Do-it-yourself in agriculture

PLANTS MAY START FIXING THEIR OWN NITROGEN, SAYS **S ANANTHANARAYAN**

Plant growth is the heart of food production and also the engine of elimination of atmospheric carbon dioxide. Plants do this by turning the carbon in carbon dioxide into hydrocarbons, which are sources of energy for animals. But the process itself uses energy and the laws of physics say the process is not fully efficient, that the energy used is always more than the energy stored. But the energy used is sunlight, which, so long as there is the sun, comes for free.

But plants need more than just carbon, water and sunlight to get along — they need chlorophyll and a host of other agents, all of which need the element, nitrogen, to be formed. Now nitrogen is abundant, it forms the greatest part of the air in the atmosphere, but this is not nitrogen in the form that plants can use — atmospheric nitrogen is "inert" and needs to be "fixed" to become available, another process that takes a lot of energy. One time this happens is by lightning flashes during storms and, more regularly and to a great extent, by micro-organisms like bacteria, and how fast it takes place may be a factor that limits how fast plants can grow.

With the increasing population and demands on agriculture, the last century or so has seen great use of synthetic, chemical fertiliser to pack the soil with usable nitrogen, as also small quantities of other soil nutrients

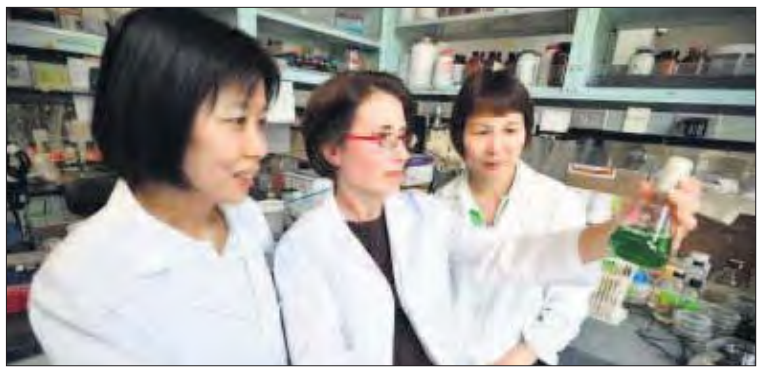
Professor Himadri Pakrasi

like phosphorus, potassium, calcium, magnesium, some metals, etc. The trouble is that delivery of synthetic fertiliser to the plant is wasteful and leads to contamination of the soil and pollution of waterways, and is also needed in great quantities. The production, which is energy intensive, has to be through the use of fossil fuels, which adds to atmospheric pollution. It is in this context that the preliminary work of Professor Himadri Pakrasi and his team at Washington University, St Louis, towards enabling plants to create their own fertiliser, right where it is needed, has been received with great interest.

The reason why this is not obvious is that that creating food and usable nitrogen are opposing processes. The first extracts carbon from carbon dioxide and releases oxygen. The second needs a strictly oxygen-free environment. All bacteria, of a class called blue-green bacteria or *cyanobacteria*, have the capacity to trap the energy of sunlight and use it for synthesis, usually of hydrocarbons, using carbon dioxide. But some bacteria use stored energy to fix nitrogen. It is thought that the evolutionary ancestor of all bacteria had this capacity, but it was lost — maybe with the rise in oxygen levels.

And then there are some bacteria that can do both. As opposing processes, they need to be separated, either by being done in different places or at different times. One cyynaobacterium, *Cyanothece 51142*, which Pakrasi and his team have studied now for 10 years, does it by allotting times slots. During the day, it uses photosynthesis to create and store available carbon. And at night, it burns the carbon to use up the oxygen, so that it can start creating usable nitrogen. Both processes are really ways of pulling carbon or nitrogen atoms out of the stable niches of secure bonding — carbon with two oxygen atoms, as CO_2, and nitrogen as N_2, two nitrogen atoms bound to each other. For the second process to occur, a chemical group needs to bond with the tightly held nitrogen atom, a job it cannot do if there are ready-to-mingle oxygen atoms coming in the way.

The St Louis team is now discovering ways to transfer the genetic coding in *Cyanothece 51142*, which makes it able to fix nitrogen, on to another suitable bacterium, so that the new organism can do this, too. Developing the genetic engineering tools that can do this would be the "proof of principle" that nitrogen fixing ability could be built into plant cells, the traditional factories of photosynthesis.



Nancy Duan, Michelle Liberton and Lingxia Zhao

"That would really revolutionise agriculture," says Pakrasi, PhD, Myron and Sonya Glassberg/Albert and Blanche Greensfelder Distinguished University Professor in Arts and Sciences and director of the International Center for Advanced Renewable Energy and Sustainability at Washington University.

Cyanothece 51142 happens to be a suitable starting point, because the genetic coding for its nitrogen-fixing ability consists of a panel of some 30 genes, placed together and activated by common signals — a formation that could be more easily transferred to another genome. The target bacterium, *Synechocystis 6803*, is the best-studied strain of cyanobacteria. "Not only has its genome been sequenced, it is naturally 'transformable' and able to integrate foreign DNA into its genome by swapping it with similar native strands of DNA," says the notice sent out by Washington University.

The (US) National Science Foundation just awarded Pakrasi and his team more than $3.87 million to explore this idea further. The grant will be administered out of I-Cares, a university-wide centre that supports collaborative research in the areas of energy, the environment and sustainability. This award is one of four grants funded by the National Science Foundation jointly with awards funded by the Biotechnology and Biological Sciences Research Council in the UK.

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The Haber process

Seventy-eight per cent of the atmosphere consists of nitrogen. But in free nitrogen, the atoms are held together by strong triple bonds and the nitrogen is not available for plants to use. The chemical bonds need to be broken and replaced less securely by creating new compounds.

The Haber process, also known as the Haber-Bosch process, combines nitrogen and hydrogen at high temperature and pressure, in the presence of catalysts, to form ammonia (NH_3). The process was discovered by Fritz Haber and scaled up for use in industry by Carl Bosch.

The process does not work unless high pressure is used. But high pressure leads to high costs in the form of equipment and energy for pumps and compressors. The compromise is, hence, at about 200 atmospheres, and yield of some 15 per cent at every pass. The yield could be better if the ammonia were skimmed off, but this is difficult at the high pressure. Ammonia is extracted by cooling the gases when they leave the reactor, so that ammonia liquefies, while the free nitrogen and hydrogen are reused. The cooling for separation of ammonia is another major cost of the process.

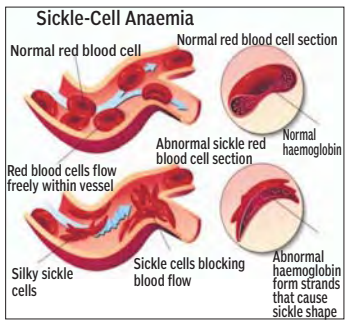
Getting plants to fix nitrogen, instead of doing it in factories, would be at some cost to the plants. A part of the carbohydrates created would get consumed b y the plant. There would be release of CO2, too.

But the biological process would be more efficient. And, what is much more important, there would be no waste of fertiliser and pollution of runoff irrigation water.

PLUS POINTS

Bad blood

Sickle Cell Anaemia is a hereditary blood disorder characterised by abnormal, rigid, sickle-shaped red blood cells. It occurs due to mutation



in the haemoglobin gene. The sickle-shaped red blood cell tends to die early. As the bone marrow cannot make new blood cells fast enough, the patient becomes anaemic and usually has a short life.

A study published in *PLoS Medicine* on 16 July states that by 2050 almost half a million babies are likely to be born with SCA compared to 305,800 in 2010. Nigeria, the Democratic Republic of Congo and India will be the most affected, notes the study conducted by Frederic Piel and colleagues at the University of Oxford and Imperial College in the UK and Kemri-Wellcome Trust Research Programme in Kenya. "Children with SCA usually die undiagnosed in their first years of life," says Piel. "Appropriate health care will be needed early in life of these individuals to avoid future complications."

"The most important thing is to create public awareness of SCA and how it is inherited. Second, there should be a population screening of teenagers and adults to detect the carrier of the gene," says Isaac Odame, medical director of the Global Sickle Cell Disease Network. "Once that is done, counselling sessions should be provided to help prospective parents avoid pairing with each other. Finally, every country should have a coherent national strategy for the management and control of SCA as recommended by the World Health Organisation."

"Preventive and curative measures for SCA are available but very costly," says SL Kate, head of the Sickle Cell Department at Maharashtra Arogya Mandal in Pune. "Simple, reliable and free diagnostic tests should be made available for poor people," he says.

PRANAV DADHICH/CSE-DOWN TO EARTH FEATURE SERVICE

Caviar & chocolate

The rules of combining flavours seem, on the surface, more of an art than a science. Some pairings seem universal in their appeal (strawberry and cream for example) whilst others don't spread much beyond a specific culture (America's love for 'peanutbutter and jelly' sandwiches for example).

New research however, has tried to find out what chemical rules might underlie our culinary predilections. Published in *Nature*, the paper aims

to determine "whether there are any general patterns that determine the ingredient combinations used in food today or principles that transcend individual tastes and recipes".

This "food pairing" hypothesis has been used by the scientifically-inclined chefs to search out novel combinations, with the paper citing the pairing of white chocolate and caviar as an example.

Although the two do not seem natural bedfellows, there is some appeal in serving them together as they both contain "trimethylamine and other flavour compounds".

In order to better visualise the research, *Scientific American* have created an interactive chart showing all the various chemical links between foods. Each blue dot represents one of 381 different ingredients, split between 14 categories including "alcoholic beverage", "fish or seafood", "plant" and "spice".

The size of the dot shows how popular the food is (how often it appeared in the sample database of recipes) and the red lines indicate similar chemical flavour compounds. Foods at the top of the page are similar to lots of others, ones at the bottom of the page are unique in their flavour.

JAMES VINCENT/THE INDEPENDENT

CLEAVED AND JOINED BACK

TAPAN KUMAR MAITRA EXPLAINS HOW HOMOLOGOUS RECOMBINATION IS INITIATED BY SINGLE-STRAND DNA EXCHANGES

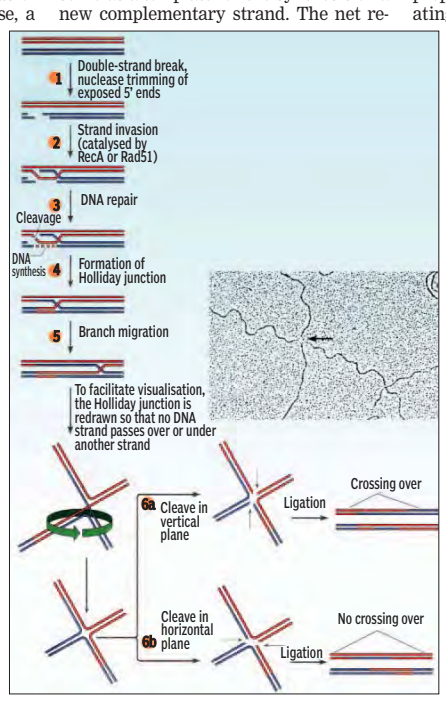
A homologous recombination is more complicated than can be explained by a simple breakage-and-exchange model in which crossing over is accomplished by cleaving two double-stranded DNA molecules and then exchanging and rejoining the cut ends. Robin Holliday was the first to propose the alternative idea that recombination is based on the exchange of single DNA strands between two double-stranded DNA molecules.

According to a current model of how such single-strand exchanges could lead to genetic recombination, the initial step is the cleavage of one or both strands of the DNA double helix. In either case, a single broken DNA strand (1) derive from one DNA molecule "invades" a complementary region of a homologous DNA double helix, displacing one of the two strands (2). Localised DNA synthesis and repair (3) and (4) ultimately generate a crossed structure called a Holliday junction, in which a single strand from each DNA double helix has crossed over and joined the opposite double helix. Electron microscopy has provided direct support for the existence of Holliday junctions, revealing the presence of DNA double helices joined by single-strand crossovers at sites of genetic recombination.

Once a Holliday junction has been formed, unwinding and rewinding of the DNA double helices causes the crossover point to move back and forth along the chromosomal DNA (5). This phenomenon, called branch migration, can rapidly increase the length of single-stranded DNA that is exchanged between two DNA molecules. After branch migration has occurred, the Holliday junction is cleaved and the broken DNA strands are joined back together to produce two separate DNA molecules.

There are two ways in which a Holliday junction can be cleaved and rejoined. If it is cleaved in one plane, the two DNA molecules that are produced will exhibit crossing over — that is, the chromosomal DNA beyond the point where recombination occurred will have been completely exchanged between the two chromosomes (6a). If the Holliday junction is cut in the other plane, crossing over does not occur but the DNA molecules exhibit a noncomplementary region near the site where the Holliday junction had formed (6b).

What is the fate of such noncomplementary regions? If they remain intact, an ensuing mitotic division will separate the mismatched DNA strands and each will serve as a template for the synthesis of a new complementary strand. The net result will be two new DNA molecules with differing base sequences, and hence two cells containing slightly different gene sequences in the affected region. This is the situation occasionally observed in Neurospora, where two genetically different cells can arise during the mitosis following meiosis. Alternatively, a noncomplementary DNA region may be corrected by excision and repair. The net effect of DNA repair would be to convert genes from one form to another — in other words, gene conversion.



A key enzyme involved in homologous recombination was first identified using bacterial extracts that catalyse the formation of Holliday junctions. Such extracts contain a protein called RecA, whose presence is required for recombination. Mutant bacteria that produce a defective RecA protein cannot carry out genetic recombination, nor are extracts prepared from such cells capable of creating Holliday junctions from homologous DNA molecules. The RecA protein catalyses a "strand invasion" reaction in which a single-stranded DNA segment displaces one of the two strands of a DNA double helix. In catalysing the strand invasion reaction, RecA or Rad51 first coats the single-stranded DNA region; the coated, single-stranded DNA then interacts with a DNA double helix, moving along the target DNA until it reaches a complementary sequence with which it can pair.

In addition to being involved in genetic recombination, strand invasion also plays a role in certain types of DNA repair. Double-strand breaks in DNA are often repaired by a process that takes advantage of the fact that if one chromosome incurs a double-strand break, the intact DNA molecule of the homologous chromosomal DNA can serve as a template for guiding the repair of the broken chromosome. In such bases, single DNA strands from the broken DNA molecule invade the intact DNA molecule and find a complementary region to serve as a template for repairing the broken 5NA.

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Too tempting to ignore

165,000 APPLY FOR MISSION TO COLONISE MARS — DESPITE NO CHANCE OF THEM EVER RETURNING TO EARTH. **STEVE CONNOR** REPORTS

A one-way ticket to another planet where there is no air, no water or food – and certainly no return home – may not sound like a lot of fun, but for 165,000 people the opportunity to live permanently on Mars has been too tempting to ignore.

The organisers of the ambitious Mars One mission to send a group of four men and women to Mars in 2023 said they were surprised by how many had offered to become the first astronauts to land and live on the Red Planet, even though the privately-financed plan did not involve a return journey because of the technical difficulty and cost of equipping and fuelling the trip home. Each applicant has had to pay,

An artist's rendition of a Mars settlement

on average, about £17, depending on their country, to cover the administrative fees of the selection process, but even this small financial penalty has failed to dissuade the tens of thousands who dream of going to Mars – even though it also involves the possible nightmare of never returning.

The final tally could rise even higher before the deadline for applications is reached at the end of the month, but already the quality of the applicants is very high, according to Dutch entrepreneur Bas Lansdorp, who dreamed up the scheme last year after seeing the viewing figures and income stream of the London Olympics.

"We're not looking for individuals, we are looking for perfect teams. We want individuals who fit into certain teams of people going to Mars. They must be healthy, smart enough to learn new skills and with a character and mindset that can function in a small group," he said.

A selection committee will begin to sort the applicants in the coming weeks and shortlists will be drawn up in the coming year. The plan is to build teams of four people, each from a different continent, who will live and train together for seven years before the first manned launch in 2022, arriving the following year. The estimated £4 billion cost of the mission will be met by television rights and other kinds of media sponsorship and spinoffs, but even though creator Paul Römer sits on the Mars One board, the plan is to create a media event that is more like an Olympics spectacular than a TV reality show, Lansdorp said. "I really don't think that anything other than that is shootable for a mission to Mars. It should be exciting, inspiring and beautiful, just like the Olympics."

Nearly a quarter of the 165,000 applicants live in the USA and include people such as 27-year-old Carl, a computer programmer who describes himself as "logical, loving and awful good". The space nations of China, Brazil, India and Russia come next on the list of the 140 countries where the applicants live.

Rockets carrying cargo, food and living modules will be sent to Mars a few years in advance of the manned landing and a further crew of four people will arrive two years after the first astronauts have landed, Lansdorp said. But perhaps the biggest obstacle will be the psychological anxiety of knowing there is no return. "They will only step into the rocket if they really want to go. The risk is theirs and they cannot change their minds," Lansdorp said.

THE INDEPENDENT