require a second green revolution. But that may involve the most audacious feat of genetic engineering yet attempted, says Christopher Surridge.



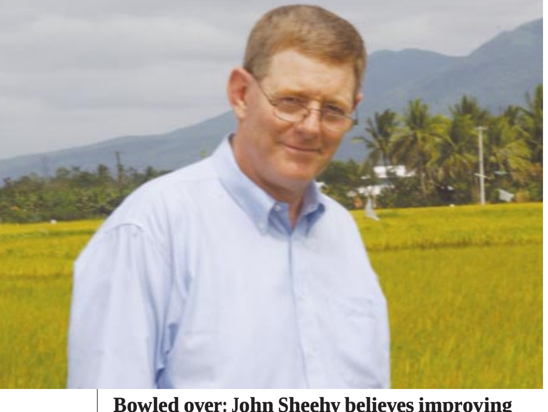
A. JAVELLANA/IRRI

Reaching a plateau: millions of people rely on rice, but the crop is nearing its limit in efficiency.

First, some sobering facts and figures: over the next 50 years, the world's population is expected to increase by a third, to some 8 billion. Much of this rise will be in southeast Asia, where half the population is already poorly nourished.

Global production of rice (*Oryza sativa*), the world's most important staple crop, has risen threefold over the past three decades — and if similar progress could be maintained, there should be enough food to meet the rising demand. But here's the rub: yields are fast approaching a theoretical limit set by the crop's efficiency in harvesting sunlight and using its energy to make carbohydrates.

So to feed the world, rice may have to be re-engineered at the biochemical level. "The only way to increase yields and reduce the use of nitrogen fertilizers is to increase photosynthetic efficiency," argues John Sheehy, an ecologist at the International Rice Research Institute near Manila, the Philippines, who specializes in modelling crop yields. He calculates that a boost of 20% should do the trick.



A. JAVELLANA/IRRI

Bowled over: John Sheehy believes improving rice photosynthesis is vital for food production.

Easily said, rather more difficult to do.

The first green revolution, which began in the late 1960s, depended on dwarf varieties of rice, wheat and maize that, put simply, made more grain for less stem. It has since been shown that this relied on breeding mutants that do not produce, or fail to respond to, the plant hormone gibberellic acid.

Grain power

Now we can add the techniques of genetic engineering to the old methods of mutation and traditional plant breeding. The modified crops created so far generally involve the addition of a single gene — for a natural insecticide, say, or resistance to a herbicide. For geneticists, this is like improving a car's performance by adding an aerodynamic spoiler or changing the tyres. But boosting a plant's photosynthetic efficiency will involve manipulating a whole suite of genes. By the same analogy, it's like supercharging a car's engine by fitting a new fuel injection system.

Thankfully, evolution provides plant scientists with precedents. On at least 30 separate occasions[1], different plant lineages have evolved to use the Sun's energy more efficiently, making sugars in a two-stage process known as C_4 photosynthesis. Exactly when this trick first evolved is unknown, but from about 10 million years ago, falling concentrations of carbon dioxide in the atmosphere gave plants using C_4 photosynthesis an important selective advantage. The ancestors of maize were among these plants. But rice, wheat and most other cereals all use conventional C_3 photosynthesis.

The terminology refers to the number of carbon atoms in the first molecule created when atmospheric CO_2 is assimilated by the plant. In C_3 plants, CO_2 reacts with ribulose 1,5-bisphosphate (RuBP), catalysed by an enzyme called ribulose 1,5-bisphosphate carboxylase–oxygenase (Rubisco). The product is phosphoglycerate, an organic molecule with a backbone of three carbon atoms, which is then used to build sugars and more complex carbohydrates (see diagram, opposite).

In C_4 plants, CO_2 is assimilated using a different enzyme — phosphoenolpyruvate carboxylase (PEPC). This attaches CO_2 to the three-carbon compound phosphoenolpyruvate (PEP) to produce oxaloacetate, a molecule that contains four carbon atoms. Oxaloacetate can be converted into a series of other four-carbon products, including malate, citrate and aspartate. These four-carbon molecules are broken back down into three-carbon compounds and CO_2, which is then fed to Rubisco.

This two-stage process is more efficient because it allows C_4 plants to boost the levels of CO_2 reaching Rubisco. PEPC has a stronger affinity for CO_2 than Rubisco, and so can work better at low concentrations of the gas. In addition, Rubisco is inhibited by oxygen — an unavoidable product of the light-harvesting stage of photosynthesis — whereas PEPC is unaffected.

C_4 plants shield Rubisco from the debilitating effects of O_2 by packaging the enzyme's activity into specialized cells, isolated from the O_2-producing reactions of photosynthesis. In maize these cells surround a leaf's veins, and are called bundle sheath cells. PEPC operates happily in 'mesophyll' cells, found in the rest of the leaf, immune to the build up of O_2. Four-carbon molecules are then shunted across to the bundle sheath cells, where they are broken down to create locally high concentrations of CO_2. This allows Rubisco to work with maximum efficiency — and has knock-on effects that lessen the waste of nitrogen, used to make amino acids, and of water.

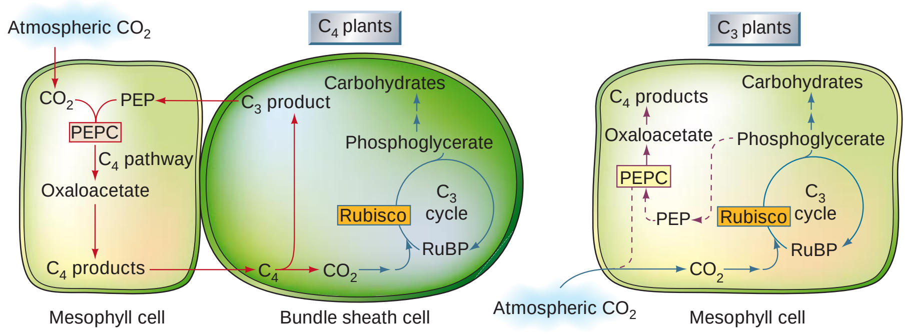
Separate lives

In C_4 plants, the separation of photosynthesis into two pathways operating in different types of cell involves what is dubbed a 'Kranz' anatomy. This is strikingly evident in maize, where alternating bands of mesophyll and bundle sheath cells form stripes running the length of the leaf.

Given that it would involve major changes in both biochemistry and anatomy, turning rice into a typical C_4 plant is no trivial matter. But that hasn't stopped some plant scientists from tinkering with the genes that control photosynthesis reactions in an effort to imbue rice with some C_4 characteristics.

Photosynthesis in maize differs from that in rice in the activity of seven enzymes. Genes for some of these enzymes are present in the *O. sativa* genome — drafts of which were published last week[2,3]. But some of the rice versions — including that for PEPC, which in rice can make oxaloacetate from the products of the C_3 pathway — may not be suitable for bona fide C_4 photosynthesis. So researchers led by Maurice Ku of Washington State University in Pullman are attempting to introduce key genes from maize into rice.

In 1999, Ku collaborated with two Japanese groups, led by Makoto Matsuoka of Nagoya University and Mitsue Miyao of the National Institute of Agrobiological Sciences in Tsukuba. Together, they used *Agrobacterium tumefaciens* — a bacterium that can insert foreign sequences into the genomes of its hosts — to introduce the maize gene for PEPC, including its regulatory sequences, into two cultivars of rice. They found that transformed plants containing multiple copies of the gene produced two or three times as much PEPC as ordinary maize. Encouragingly, photosynthesis in the transformed plants was inhibited less by oxygen than in control rice plants that had not taken up the gene[4].



Supercharged: two-stage C_4 photosynthesis is more efficient than the C_3 version found in most plants.

This was merely the first step. Ku and his colleagues are also working with the maize genes for two other enzymes, pyruvate orthophosphate dikinase (PPDK) and NAD malate decarboxylase. The former is involved in the production of PEP; the latter breaks down four-carbon molecules to feed CO_2 to Rubisco. Ku's team plans to make transgenic rice plants that express each of the three genes, and then interbreed them to incorporate all of the genes into the same plants.

Rice plants expressing either the maize gene for PEPC or PPDK seem to have a photosynthetic capacity about 30% greater than normal plants[5], and in preliminary field trials have produced 12% and 35% more grains, respectively. Ku's team has also made plants expressing both genes. These have double the photosynthetic capacity of normal rice[5] — and, in small-scale field trials in China and South America, they have produced up to 90% more grains. "These plants are also more vigorous and uniform, more tolerant to stress conditions, and flower four to six days earlier than non-transformed controls," claims Ku.

Maurice Ku is trying to add maize genes to rice.

IRRI

Such results make it sound as if the second green revolution is already in full swing. But most experts urge caution, because the gains seen by Ku and his colleagues may actually have little to do with the acquisition of C_4 photosynthesis — instead, they might be a result of improved stress tolerance. The production of PEPC can increase dramatically in normal rice plants grown under stressful conditions. And the most spectacular gains in yield seen in the field trials came in plants grown in the stressful environment of South American soils, which have a high salt concentration and where ultraviolet wavelengths make up a significant proportion of the sunlight.

Ku and his colleagues also note that the introduced genes increase the opening of pores called stomata on the surface of the plants' leaves[4,5] — which would increase the amount of CO_2 available for photosynthesis even in the absence of a functional C_4 pathway. Still missing, say plant scientists, is any biochemical proof that the transgenic plants are concentrating CO_2 at Rubisco using four-carbon molecules. "The evidence is not there yet," says plant physiologist and ecologist Barry Osmond, president of Columbia University's Biosphere 2 Center in Oracle, Arizona. "It comes down to keeping an open mind but not overstating things."

Division of labour

Even if the full complement of C_4 enzymes can be transferred to rice, reaping all the benefits requires PEPC to be kept physically separate from Rubisco to prevent the latter from being inhibited by oxygen — which brings us back to Kranz anatomy. Unfortunately, plant scientists do not yet know how to conjure up this anatomical arrangement. Although the genes for the C_4 photosynthetic enzymes are well known, those responsible for directing the development of Kranz anatomy remain mysterious.

E. KU

But the latest research suggests that C_4 and C_3 plants are more similar than was once thought — which has boosted hopes of finding a simple genetic switch to convert rice to a Kranz anatomy. In January this year, British researchers reported the discovery of C_4 characteristics hidden in tobacco (*Nicotiana tabacum*), a C_3 plant[6]. Julian Hibberd of the

Could a simple genetic switch make rice capable of meeting the world's food needs?

A. JAVELLANA/IRRI

Sowing the seeds: the International Rice Research Institute is backing the effort to boost rice yields.

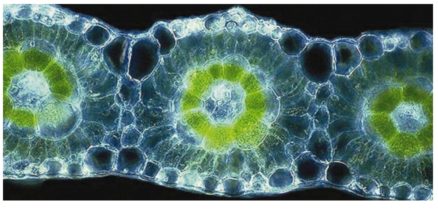
University of Cambridge and Paul Quick of the University of Sheffield studied photosynthetic cells surrounding vessels in tobacco stems — the same type of cells that give the veins of celery stalks their prominent green colour. The researchers found active C_4 enzymes, and showed that the cells broke down malate to release CO_2, just like the bundle sheath cells in maize leaves.

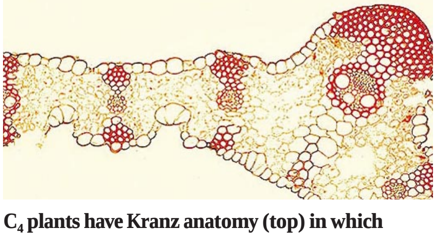
Trigger happy

That even archetypal C_3 plants contain cells functionally equivalent to the bundle sheath cells of maize helps to explain why C_4 photosynthesis has been able to evolve repeatedly. Intriguingly, it also suggests that if plant scientists can find the genetic triggers to induce the cells studied by Hibberd and Quick to develop in rice leaves, it may not even be necessary to engineer in the genes for C_4 enzymes.

There are precedents for finding simple genetic changes that underlie a major anatomical shift. For example, domesticated maize (*Zea mays* ssp. *mays*) arose from a wild relative called teosinte (*Z. mays* ssp. *parviglumis*) in Mexico between 5,000 and 10,000 years ago. Teosinte is a bushy plant with a lot of branching stems, each carrying many male flowers and small female ears. In maize, these side branches have all but disappeared, leaving a single stem with one male flower and several large, grain-bearing cobs. This transformation was achieved largely through changes in a single gene, *teosinte branched1* — mutations in which can restore maize to an ancestral, teosinte-like, appearance[7].

M. RICKETTS/UNIV. SYDNEY





C_4 plants have Kranz anatomy (top) in which different photosynthetic pathways take place in bundle sheath cells (bright green) and mesophyll cells, a distinction not seen in C_3 plants (below).

But what are the genetic switches that have given rise to Kranz anatomy? Studies of maize may again prove useful. Although maize leaves have a well-defined Kranz anatomy and C_4 photosynthesis, other leaf-like organs, such as the husks that wrap around developing cobs, do not. Instead their cells are more homogeneous and use C_3 photosynthesis[8]. Jane Langdale of the University of Oxford suspects that this pattern is the 'ground state', with the C_4 mesophyll and bundle sheath cells being more differentiated variations.

Golden nuggets

Langdale has identified a family of genes, dubbed *G2-like* (*Glk*), that is involved in the development of chloroplasts — the subcellular organelles in which photosynthesis occurs. The genes are named after the first member of the family, *Golden2* (*G2*), which was discovered in maize. Their sequences suggest that they encode proteins that act as transcription factors, regulating the expression of other genes[9].

In maize leaves, *G2* is expressed primarily in bundle sheath cells whereas another member of the family, *ZmGlk1*, is active in mesophyll cells[10]. But in maize husks — and in rice leaves — the two main *Glk* genes are expressed at similar levels in all cells. If the differential expression of *Glk* genes is crucial to establishing Kranz anatomy, it should be possible to identify the genes responsible for controlling the *Glk* genes' activity, and to engineer them into rice.

More exotic plants might provide further clues. The viviparous spikerush (*Eleocharis vivipara*), a leafless sedge that lives in the swamps of Florida, is a C_3 plant when submerged. But when it grows above the water, its stems develop Kranz anatomy and full-scale C_4 photosynthesis[11,12]. Buried in such amphibious plants might be a master switch to turn on C_4 characteristics.

It may not even be necessary to engineer Kranz anatomy into rice. *Hydrilla verticillata* is an aquatic C_4 plant that keeps PEPC and Rubisco separate, not in different cells, but by operating the former in the cytoplasm of mesophyll cells, and the latter in its chloroplasts[13]. *Borszczowia aralocaspica*, which lives in salty depressions in the semi-deserts of central Asia, performs a similar trick by packaging the two enzymes into different chloroplasts[14].

Hydrilla and *Borszczowia* are probably evolutionary halfway houses between typical C_3 and C_4 forms. "This may offer an alternative to engineering C_4 using Kranz anatomy," says Gerald Edwards of Washington State University, who led the team that discovered the compartmentalization in *Borszczowia*. But that still means identifying the genes responsible and engineering rice to develop in a similar fashion.

Given such obstacles, turning rice into a C_4 plant is an endeavour that could take a decade or two. Aside from the scientific challenges, funding could become an obstacle. Japan is the most significant benefactor for rice research, and its largesse will be needed to support efforts to re-engineer the crop's photosynthetic efficiency. But given the present state of the country's economy, there is currently talk of Japan slashing its support for the International Rice Research Institute, which is the natural home for the project.

Many of the non-governmental bodies committed to fighting world hunger, meanwhile, remain vehemently opposed to plant genetic engineering — which they see as a method of enriching multinational companies, rather than a means of achieving food security for developing countries.

But enthusiasts argue that supercharging photosynthesis in rice to achieve a second green revolution might be just the project to change hearts and minds. "This is the next frontier," Sheehy asserts. ■

Christopher Surridge is a senior biology editor at *Nature*.

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