

DIY in agriculture

Plants may start fixing their own nitrogen, says S. Ananthanarayanan.

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 and 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 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 increasing population and demands on agriculture, the last century or so has seen great use of synthetic, chemical fertilizer, to pack the soil with usable nitrogen, as also small quantities of other soil nutrients like phosphorus, potassium, calcium, magnesium, some metals, etc. The trouble is that delivery of synthetic fertilizer 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 use of fossil fuels, which adds to atmospheric pollution. It is in this context that the preliminary work of Prof Himadri Pakrasi and his team at Washington University, St Louis, towards enabling plants to create their own fertilizer, right where it is needed, has been received with great interest.

The reason why this is not obvious is that that creating food and creating 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 rise in oxygen levels.



Prof Himadri Pakrasi



Nancy Duan, Michelle Liberton and Lingxia Zhao

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 cyanoobacterium, ***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 the bacterium, ***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. "That would really revolutionize agriculture," says Pakrasi, PhD, the Myron and Sonya Glassberg/Albert and Blanche Greensfelder Distinguished University Professor, in Arts & Sciences, and director of the International Center for Advanced Renewable Energy and Sustainability (I-CARES) at Washington University in St. Louis.

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 center 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 United Kingdom.

Haber Process

78 % 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 (NH₃). The process was discovered by Fritz Haber and scaled up for use in industry by Carl Bosch.

The process does not progress 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% 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 by the plant. There would be release of CO_2 , too. But the biological process would be more efficient. And, what is much more important, there would be no waste of fertilizer and pollution of runoff irrigation water.