Impossible dream: Self-fertilising cereal crops?

Is it possible to create cereal crops that provide their own nutrition? In a bid to find out, *CPM* talks to researchers attempting to transfer the nitrogen-fixing abilities of legumes into cereals.

By Mike Abram

Researchers are attempting to do what would seem unlikely, if not impossible: transfer the ability to fix nitrogen in legumes into a much broader range of crops including cereals.

And while the reality of cereal crops that don't require any or much less additional fertilisation is still at least 10-20 years away commercially, the past two decades of research provides optimism that it isn't just a pipe dream.

Much of the research since 2013 has been through a multi-national project, Enabling Nutrient Symbioses in Agriculture (ENSA), led by Prof Simona Radutoiu from Aarhus University. The main purpose of the project, which is currently grant funded from Bill and Melinda Gates Agricultural Innovations, is to make global agriculture more sustainable and equitable, explains researcher Dr Sebastian Schornack, a senior group leader based at the Sainsbury Laboratory at the University of Cambridge.

"To achieve that, we want to expand the use of beneficial microorganisms for the delivery of nutrients essential for crop production."

As is becoming increasingly obvious to farmers across the world, symbiotic relationships between plants and microorganisms can be very helpful, including for supplying key nutrients for plant growth. But taking advantage of those interactions is less necessary when growing crops in fertiliser-rich environments, suggests Sebastian.

REDUCING THE SYNTHETICS

Rising societal pressure on the use of nitrogen fertiliser amid awareness of the environmental damage its excessive use can cause, plus an acknowledgement of the high energy and emissions costs of making such products, means reducing synthetic fertiliser is now a key target for many growers.

In addition, there are many parts of the world where farmers don't have ready access to fertilisers because of cost or distribution limitations, or there are nutrient-poor soils, adds Sebastian. "We require alternatives to synthetic fertilisers and one solution is to improve the way crops engage with microorganisms for fertilisation." ▶

ENSA project purpose

Researcher Dr Sebastian Schornack says a significant part of the ENSA project is to expand the use of beneficial microorganisms for the delivery of nutrients essential for crop production.

Cowpea trials

Cowpea, as in black-eyed pea, has been used as a target crop for the research.

▶ The ENSA project is exploring a number of different routes to achieve this, including whether it's possible to transfer nitrogen-fixing root nodules found in legume plants to other crops, particularly cereals.

Understanding how nitrogen fixation happens in legumes is crucial to that longer-term goal, with it also helping researchers to understand how to improve the process in legume crops as well. "We know from research and the natural variation found in plants, that it is possible for this to work better than it does currently," comments Sebastian.

One reason is that root nodule symbiosis hasn't been a trait that breeders have actively tried to improve through plant breeding in legume crops, he suggests. "So there's a lot of potential for improvement in our existing crops."

Sebastian's research group has already discovered in a model legume crop, Medicago truncatula or barrel clover – that by switching off a single gene using gene editing techniques the plants go into overdrive and fix more nitrogen.

In that model crop, nitrogen fixing activity is increased by 25-30% by deactivating this single gene, which could also be achieved through traditional mutagenesis breeding, while shoot biomass increases by 10-15% when grown on nutrient-poor soil under experimental conditions.

GENE SWITCH OFF

The next step is to switch off identified candidate genes in the target crops of soybean and cowpea (as in black-eyed pea). Finding those genes in cowpea was more complicated than in soybean, notes Sebastian. "In soybean, because it's such an important crop globally, you can benefit from existing knowledge. However with cowpea, because it's much less well understood, we had to do more groundwork."

That included growing cowpeas to find out which genes were active when root nodules were present – a step that was already known with soybeans, as well as the model crop. "We're hopeful, although we still have to test this, that turning off identified genes in our target crops will lead to more protein in each seed and therefore higher value legumes," says Sebastian.

Other legume crops could also benefit from this approach, he says. "Barrel clover is very closely related to peas, fava beans and clover – more so than soybean or cowpea – so it would be perhaps more straightforward to implement such a trait in these crops."

Progress is also being made on the longer term aim of transferring nitrogen fixing nodules into cereal crops. "The nodules are an elaborate home for the bacteria," highlights Sebastian.

He says that's required because of what's known as the oxygen paradox: a common feature of all microbial nitrogenase enzymes that fix nitrogen is they are broken down in the presence of oxygen, yet a lot of energy is required to break the stable triple bonds between nitrogen atoms to fix nitrogen into ammonia. This presents a conundrum – how can aerobic respiration be maintained to produce the energy required to fix nitrogen while protecting the nitrogenase from oxygen?

But, leguminous plants have come up with an ingenious solution to remove oxygen in their nodules so the bacteria can do their job, says Sebastian.

Within the nodules, rhizobia colonise the cells in the centre and become encapsulated by a plant membrane. They're further protected from free oxygen diffusing into this area by a zone of densely packed tissue.

TRANSPORTING OXYGEN

An oxygen carrier, leghaemoglobin, which gives the nodules their pink colour, then transports oxygen to the bacteria so they can respire and produce the energy required to fix nitrogen without it encountering the nitrogenase enzyme.

Understanding all the processes involved in forming these nodules, including how legumes recognise nitrogen-fixing rhizobia bacteria in the first place, how those rhizobia infect legume roots, what triggers the nodules to develop, as well as creating that perfect environment for nitrogen fixation will be crucial for transferring the ability to cereals, stresses Sebastian.

An initial key discovery made by ENSA researchers is that cereal crops have inherited processes involved in forming relationships with arbuscular mycorrhizal fungi – evolutionary the earliest beneficial microbial association in plants. "This means cereal crops can recognise beneficial microbes, but what they don't have is the genetic trigger to produce the actual nodule," he adds.

"So one of the major challenges for ENSA researchers now is to transfer from legumes the ability to build nodules in cereals."

The researchers can potentially take advantage of genetics already present. "For example, ENSA research has shown that the ability to build nodules shares some of the same triggers with producing lateral roots," continues Sebastian.

That means it could be possible to either transfer the relevant genes from legume crops into cereals using a GM approach, or use gene editing to modify the trigger to produce lateral roots in cereals to instead produce nodules, he suggests.

But that would just be one step in creating a self- ▶

Root nodules

Leguminous plants have an 'ingenious' solution to remove oxygen in their nodules so the bacteria can do their job.

fertilising plant. "You would still require the nodules to be colonised by the bacteria at the right time and create an environment that allows them to efficiently fix nitrogen. "It's a multi-step process that requires input from various research groups. It's like a big building project."

Another strand of the ENSA project is looking at how plants obtain phosphorus, water and micronutrients through the association with arbuscular mycorrhizae fungi. These associations can form in most crop plants but few crops fully benefit, says Sebastian.

"It's not just nutrients the plant can gain from this symbiosis, but also resilience to pathogens both in their roots and sometimes in their shoots."

But applying phosphate fertilisers suppresses these associations and with it the beneficial effects, so researchers in ENSA have modified barley plants to support mycorrhizal association even when nutrient fertiliser is applied.

"The plants might not necessarily benefit from the symbiosis by getting nutrients from it, but they might be fortified from diseases, and if nutrient availability varies across the field, it could also buffer that as well," he concludes.

Can free-living bacteria supply nitrogen?

Evidence could suggest otherwise

Evidence that inoculating cereal crops with free-living or endophytic bacteria strains will result in agriculturally significant amounts of nitrogen being fixed from the atmosphere is lacking, according to a new research paper authored by scientists from Wageningen University, the James Hutton Institute and two Australian universities.

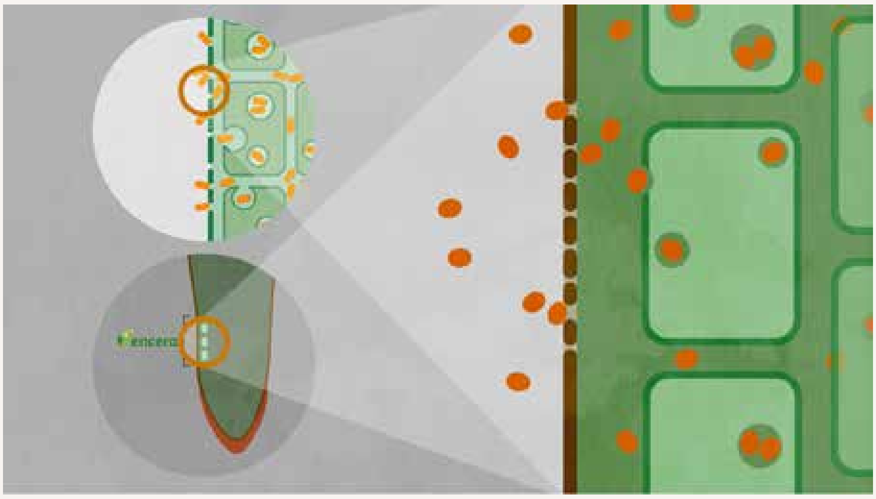
A plethora of bacterial products claiming to increase growth and yields in cereals and other crops through stimulating nitrogen fixation are now available, but simply demonstrating the bacteria are present on roots or inside a non-legume plant doesn't provide evidence for significant nitrogen fixation, suggest the paper's authors, led by Ken Giller from Wageningen University.

Among the bacterial inoculants reviewed in the paper are Methylobacterium symbioticum marketed as BlueN by Corteva and Gluconacetobacter diazotophicum (Gd), sold as Encera in the UK and Europe by Azotic Technologies.

Claims about the nitrogen-fixing ability of both, according to the paper's authors, don't stack up. This is because genome sequencing of the Methylobacterium strains suggests that while there are some that have the capability to make a functional nitrogenase enzyme to fix nitrogen, the ones used within BlueN don't have these genes and therefore lack the capacity to fix nitrogen, the paper claims.

The authors also pull apart research around the Gd bacteria in Encera, suggesting a paucity of field data indicating colonisation of plant cells by Gd in target crops even after inoculation, and a lack of supportive evidence for claims of yield responses due to nitrogen fixation.

In the authors' opinion, 50 years of research more broadly suggests that responses to microbial inoculants are due to plant promotion effects other than nitrogen fixation, such as producing plant hormones or through the stimulation of root growth leading to improved efficiency of nutrient uptake.



Colonising plant tissue

According to Azotic Technologies, the strain of Gd in the firm's products colonises live plant tissues and promotes plant growth via nitrogen fixation by the bacteria.

Not surprisingly, both Corteva and Azotic Technologies reject the claims around their products. A Corteva spokesperson told *CPM*: "Corteva has conducted multiple studies confirming that Methylobacterium symbioticum SB23 – the bacteria on which Utrisha N/Blue N is based – is capable of fixing nitrogen and improving nitrogen use efficiency, yield and quality in colonised crops.

"Further, since its launch in 2021, Utrisha N/Blue N has been well received by farmers with demonstrated success in improving quality and yield performance on row crops and specialty crops."

Azotic Technologies R&D Director Adriana Botes pointed to recent peer-reviewed papers, including one by Phil Hill from the University of Nottingham, which she says proves the strain of Gd in Azotic products colonises live plant tissues and promotes plant growth via nitrogen fixation by the bacteria.

The firm has also conducted experiments comparing strains of Gd modified so that it can't fix nitrogen versus the Gd strain used in Azotic products, which show benefits from using the strain can't be purely hormonal plant promotion effects and must also come from nitrogen fixation by the bacteria.

Field trials also back up the claims that nitrogen is being fixed and contributing to yield responses, says global trials manager Tom Harries. "Multiple trials on different crops, where nitrogen rates are reduced and Gd applied, show yield responses above where Gd isn't used.

"When you look at the trials and in the lab when we knock out the nitrogen fixing part of Gd, it's providing strong weight of evidence that nitrogen is being fixed and contributing to yield," he says.