SERGE BLOCH

AGRIBIOTECHNOLOGY

Blue-sky rice

Rice is a staple food, but production is not keeping pace with the rise in global population. So scientists are dreaming big and aiming high to change the future for this crucial grain.

BY LEIGH DAYTON

Researchers looking for the next big thing in rice studies have to deal with all sorts of obstacles, including, surprisingly, salt-water crocodiles. The swathes of wild rice growing in the tropics of northern Australia are thought to harbour game-changing genetics, but they also keep some unpleasant company. "It is a little bit tense," confesses Robert Henry, a plant geneticist at the University of Queensland in Brisbane, Australia, who is also director of the University's Queensland Alliance for Agriculture and Food Innovation. "The crocs might explain why the rice is under-studied."

For Henry and his colleagues, Australia's wild rice is a potential treasure trove, a source of ancient genetic diversity that could hold the key to protecting rice crops worldwide from a fungal disease that happens to be one of rice's biggest natural enemies. The team is not alone in going beyond the basic nuts and bolts of rice research. Worldwide, researchers increasingly recognize that wholesale application of fertilizers, bombardment with fungicides and other conventional approaches to creating bigger, better rice plants are so last century. "We need another green revolution to meet demands for food," says Robert Furbank, a plant physiologist with the Commonwealth Scientific and Industrial Organisation (CSIRO) near Canberra.

Not only are these rice researchers calling for revolution, they are working on one. They are applying the tools of genetics, bioinformatics, plant physiology and even evolution to the task. Plant geneticist Allen Good at the University of Alberta in Canada says that they are thinking visionary science and betting on high-risk research that may or may not pay off, but if it does, it would be, well, revolutionary.

It is definitely time for big ideas. Rice is a world staple, but production is struggling to keep pace with population growth. The agricultural system itself is in peril; climate scientists warn that the shift in the planet's weather systems will make clean water — the stuff of life for rice — increasingly scarce. Sea-level rise is already increasing rice-killing salinity in the deltas of Asian rivers, the richest rice-growing areas of the world. Added to these pressures is the fact that, worldwide, agricultural land is being converted to housing or industrial uses at a breakneck pace.

As Furbank notes, science has come to the rescue before. The first green revolution — conceptually kick-started in the 1940s and 1950s by the late US plant scientist Norman Borlaug and Indian rice geneticist M. S. Swaminathan, founder of the M. S. Swaminathan Research Foundation — delivered higher-yielding strains of rice through selective breeding for useful traits such as plant size and productivity. Combined with public policies that promoted farmer training programmes in developing nations and the widespread application of artificial nitrogen fertilizer, the improved strains doubled wheat and rice yield in Asia and Latin America in the 1960s and 1970s.

So in laboratories, fields and wetlands around the world, teams of scientists are tackling the big challenges of rice research: novel ways to exploit the energy of the Sun; new technologies to help rice plants to kick the fertilizer habit and feed themselves; and dramatically improved resistance against devastating diseases.

PHOTOSYNTHESIS SHIFT

The big idea started over a few beers in the early 1960s. At the time, Australian scientists Hal Hatch and Roger Slack were both employed in the laboratory of the Colonial Sugar Refining Company in Brisbane. The pub-time conversation got around to a mutual curiosity: how did the cane plant produce and store so much sugar? While investigating this seemingly simple question, they made one of the most important discoveries in the history of plant science: the C_4 photosynthetic pathway[1]. This is the process by which cane and nearly one-fifth of all plant species convert inorganic carbon dioxide from the air into an organic compound, sugar (see 'Photosynthesis variants').

The C_4 pathway is more efficient than the alternative C_3 pathway — well-known to Hatch and Slack — because it wastes less light energy when fixing CO_2. And as an added benefit, C_4 plants need less water and nitrogen to fuel the process. In short, C_4 plants do more with less. The C_4 pathway was later found in other crop plants, including maize (corn), millet and sorghum — but not rice.

Scientists reasoned that if they could turn C_3 rice into C_4 rice, more of the crop could be produced, and produced more sustainably, even in a hotter, dryer, more crowded world. The International C_4 Rice Consortium (ICRC), headquartered at the International Rice Research Institute (IRRI) in Los Baños, the Philippines, estimates that the supercharged rice could support yield increases of a remarkable 30–50% with the same amount of water and fertilizer used on current C_3 crops.

As pointed out in 2013 by John Sheehy, former head of the Applied Photosynthesis and Systems Modeling Laboratory at IRRI and the father of the institute's C_4 project, the conversion from C_3 to C_4 occurred in evolution multiple times in the past 60 million years in plants growing in warm, semi-arid regions of the planet. Because rice can thrive in such climates, it is seen to be a good candidate. Sheehy notes that they are imitating nature, not inventing something from scratch.

Still it is not an easy task. "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 twenty-first century," write Sarah Covshoff and Julian Hibberd, scientists at the University of Cambridge, UK[2]. Fortunately, this was recognized early on, not just by Sheehy but by the US-based Bill & Melinda Gates Foundation. To understand the foundation's part in the process, it is necessary to turn the clock back seven years. It was after inviting global experts, including Furbank and Hibberd, to a workshop in 2007 that Sheehy took a group of scientists and a proposal to the philanthropic foundation. The result was a US\$20-million grant and establishment of the ICRC in 2008. Today, 22 teams from 9 nations collaborate on different aspects of the endeavour. "The project aims to enhance photosynthesis in crops like rice and wheat by giving them the genes that allow crops like maize to be so much more productive," says plant physiologist Paul Quick, who took over as project leader in 2009 when Sheehy retired.

According to Quick, there are two key components of the project. First, the identification and introduction of C_4 genes into C_3 plants. "We've already added 6 of the 12 genes we think are necessary," he says. The second involves changing the anatomy of the rice leaf to introduce specialized cells that allow the plant to break down CO_2 in the absence of oxygen, making the process more efficient. Unfortunately, the researchers do not yet know the underlying genetics for this stage. "So we have a gene-discovery programme that uses bioinformatics and mutagenesis to identify those genes," says Quick. A number of candidate genes are currently being put through

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Rice blast disease can decrease yield.

their biochemical and anatomical paces.

What is the timeline for seeing the first crop of C_4 rice planted? Furbank says he expects a prototype crop within three years, but his best guess is that it will take another 15 years before C_4 rice is ready for cultivation in farmers' fields.

DIY FERTILIZER

Rice research is usually associated with year-round warm climates, so an institute in Alberta — where it gets cold, really cold — seems an unlikely place to investigate the food crop. The likelihood of seeing rice flourishing on the prairie soil is as sub-zero as the temperature in winter. Yet scientists at the University of Alberta have taken up another of the big challenges of rice research — boosting the uptake and use of natural nitrogen. If they can meet the challenge, the impact could be on a par with successful C_4 conversion.

When asked, "What are the most important jobs of nitrogen?", Good responds: "You name it. It's the key nutrient." Without nitrogen, plants such as rice will not grow and produce plump grains. The more efficiently plants take up nitrogen from the soil, the higher the crop yield.

There is already one proven solution to a lack of nitrogen in soil: fertilizer. But, write Good and his associate Perrin Beatty, although applying fertilizer "revolutionized crop yield and food production worldwide", the advance came at a "heavy economic and environmental cost"[3]. In terms of cost, over the past 5 years the worldwide price of commercial fertilizer has ranged from US\$0.60 to \$1.00 per kilogram. Given that 80–100 kilograms of fertilizer are typically used on each hectare of rice, that soon adds up. Worse, much of the added nitrogen escapes into the soil and waterways. Because plants take up an average of 30–50% of the total available nitrogen, the excess nitrates can cause environmental damage, including algal blooms on lakes and coastal waters and acidification of soil, which, in turn, makes some land unsuitable for crops. The application of fertilizer also results in the release of nitrous oxide, a potent greenhouse gas.

Good's group started looking at nitrogen largely by accident. While investigating stresses from drought and shortfalls of oxygen, the team discovered that crop plants that over-produce alanine aminotransferase (AlaAT), an enzyme that catalyses the transformation of amino acids, have enhanced nitrogen uptake. The researchers showed[4] that if they insert a barley *AlaAT* gene, and a promoter — a molecular on–off switch — into canola, the plants use nitrogen more efficiently than control plants. The team has expanded its canola work, inserting *AlaAT* into rice and other cereal crops. The group has licensed its technology to agritechnology firm Arcadia Biosciences in Davis, California. Field trials with the enhanced canola showed that it uses two-thirds less nitrogen fertilizer than the conventional variety to generate the same yield.

Arcadia is keeping further findings close to its corporate chest, but Good understands that field trials of rice — as well as barley and wheat — show mixed results. Some plots thrive with minimal fertilizer, whereas others do not. It is likely, Good says, that the plant works differently in different environments, and soil condition could be a key variable. He suspects that the inconsistent results are slowing progress in the field trials. He adds that a negative attitude towards genetic modification in some communities may also be a roadblock to commercialization (see page S55).

> ***"We need another green revolution to meet demands for food."***

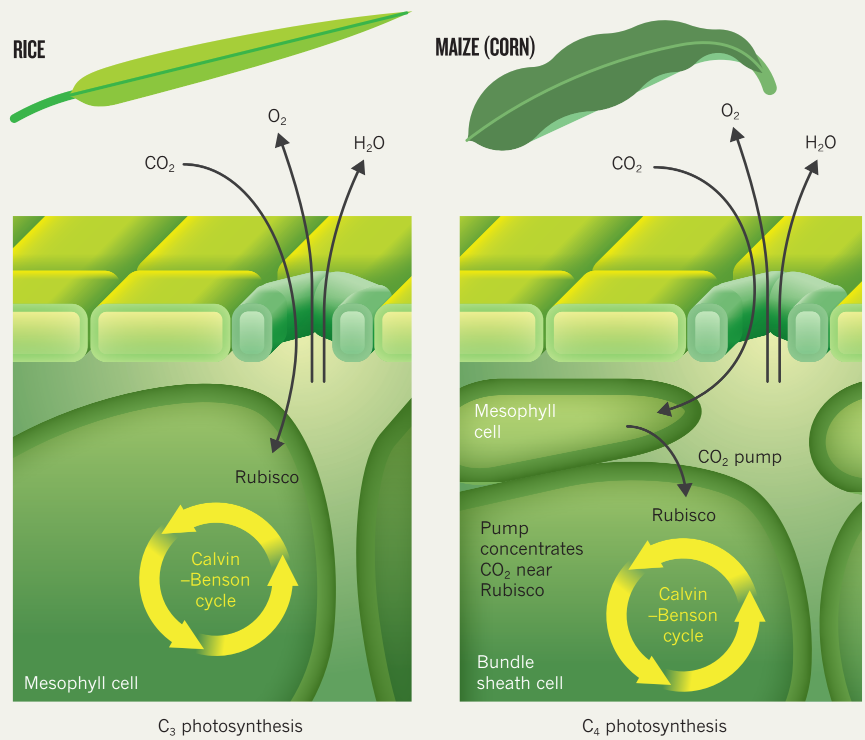
The timeline for nitrogen-efficient rice is longer than for C_4. "I honestly don't think we'll see commercial rice crops for 20 to 30 years," Good says. But he is confident that researchers will at least discover the environmental circumstances that determine whether *AlaAT*-enhanced rice performs well. And he is certain that the research will reveal "quite cool" findings along the way, even if his team has to struggle constantly for funding. "That's why every bit of grant money we get goes towards researching NUE [nitrogen use efficiency] in cereal crops. It's too visionary and too high risk for conservative funding agencies."

In the United Kingdom, plant scientist Edward Cocking is wielding bacteria instead of genes in the quest to help rice plants thrive with less added fertilizer. The University of Nottingham's Centre for Crop Nitrogen Fixation, of which Cocking is director, is part of an international network that is exploring ways to encourage cereal crops, such as rice, to attract nitrogen-fixing bacteria to take up residence in cells of the plant, from roots to leaves.

The idea was initiated by Borlaug, who observed decades ago that legumes, unlike cereal crops, have evolved a symbiotic relationship with a soil bacterium called rhizobia that fixes nitrogen inside specialized root nodules.

PHOTOSYNTHESIS VARIANTS

All green plants use the photosynthesis enzyme Rubisco to harvest carbon from carbon dioxide. C_4 plants have special 'bundle sheath' cells that concentrate carbon dioxide around the enzyme while blocking out oxygen, making the process more efficient.



SOURCE: D. R. TAUB *NATURE EDUCATION KNOWLEDGE* **3**, 21 (2010). AVAILABLE AT GO.NATURE.COM/5SQC5W

Because rice lacks such nodules, using a bacterium to fix nitrogen seemed like a hopeless task. But in 1988, Brazilian agronomist and microbiologist Johanna Döbereiner discovered a different type of bacterium, *Gluconacetobacter diazotrophicus*, in sugar cane that fixes nitrogen from the air, no nodules required. Why not try it with rice and other cereals, thought Cocking. "This was my 'a-ha' moment," he recalls, although it took a while to put the pieces of the puzzle together.

Cocking set to work about ten years ago. The result is N-Fix, a method of putting *G. diazotrophicus* into the cells of plant roots. Once there, the bacterium colonizes all the cells of the plant. "It's environmentally friendly and can be applied to all crops," says Cocking. His group has already proved the concept in the laboratory and greenhouse with maize, rice, wheat, oilseed rape and tomatoes. The team is currently testing different delivery systems, among them coating seeds with *G. diazotrophicus* and inoculating seedlings with the bacterium.

The University of Nottingham has licensed the N-Fix technology to Azotic Technologies based in Chorley, UK, a company committed to commercial-scale nitrogen fixation. Field trials conducted in 2013 on wheat and oilseed rape showed that *G. diazotrophicus* provided between one-quarter and one-half of the recommended amount of nitrogen-rich fertilizer treatments, meaning that additional fertilizer use can be dramatically reduced. Azotic has not yet tested N-Fix with rice, but Cocking says discussions are under way about possible trials in Asia, particularly China, and parts of Africa. "The aim is to bring N-Fix to the market for farmers in the next few years," says Cocking.

ANCIENT GENES, NEW ADVANCES

At the end of the wet season, wild rice flowers in lagoons, waterways and wetlands of Australia's Cape York peninsula at the far north of Queensland. "In many places it's growing in very large populations, occasionally as far as the eye can see," says Henry. The wild species flourishing in the very north of Australia are ancient. They are descendants of plants that lived 184 million years ago on the supercontinent Gondwana, which included the landmass of what is now Australia. It is even possible that Australia is the birthplace of rice, says Henry. Moreover, Australia's wild rice was neither domesticated nor interbred with domestic rice, as was Asian wild rice. That's why Henry and his teammates from the Queensland Alliance for Agriculture and Food Innovation put on their waders and risk encountering crocodiles to explore the wetlands. It is essential to collect samples from as many populations of wild rice as possible. "We're trying to do the genetics and understand reproductive biology, and identify species, pathology and the diseases and resistance that might exist," explains Henry.

The results gathered by Henry's team may help a team of international researchers studying blast disease. Many rice scientists consider blast, a fungal disease that affects crops in more than 80 countries to be the world's most important rice disease. The severity of a blast infection varies with year and location, and even within a field, depending on environmental conditions and crop management — yield losses can be as high as 50%.

But evidence is building that Australian wild rice plants have lived, apparently disease-free, with blast for thousands of years[5]. The wild rice is related closely enough to domesticated rice for cross-breeding without genetic tinkering. Clearly, the prospect of pinpointing blast-resistant genes and breeding them into domesticated rice is exciting. Plus, there is plenty of genetic diversity on offer that could improve other traits such as drought resistance. Henry's group has so far identified four species of Australian wild rice, including two newly discovered, unnamed species, suggesting even greater potential that is still untapped. These and other discoveries are being closely watched by the Oryza Map Alignment Project (OMAP), a rice research programme headed by geneticist Rod Wing at the Arizona Genomics Institute in Tucson. In July, OMAP researchers published the complete genome sequence of a hardy, stress-resistant African rice[6]. (For more on African rice, see page S64.)

The international team at OMAP has set itself the goal of unravelling the evolution, physiology and biochemistry of the genus *Oryza*, the group that includes domesticated Asian and African rice as well as Australian wild rice. "By understanding the entire genus at a genome level we have a whole new pool of genetic variation that can be used to combat pests and plant pathogens," Wing says. Decoding the genome is the easy part, he says. Understanding the code and applying discoveries to improve existing rice will take time.

Henry agrees that Australian rice has unique potential: as a laboratory for rice-breeding biology, as a source of disease-fighting genes, and even as a new commercial crop, but most of all as an important key to global food security. "The world has to recognize the value of this material," he says. Little wonder Henry and his colleagues are prepared to literally risk life and limb. As he says: "Don't worry about the crocs. This is the latest thing in rice." ■

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