Dr Nicolas Taylor is investigating the mechanisms that allow plants to survive in extreme conditions. In an enlightening interview, he describes what first captivated his interest in these processes and the progress facilitated by mass spectrometry.

What inspired you to research the molecular mechanisms behind plant adaptability?

I was first attracted to the study of plants as I am particularly interested in how the complex biological pathways and networks inside cells change in response to the environment. Plants, being sessile, are highly environmentally adaptable compared to an insect or a mammal that has the freedom to move away from a hostile environment.

To achieve survival, plants must have huge biochemical flexibility to maintain crucial processes like photosynthesis and respiration. These complex processes involve many enzymes, each with a different temperature optima, yet plants can coordinate them all. Similarly, when a complex enzyme such as those of the mitochondrial electron transfer chain (METC) is exposed to increasing salt concentrations, the subunits of these enzymes tend to disassociate and function is lost. However, some plants are well suited to salty soils and their enzymes do not dissociate and continue to function. My research focuses on the molecular mechanisms that overcome these fundamental biochemical limitations and how they influence plant survival.

Why do extremes of temperature and high salinity have negative effects on plants in temperate climates?

Plants grown in temperate climates are often exposed to substantial changes in temperature or soil salinity in their life cycle. A plant may experience a 20 °C temperature shift from early morning to mid-afternoon (any shift of more than 5 °C in human body temperature would be fatal). As the climate changes, it is likely that we will see an increase in the frequency and severity of extreme weather events and this will have a significant impact on agriculture. This is because crops traditionally bred and grown in temperate regions do not have the ability to acclimate to these extreme events and in most cases this will lead to complete crop loss.

The same is true for plants exposed to salinity, if crops have traditionally been bred and grown on high-quality (low-salt) soils, they do not have the metabolic flexibility to be able to adapt to an increasingly salty environment. Thus the relatively rapid increases we are seeing in salinity in our growing regions will have major impacts on production.

Can you provide an insight into how mass spectrometry can be applied to biology?

Mass spectrometry has revolutionised the study of biomolecules in biological systems, allowing the quantitative investigation of many proteins, lipids and metabolites simultaneously. This allows us to assess how the entire proteome (all the proteins), lipidome (all the lipids) and metabolome (all the metabolites) of a plant change in response to variations in its environment. This has led to the emerging research fields of plant proteomics, lipidomics and metabolomics that specialise in applying and improving the use of mass spectrometry to decipher biological questions. With the development of peptide selected reaction monitoring approaches, we can now target and quantify a specific protein in a plant as opposed to survey type methods relied on previously. This revolutionary approach has been referred to as the ‘second coming of proteomics’ due to the step change it has introduced to our research. Today we have the ability to quantify any protein, in any plant, as long as we can detect it by mass spectrometry, and with the almost yearly leaps in mass spectrometry sensitivity, it will not be long before no protein will be out of reach.

In what ways are you seeking to understand how lipid composition in the mitochondrial membrane affects respiratory enzyme activity?

Until recently, the link between membrane composition and enzyme activity within the membrane had not been established. While it was known that the lipid content of plant mitochondrial membranes varied with temperature in order to maintain membrane fluidity and integrity, it was only just shown that changes in the lipid composition of plant mitochondrial membranes can alter respiratory enzyme activity and stability.

We aim to further characterise this phenomenon by investigating how changes in lipid composition required to maintain membrane function during hot and cold events affect the enzyme activity of the METC. Harnessing the power of mass spectrometry coupled to classical plant biochemistry, we will determine the abundance of all the lipid species of the mitochondrial membrane and link this to classical in vitro activity assays of the respiratory enzyme complexes following hot and cold events.
Adapting to extremes

Combining proteomic and lipidomic approaches, University of Western Australia researchers are studying the molecular changes that enable plants to survive the severities of the environment; potentially holding the key to engineering plants capable of growing on marginal land and adaptable to future climate change.

PLANTS ARE ABLE to adapt to extreme conditions in ways that astound humans, surviving vast fluctuations in temperature and in high-salt soils. Aside from being biologically fascinating, this has important implications for society. Worldwide, the amount of land available for crop production is in decline, but the demand for food is increasing. In fact, the Food and Agriculture Organization of the United Nations (FAO) estimates that the global demand for food will increase by 70 per cent by 2050, thereby intensifying the need to utilise low-quality agricultural (marginal) land to grow crops.

Understanding the molecular mechanisms that permit plants to grow in non-optimal conditions could open the doors to the selective growth of plants able to thrive on such marginal land, helping meet the production levels necessary to cope with future food demands.

As part of an Australian Research Council (ARC) Future Fellowship, Dr Nicolas Taylor is studying the complex biological pathways within plant cells that enable them to respond to the external environment, investigating the molecular mechanisms of tolerance to both temperature and salt in a model plant, Arabidopsis, and in wheat. Applying proteomic and lipidomic approaches, his team is revealing the role an important organelle – the mitochondrion – plays in these adaptive processes.

PROTEIN RESPONSE

One of the most common environmental challenges plants face is changing temperature. Temperature fluctuations affect the vital metabolic processes of photosynthesis and respiration and can also impact cellular maintenance and the synthesis of biomolecules. Indeed, thermal stress events are a major agricultural problem across the globe and a significant environmental factor – second only to water availability – limiting the productivity and distribution of plants.

Temperature stress can cause a host of changes – anatomical, physiological and biochemical – that impede plant growth and development. However, plants have evolved a wide range of responses to cope with this stress. These tolerance mechanisms enable plants to survive a wide range of temperatures, through a process called thermal acclimation. When exposed to a sub-lethal change in temperature during growth, plants can adjust their metabolism to survive in more extreme conditions. Amazingly, their respiratory and photosynthetic rates remain similar to those grown at normal temperatures, yet the molecular mechanisms underlying this response are unresolved.

However, there are indications. Proteins within the plant mitochondrial electron transfer chain (METC) which drives respiration – the set of reactions that produce the cellular energy source adenosine triphosphate (ATP) – have been shown to respond to changing temperature. This raises the question of whether specific changes in the abundance of certain proteins enable metabolic homeostasis. Taylor, in a project continuing through to 2017, hopes to answer this question by investigating the precise role of these proteins in respiratory acclimation.

A LIPID PARTNER

It is not only proteins that are important. The other key component of membranes, lipids, also play a role in this response. Although it is an established fact that the lipid content of plant membranes varies with temperature to maintain membrane structure, recent research has shown that these changes can also alter the activity of respiratory enzymes. It therefore seems that changes in the lipid composition of mitochondrial membranes during temperature fluctuation could be bifunctional, maintaining both membrane fluidity and respiratory function.

To investigate this further, Taylor will conduct a number of lipidomics studies to assess both characterised and still uncharacterised lipids.

Considering climate

The temperatures in which plants grow are changing as a result of global climate change, with increasing mean temperatures and also greater extremes. In Australia extreme thermal events are thought to cost the wheat production industry millions of dollars a year.

In the future, climate will vary further and conditions will become more severe. Understanding how plant growth responds to different temperatures is critical for predicting the impact of climate change on life on Earth. Taylor hopes his work will provide the knowledge needed to breed certain traits so plants can survive in this new environment.
mitochondrial lipids. These lipid and protein studies work in synergy, enabling Taylor to build a picture of the abundance of enzymes within the inner mitochondrial membrane at a specific temperature, alongside that of lipid species. Coupling the two, he aims to elucidate the precise impact of changing lipid composition is enzyme function.

**SHIFT IN THINKING**

Since proteomic approaches emerged 10 years ago, researchers across the world have utilised a multitude of approaches to determine if the proteins of the large respiratory complexes of the METC change in response to the environment, as explained by Taylor: "Many believed this to be the case, but no evidence could be found to support this theory. Some researchers resigned to the fact that these complexes were stable and the changes in its activity were due to changes in the availability of substrates".

Taylor changed this thinking in 2010 when his team was able to characterise the abundance of over half of the known proteins in the METC following stress treatments. The most comprehensive study to date, it elucidated the fact that the METC actually significantly alters its protein composition in response to the environment. This was a shock to the field, and also changed how Taylor thought about the environment. This was a shock to the field, and now plans to utilise these to gain a comprehensive understanding of how the METC enzymes will enable us to identify the crucial components that allow plant mitochondrial metabolism to be maintained," he explains. Taylor hopes that this information, when combined with that of other plant scientists, will build a framework of enzymes essential for plant survival under extreme conditions. "Ultimately, I hope this knowledge will contribute to programmes to breed or engineer plants with alterations in these enzymes that are more thermally or salinity tolerant,” he concludes.

**SURVIVING IN SALT**

Alongside the challenges of temperature, Taylor is also interested in what happens to plants when they encounter high concentrations of salt — a growing challenge in agriculture. "Salinity is becoming an increasing problem worldwide, caused by a rising water table or from decades of irrigation," Taylor explains. So, like temperature, understanding how plants react to salinity is also critical to future food security.

Recently, Taylor revealed a protein that may be key in protecting mitochondrial metabolism during salt exposure. Through a series of studies, his group identified a particular form of a mitochondrial enzyme that may confer resistance to high salinity. Mitochondrial manganese superoxide, dismutase (MnSOD) detoxifies superoxide, a potentially harmful reactive oxygen species (ROS), and when overexpressed in Arabidopsis is critical to salinity tolerance. "Interestingly, in wheat we observed that one particular isoform of this enzyme was more highly expressed in salt-tolerant plants," Taylor adds.

Taken together, these findings indicate that higher levels of MnSOD during exposure to salt allow the plant to survive by detoxifying ROS. It is unlikely that MnSOD alone is the ‘silver bullet’ to enhance wheat’s tolerance to salinity, but used in concert with other proteins from further studies it has huge potential to contribute to more tolerant wheat lines.

**NOVEL BREEDING PROGRAMMES**

Taylor’s work over the coming years will provide important information on the mechanisms that allow plants to adapt their respiration to temperature change. This is critical considering that the future will likely bring a warmer climate and more extreme temperature events.

His team has developed a number of innovative mass spectrometry approaches, and now plans to utilise these to gain a comprehensive understanding of the changes in metabolites, proteins and lipids within plant mitochondria in response to extremes of temperature and salt. "Linking this information with measurements of respiration and the activity of METC enzymes will enable us to identify the crucial components that allow plant mitochondrial metabolism to be maintained,” he explains. Taylor hopes that this information, when combined with that of other plant scientists, will build a framework of enzymes essential for plant survival under extreme conditions. "Ultimately, I hope this knowledge will contribute to programmes to breed or engineer plants with alterations in these enzymes that are more thermally or salinity tolerant,” he concludes.

**INTELLIGENCE**

**PROTEOMIC AND LIPIDOMIC APPROACHES TO STUDY THE ROLE OF PLANT MITOCHONDRIA IN THERMAL ACCLIMATION AND SALINITY TOLERANCE**

**OBJECTIVES**

- To determine how the abundance of mitochondrial proteins and membrane lipids in Arabidopsis and wheat vary in different tissues and their effect on respiration rate and ATP production
- To assess the abundance changes of mitochondrial proteins and membrane lipids of Arabidopsis and wheat during thermal acclimation and salinity treatment, to identify key components that limit mitochondrial respiration and ATP production
- To characterise the abundance of mitochondrial membrane lipids and proteins in Arabidopsis ecotypes and wheat varieties with high and low respiratory rates following thermal acclimation and shock to identify key components that limit mitochondrial respiration and ATP production

**KEY COLLABORATORS**

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