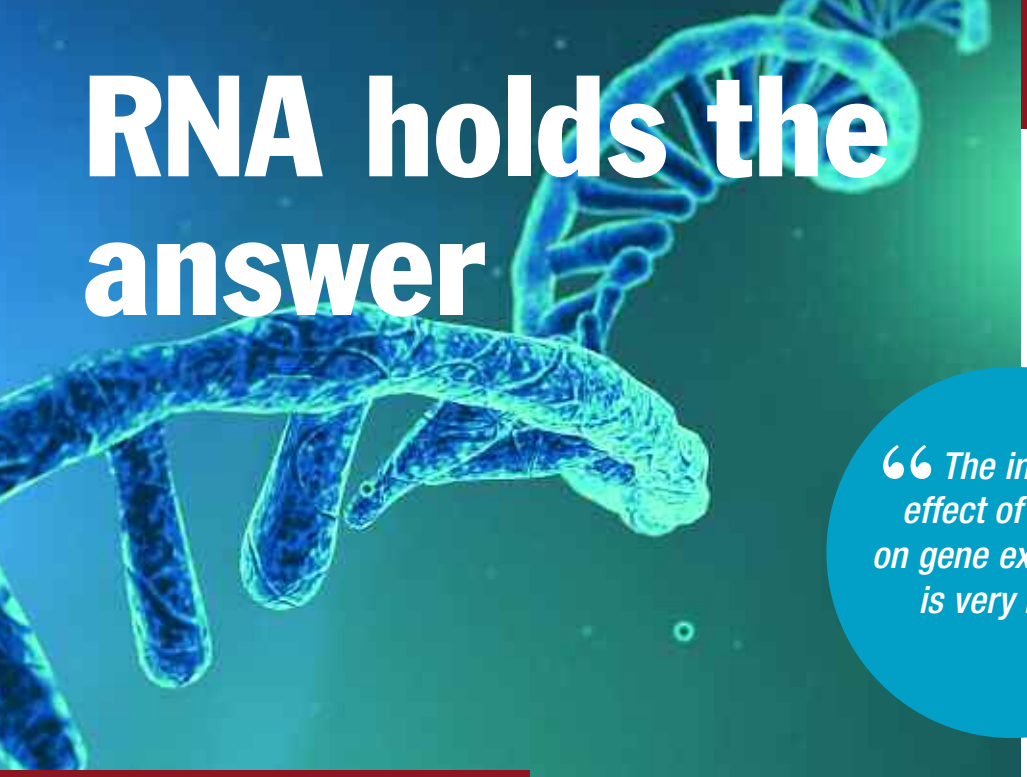
RNA holds the answer



> *"The initial effect of Bio 20 on gene expression is very rapid."*

Research briefing

The 'Central Dogma' is the process by which the instructions in DNA are converted into a functional product and understanding how biostimulants effect this process in plants could help answer how they work. *CPM* finds out more.

By Lucy de la Pasture

So how does it work? That's the question that's been asked about biostimulant products for decades. Advances in molecular biology and 'omics' technologies mean that this question can be answered with a degree of certainty for the first time.

Up until very recently it's been the results of field trials and glasshouse studies that have furthered the understanding of benefits biostimulant products can bring, explains David Booty, technical development manager at Omex.

"One of the problems with biostimulant products is that they perform better in some circumstances than in others and that's made it very difficult to understand how to get the best out of them in the field."

To help bridge this gap in knowledge, Omex commissioned Novogene in Cambridge to carry out an RNA-sequencing transcriptomics study using its globally best-selling biostimulant/nutritional product, Bio 20.

"Genetic analysis techniques have become faster and more affordable in recent years, and the specific functions of gene groups are better understood. That means it's become possible to investigate in detail the effect of Bio 20 at a genetic and biochemical level. These results should help explain why we get the benefits we've seen in the field after application," explains David.

Genetic testing

Bio 20 contains a seaweed extract in combination with both macro and micronutrients. It has been extensively put through its paces in trials, which have shown Bio 20 helps improve establishment, growth and yield, rooting, and resistance to stress. In the field it's gained a reputation for helping crops cope with drought stress and this effect has also been proven in glasshouse trials (see Research Briefing article, *CPM* Sept 2021 for full details).

Genetic testing and the role of messenger RNA (mRNA) is something we've all become more familiar with during the course of the COVID pandemic and the consequent development of novel vaccine technologies (Pfizer BioNTech and Moderna) where mRNA is used to provide instructions to the recipient's immune system.

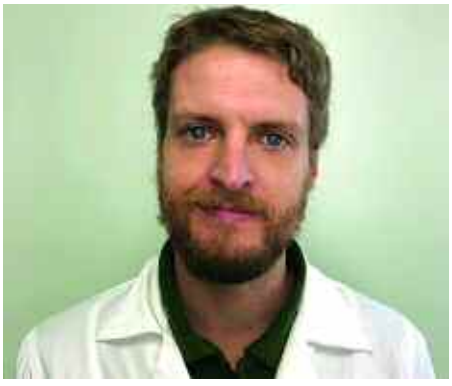
RNA transcriptomics can be used to discover the set of instructions to cells that are being kicked into operation after application of Bio 20. Omex trials officer Tom Pitcher explains how this study can help uncover how a biostimulant is acting on crop genes.

"Genetic information is stored as DNA in the nucleus of every cell. A species has thousands of genes (approx. 95,000 in wheat) but only a few are active at any one time in a cell. In transcription, the information in the DNA of every cell is converted into small, portable RNA messages. When a gene is active it produces this mRNA, and it's this that provides the template which leads to a particular protein being made. Each protein has a specific purpose, such as for structural strength, chemical reactions, storage or signalling," he explains. "RNA-sequencing is a technology that allows us to read all the mRNA that is present in a cell or plant organ and shows gene expression — which genes are active and which proteins are being made."

To assess the effects of Bio 20, 40 wheat seedlings were germinated in compost. They were then transplanted to 10cm pots 18 days after planting where they were allowed to grow on for a further 58 days after. After this time, 20 of the plants were sprayed with Bio 20 and the other half left untreated.

Three hours after spraying, ten sprayed and ten unsprayed plants were removed from the soil. The root tips (last 2cm) of each plant were cut and cleaned, immersed in a special solution and frozen to preserve the RNA in the sample. The remaining plants, ten sprayed and ten untreated were removed and sampled in the same way 24 hours after spraying.

RNA sequencing was done via Illumina platforms, based on the sequencing by synthesis (SBS) mechanism, explains Tom. "In this technique, RNA is extracted from the



Messenger RNA provides the template which leads to a particular protein being made, explains Tom Pitcher.

cells and purified, then the RNA is broken into fragments and converted to DNA.

"The sequencing method uses building blocks of DNA, which are modified with fluorescent tags. The fluorescence is detected by the sequencing machine as each building block is added. Thousands of DNA strands are sequenced simultaneously with high precision."

The raw data from the machine is converted to huge numbers of genetic sequences, he adds. The results were analysed and were found to be incredibly accurate — with an error rate in the range of 0.02 – 0.03% (i.e. the accuracy was above 99.97%) for all positions along the transcriptome, and for all samples.

"The genetic sequences are aligned to a reference genome — in this case the wheat genome. For each sequence, an algorithm finds what gene or part of the genome it came from. Based on the number of sequences mapped to each gene, the activity level of each one can be compared to identify the differential gene expression.

"Since the function of most genes is known, it's possible to identify which metabolic processes within the cells are being switched on or off," explains Tom.

So the results give an insight into the differential gene expression in the two sets of wheat root samples, with or without Bio 20 at three and 24 hours after application. The results showed there was a marked difference at the two different sampling timings in the gene expression, comments Tom.

"Comparing the treated with the untreated samples at three hours after treatment application, 723 genes were significantly upregulated and 403 were significantly downregulated. Comparing the samples at 24 hours after treatment, there were far fewer significantly changed genes. This implies that the initial effect of Bio 20 on gene expression is very rapid," he explains.

Having established which genes Bio 20 has an effect on, what are the main functions of the metabolic pathways where such big changes in gene expression were seen in a very short space of time after application?

This was determined using a functional analysis using two databases of gene function, explains Tom. These were gene ontology, which uses a major bioinformatics classification system, and the Kyoto Encyclopaedia of Genes and Genomes (KEGG) — a collection of manually curated databases containing resources on genomic, biological-pathway and disease information. A protein-protein interaction network was also constructed.

The gene ontology analysis found that the most upregulated genes, three hours after Bio 20 application, were related to catabolic (breaking down) and metabolic processes involving: glucosamine-containing compounds; amino sugars; cell wall macromolecules; and chitin.

Using the KEGG analysis it was discovered that nitrogen metabolism; alanine, aspartate and glutamate metabolism; glyoxylate and dicarboxylate metabolism and ribosome biogenesis were all upregulated three hours after Bio 20 was applied.

Metabolic pathways

So what does this mean? David explains that the most significant metabolic pathways influenced by Bio 20 were responsible for nutrient assimilation and usage; plant growth and plant defence mechanisms, according to the results.

"It helps explain our belief that one of the reasons Bio 20 is so successful is because it's a combination of biostimulant and nutrients.

"A biostimulant alone doesn't have such a strong benefit in a drought situation as we've been seeing with Bio 20 in trials. What the result of the transcriptomics tells us is that in this situation, it's like turning a light on without a bulb in the socket — you need that bulb to make use of the electricity and get light.

"In Bio 20 the biostimulant component is switching the light bulb on — gives the instruction to cells to take up more nutrients — and the nutritional elements are available for uptake via the leaves if the roots aren't able to function properly due to drought," he explains.

That doesn't mean timing isn't important and David advises growers not to wait until crops are flagging before applying Bio 20 to help alleviate drought stress.

"It's best to use it at the beginning of a stress event because once a crop has flagged then it has already lost potential — plants under stress will shut down and will even switch their energy into reproductive growth. That means if the weather turns dry and is forecast to stay dry, then that's the time to apply Bio 20. If the dry period is prolonged, then application could be repeated two weeks after the first application if necessary."

Targeting the right crops is just as important as the right timing if the greatest benefit is to be achieved, he adds.

"It's small, spring-sown crops that will be the most responsive because they have few reserves to withstand a drought, so they will suffer the most. Conversely, if spring crops are fast growing in ideal conditions, then you'd be unlikely to see any significant benefit from applying Bio 20. These plants are able to fend for themselves."

The most significant metabolic pathways influenced by Bio 20 were responsible for nutrient assimilation and usage; plant growth and plant defence mechanisms, says David Booty.

The enhancement of metabolic pathways connected to the plant's defence responses is also worth noting, comments David. "We've long seen a correlation between crop health and how a crop tolerates or resists diseases. The findings of the transcriptomics study seems to back up this observation." ■

Research briefing

To help growers get the best out of technology used in the field, manufacturers continue to invest in R&D at every level, from the lab to extensive field trials. *CPM* Research Briefings provide not only the findings of recent research, but also an insight into the technology, to ensure a full understanding of how to optimise its use.

CPM would like to thank Omex for sponsoring this Research Briefing and for providing privileged access to staff and material used to help bring it together.

Omex operate in over 85 countries around the world, researching and developing a range of liquid fertilisers, including suspension, solution and foliar fertilisers. Omex work with farmers to help sustainably feed a growing population, promoting plant health and crop nutrition to achieve optimum yields. The company is celebrating its 46th year as a family run business.