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Metabolites hold the key

Technical
Biostimulants

Biostimulants have been used on crops for decades but the mechanisms they employ are only just beginning to be understood. *CPM* finds out how studying metabolites is helping elucidate biostimulant modes of action – a knowledge which could make them a precision tool in the future.

By Lucy de la Pasture

Few would argue that biostimulants show promise and that’s reflected by the rapid growth of the industry. But with various degrees of scientific rigour underpinning the c300 products currently available in the UK and variable performance in the field, using them successfully can be somewhat hit or miss.

Part of the problem is the limited fundamental research into their modes of action, says Dr Fidele Tugizimana, biochemist at the University of Johannesburg and for the Omnia Group, the biggest fertilizer producing company in South Africa. He’s working together with the University of Edinburgh on a project, jointly funded by Innovate UK/Department for International Development (DFID) and Omnia, that’s investigating the relationship of plant growth-promoting rhizobacteria (PGPR) and maize using metabolomics.

Most biostimulant studies look at physiological and phenotypic measurements and agronomic observations and draw conclusions on how well a product is working based on this information. Metabolomics offers a different perspective.

Biochemical reactions

“Metabolomics applies different sciences to try to understand the metabolism of a system. It looks at the metabolites, which are involved in different biochemical reactions and this helps us suggest the possible mechanisms that explain how a biostimulant product may be acting on the metabolism of plants.

Fidele describes the metabolome as the ‘chemical space and language of metabolism’, which carries the imprint of genetic and environmental factors and is more sensitive to disturbances to the metabolic flux than either the transcriptome (mRNA) or proteome (proteins).

“In other words, genes and mRNA provide information on the potential of what can happen, enzymes show what could happen, but metabolites show what is actually happening in the plant — at both a cellular and molecular level,” he says.

Dr Karl Burgess, senior lecturer in biological mass spectrometry at University of Edinburgh, adds that the genome doesn’t change. “That’s the beauty of the genome and it gives the whole potential of an organism, so it’s a baseline from which everything else springs. There’s a ‘central dogma’ — a chain that goes from the genome through the transcriptome to the proteome, and then to the metabolome. At each stage, you get closer to the phenotype or what’s actually happening to the organism.”

Practically the field of metabolomics can be a challenging one as changes in the metabolism can happen very quickly. Over the years, it’s become easier as the instruments relied upon — mass spectrometers — have become more capable, with the capacity to analyse hundreds of thousands of molecules at any one time, explains Karl.

“Instead of looking at one metabolite at a time, we can now look at most of them. There are always limitations, you don’t necessarily pick up everything so there are ▸

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Modern equipment is able to analyse hundreds of thousands of metabolites at any one time.

▶ gaps in the data but, in comparison to what we used to be able to analyse, mass spectrometers give us a huge raft of information.

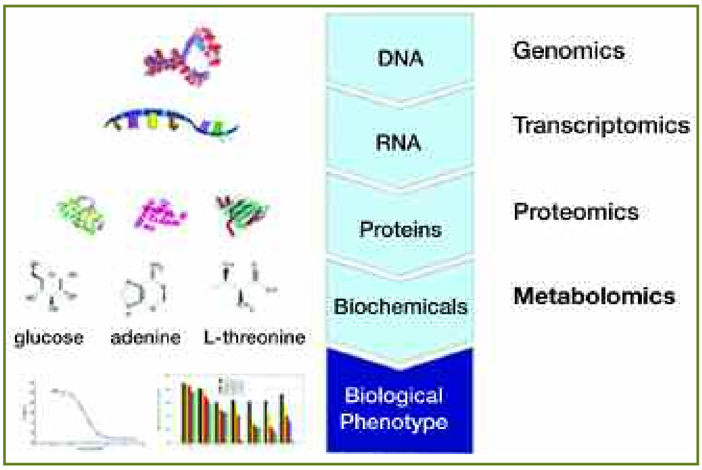
"So if a biostimulants has a mechanism that 'hits' one part of the plant's metabolism, we'll see that. Equally if it 'hits' somewhere else, then we're also know. It's this scale of information that metabolomics provides that sets it apart from other techniques," he says.

Metabolomics can be used in a very targeted way to assess how biostimulants may be interacting with the plant's metabolism, but it can also provide wider information that may pick up unexpected effects. Karl describes this as "answering the question asked and giving free stuff as well."

Most of the biostimulants products used today have been shown to have a beneficial effect in field trials by making physical assessments and then working backwards to explain why this may have happened.

"Using observations, it's possible to draw up a list of positive effects from a biostimulant product but there's a lack of understanding as to why these happen. For example, if one product sometimes works better than another, then when developing a new product it can be difficult to know which basis to formulate on going forward. These are difficult questions to answer due to the lack of basic understanding about how these products work."

By using metabolomics to study PGPRs in maize, Fidele has been able to link positive phenotypic benefits to the different molecular pathways involved and to the different molecular events, such as hormone signalling or any primary metabolisms that have changed, to lead to the beneficial traits.



The central dogma of biology shows that metabolomics gives the closest insight to the phenotypes, or what's actually happening in the plant's metabolism.

Fidele believes that gaining this fundamental knowledge about modes of action will give confidence in biostimulant products and can help to better define how to use products under different conditions.

"In trials using a microbial biostimulant, application at a certain rate produced an effect which can be visibly seen, and this correlates nicely at the metabolomics level. So from this we could conclude that at the molecular level that application rate worked. But if the conditions or the application area changed, we found that at a metabolic level a different application rate worked better.

"Such information provides the knowledge that can guide the biostimulants industry in terms of product formulation and their application," he says.

True biostimulant

Since biostimulants are defined as 'a substance or microorganism that's not a nutrient, pesticide or soil improver but has the ability to promote the health and growth of a plant through the induction of natural biological processes', metabolomics is well placed to help determine whether a product has a true biostimulant effect or not.

Use of metabolomics to study biostimulants is a relatively new application of the science and it's having to play catch up with the other plant science disciplines, where it has already been employed to elucidate metabolic pathways in plant breeding and to study defence priming to biotic and abiotic stresses.

There's emerging evidence which suggests biostimulants can act as priming agents, enabling plants to better withstand periods of abiotic and biotic stresses, says Fidele.

So what exactly is priming? Defence priming is a

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phenomenon whereby the plant immune system and abiotic defences are preconditioned, which results in a faster, stronger and more effective defence and resistance mechanisms against subsequent biotic and abiotic stresses, he explains.

"This immune-stimulation of plants, which is postulated to be an adaptive and low-cost defensive measure, is a result of interactions of plants with beneficial microbes, chemical compounds, insect herbivores or environmental cues.

"In their natural habitats, plants coexist with highly dynamic microbial communities, some of which are harmful to plant health. Environmental constraints, such as drought and extreme temperatures, negatively affect plant growth and development, so for optimal growth and development plants must have a protective immune and defence system that properly integrates both microbial signals and abiotic factors at both local and systemic levels," he explains.

Studies have revealed that plants have evolved to develop active, inducible and tightly regulated immune and defence systems that mediate interactions with diverse environments where they're faced with both biotic and abiotic stresses. It's the result of these dynamic and complex interactions that determines a plant's ability to survive.

"Generally, the first line of

Lerato Nephali says phenotypic evaluations don't guarantee a specific level of biostimulant efficacy and effectiveness under all conditions.

defence in a plant's response to biotic stresses involves preformed physical and chemical barriers — such as cutin and waxes (cuticles), cell walls, antimicrobial enzymes and secondary metabolites — to prevent or attenuate invasion by various pathogens."

When these are breached then danger signals are recognised by plants and a second line of defence is induced at both local and systemic levels. Some of the general defence responses include a highly-regulated cascade of signalling events, activation of defence-related genes, the accumulation of ROS and antioxidant mechanisms, production of defence-related metabolites and subsequent physiological and morphological changes.

Adverse environmental conditions trigger another set of responses, he explains. "One of the primary reactions to abiotic stresses involves a highly regulated and coordinated web of plant hormones such as abscisic acid, auxins, ethylene, cytokinins, gibberellins, salicylic acid, jasmonic acid and brassinosteroids.

"These phytohormones often act as primary signalling molecules that trigger complex signalling cascades, subsequently leading to stimulation of the expression of stress-related genes and the induction of physiological and morphological changes, which eventually lead to abiotic stress tolerance or resistance."

Some of the common physiological responses to major abiotic stresses include a reduction in both transpiration and photosynthesis rates, a decrease in stomatal opening and in leaf water content and a reduced relative growth rate.

"For example, when a plant is under drought stress, it may respond by root swelling to promote nutrient uptake, leaf stomatal closure and leaf rolling to prevent water loss via transpiration and a decrease in chlorophyll content," explains Fidele.

"Younger leaves may be prioritised, with a reallocation of nutrients that were stored in the older and diseased leaves to new leaves or shoots, leading to leaf abscission and a decrease in total leaf area."

Plants often stop growing to minimize the metabolic demands while under water stress and increase the biosynthesis and assimilation of metabolites such as osmolytes, he adds.

"These are also known as osmoprotectants and are low molecular weight, soluble compounds (including betaines, amino acids, polyols and non-reducing sugars (for example, glycine betaine, proline and inositol) that play fundamental roles in osmotic adjustment, and so gives cells protection against drying-out."

Meristem function

Root growth may also be arrested to ensure the functioning of the meristem so that root tissues are in a good position to grow rapidly once the plant is no longer under drought stress. Lateral root growth may be inhibited, while roots may elongate to reach water deeper in the soil profile, he explains.

"Plant metabolism generates reactive oxygen species (ROS), which are key regulators that mediate signalling pathways involved in developmental processes and the plant's responses to environmental fluctuations. This homeostatic mechanism can be knocked out of balance when a plant is under abiotic or biotic stresses which may result in oxidative stress – so plants have antioxidant mechanisms to cope with this."

Lerato Nephali, PhD student under Fidele's tutelage at the University of Johannesburg, was lead author in a paper published in the journal Metabolites last December (Nephali L et al. Biostimulants for Plant Growth and Mitigation of Abiotic Stresses: A Metabolomics Perspective. *Metabolites.* 2020 Dec 10;10 (12):505), which reviewed the knowledge surrounding biostimulants and identified the gaps where fundamental research is still needed.

It's easy to see that just in the example of drought, the plant reacts using many different metabolic pathways to counter the stress. "However, there are very few emerging metabolomic studies on the relationship between biostimulants products and the plant, and even fewer that are investigating the interaction between biostimulants, plant and abiotic stress," she says.

Maize is a staple in the diet of many Africans and biostimulants are thought to have a major role to play in a more sustainable agricultural system.

Fertiliser company Omnia is using metabolomic studies to research the use of plant growth-promoting bacteria in the maize crop.

"Most biostimulants products on the market are based on claims derived from phenotypic evaluations. These don't demonstrate whether a formulation is a bona fide biostimulant, guaranteeing a specific level of efficacy and effectiveness under all conditions," she says.

Karl believes the complexity of plant responses to the environment it's growing in, changes in climatic conditions and the multiple interactions it has with soil microbes means that a big body of scientific knowledge will be necessary to properly understand the relationship between plants and biostimulants.

Fidele describes the work done so far in the Omnia projects as 'a good start'. "We now have some understanding of the different pathways involved in maize when a PGPR is applied and this helps explain biostimulant effects, but there's a lot of work still to do.

"If we can understand the modes of action at another level, then we'll be able to prime a plant better to a stress and understand what the cost of that is to the plant. I believe as knowledge and technology keeps advancing and we understand the mechanisms involved, we'll be able to design products more smartly to get the intended effect and use them in a more precision programmed approach," he concludes. ■