Australian National University

Research School of Biology Summer Scholars ePortfolio

2017/2018

ANU COLLEGE OF SCIENCE

RESOLVING TAXONOMY OF THE *GEHYRA AUSTRALIS* CRYPTIC SPECIES COMPLEX

Audrey Miranda Prasetya, Monash University

Supervisor: Craig Moritz

Audrey Miranda Prasetya.

Genetically divergent, yet morphologically convergent?

The *Gehyra australis* cryptic species complex is a group of gecko species found in the northern areas of Australia that are 'cryptic' as they form distinct genetic clusters but are very hard to differentiate from one another phenotypically. Using previously analysed genetic data, the aim of my project is to provide corroborating evidence using morphological analyses to see whether the nine potential candidate

lineages from the genetic data are reflected in (very) subtle morphological differences.

Taxonomy is important not only for the sake of science but because the number of species is the most important indicator of biodiversity. We can't protect the environment if we don't know what is in it. With the increasing trend of global biodiversity loss due to environmental change, taxonomy provides the important resource necessary for conservationists, land managers, and decision-makers to mitigate this.

I started the project doing quantitative comparison methods where we use body measurements of each gecko specimens for important traits (i.e. snout-vent length, trunk length, etc.) and do fancy statistical comparisons for each lineage to find a combination of traits that are distinctive to a lineage (or not). We then continued with qualitative methods including staring at photos of dead geckos and recording their colouration and back pattern then squinting at head scales and chin shields in the isolated Museum room.

Our current findings seem to have enough evidence to justify the naming of several (if not all) of the candidate lineages. My findings are currently in the process of further review using additional specimens and further genetic screening from other sources. We will be looking to formally described these new species soon!

The SRS program has been an incredible research opportunity for me. The community at the RSB – especially those in the Moritz lab, have been immensely supportive by mentoring and sharing their vast repertoires of knowledge. I must say that one of the other greatest highlights of the program is being welcomed to the series of festivities and food we experience including the HDR conference, RSB 50th anniversary event, RSB-Fenner Christmas party - the list goes on. I've felt really welcomed to the lab and that I've contributed a great thing to science during my two months here at the ANU!

SCREENING FOR NOVEL ANTI-MALARIAL COMPOUNDS

Benjamin Lam, University of Melbourne

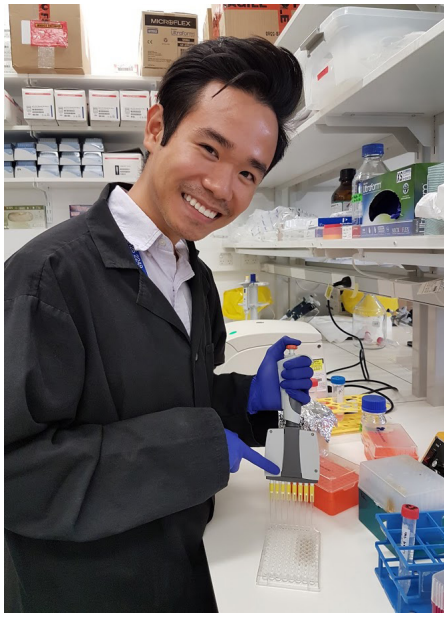
Supervisors: Adele Lehane and Dongdi Li.

My project, done in the Kirk & Lehane Lab, focused on assessing new antimalarial compounds for their ability to interfere with the malaria parasite's membrane potential. Together with Dongdi Li, I performed fluorescence measurements with the bis-oxonol dye to study changes in the parasite's membrane potential in response to experimental compounds with high time-resolution. The findings were quite interesting, to say the least. Many of the compounds were found to interfere with membrane potential; most of these had not previously been reported to disrupt any of the parasite's known regulators of membrane potential.

The results may be useful to pinpoint the specific mechanism of action of these compounds.

To be involved in drug discovery research is an exciting opportunity for me, as this is a field I have not had a chance to experience before. It not only gives breadth to my university degree in Microbiology and Immunology, but also brings a great perspective of how the work done in the lab can one day change our world for the better.

I am thankful to ANU, the Research School of Biology and the Kirk & Lehane lab for this amazing experience and I have truly had my interest in research confirmed!



Benjamin Lam.

ASSESSING THE EFFECTS OF CYTOKININ ON PSEUDONODULE DEVELOPMENT IN *M.TRUNCATULA*

Ruoxi Lin, ANU

Supervisors: Ulrike Mathesius, Jason Ng.

A nodule is a root organ that forms after the successful rhizobia infection. Forming nodules can help the plants to fix atmospheric nitrogen and subsequently promote yields. However, the nodulation process can only be found in some plants, and the majority of them are in the legume family Fabaceae. So we ask: what makes it almost exclusively seen in legumes?

By far there are three types of hormones that have been identified to play an important

role in nodulation progress: cytokinin, flavonoid and auxin. Interestingly, if we apply only these hormones to the Root nodule symbiosis (RNS) plants, they can form pseudonodules, which are morphologically similar to nodules but lack the ability to fix nitrogen. We hypothesize that the pseudonodule formation pathway should follow the same pattern nodulation, hence we can use mutants and external hormones to manipulate the pseudonodule formation and then show the mechanisms behind nodulation. For my summer projects, I mainly focused on three things:

- Evaluate the effects of cytokinin on the root-flavonoid content of M.truncatula at different time points ;

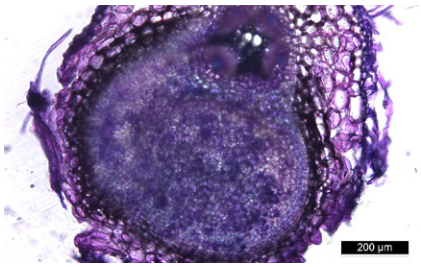
- Assess the spatial distribution of auxin in pseudo-nodulation progress using GH3:GUS construct;

- Determine the role of certain genes which perceive cytokinin in M.truncatula.

All the works were done on Medicago truncatula, a model RNS plant. Both microscopy work and Qtof-MS/MS were involved in this study. MS/MS were used to measure the concentration of flavonoid content after the roots were treated with 6-Benzylaminopurine (BAP), a synthetic cytokinin. Microscopy works were done for mapping auxin distribution and to determine whether the mutants can form pseudonodule after treated with BAP.

A few things were found at the end of this project: a. one of the flavonoid involved in nodulation progress, morin, has a significantly lower concentration 6h after treated with BAP. Based on an earlier study done in this lab, we suspect that naringenin, a flavone that is upstream to morin, might be inhibited by cytokinin, and this inhibition is crucial for nodulation; b. the GH3:GUS plant is defective in forming pseudonodule, therefore we did not get a result for our second aim; and c. SKL, NIN and NSP genes should all be upstream of the cytokinin receptor gene.

Highlights of SRS program: As a science student from ANU, SRS program let me to participate in research projects at a relatively early stage in my undergraduate study. It also provides me the opportunity to experience day to day research life, which is definitely helpful for me to see whether I fit in this kind of working environment.



Root section of M. truncatula wild type (A17) 6 days after inoculated with Sinorhizobium Meliloti. The bump at the bottom left is a nodule that is about to protrude out.

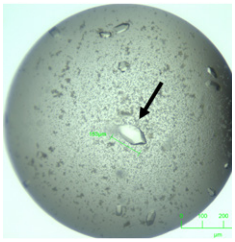
STRUCTURAL STUDIES OF FUNGAL WEAPONS

Olga Palmer, Victoria University of Wellington

Supervisor: Simon Williams, Solomon lab.

Olga Palmer.

Effector proteins are secreted by plant pathogens, such as fungi, to aid in infection of plants and to modulate plant immunity. Knowledge of the protein structure of fungal effector proteins, utilising X-ray crystallography, may help inform us how effectors function in pathogen virulence. Once we understand how these effector proteins function we can work towards creating plants that are resistant to them, reducing the devastation of these pathogens on important crops, such as wheat. To do this, we produced the protein of interest in Escherichia coli. We then lysed the E. coli to release the protein and isolated it using a series of liquid chromatography techniques, including affinity and size-exclusion chromatography. We attempted to generate protein crystals by utilising a method known as vapour diffusion in conjunction with the C3 facility in Melbourne.



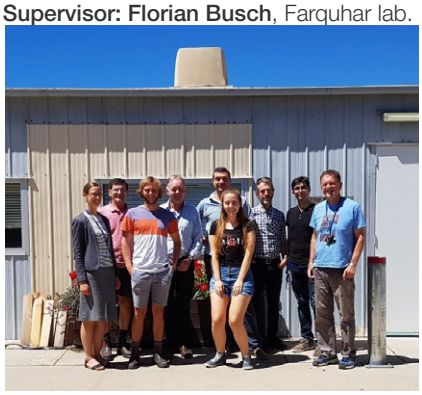
Protein crystal formation

We obtained promising protein crystal formations for some of the effector proteins we purified. These crystals will now be used in X-ray crystallography experiments and we hope to be able to derive the structure. The structure will hopefully help inform us about the function of the effector proteins. Being awarded this scholarship gave me the opportunity to work alongside experts in the field in a world-class facility. This equipped me with the core knowledge and training required for further study as a postgraduate student.

ARE MEASUREMENTS OF PLANT GAS EXCHANGE ACCURATE?

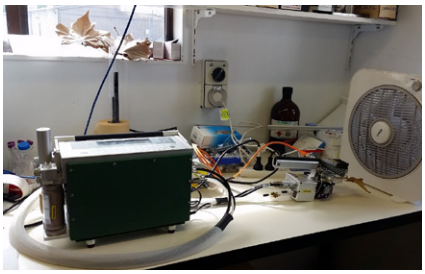
Katie Purdy, Western Sydney University

Supervisor: Florian Busch, Farquhar lab.



Katie Purdy (centre) with members of the Farquhar group.

My project aimed to examine how leaks in the Li-Cor (machine that measures plant gas exchange) chamber affect measurements of photosynthesis. This is important because measurements taken by the Li-Cor are frequently used in models to draw conclusions about how the plant's biochemistry is working and to make predictions about growth and plant productivity, from the leaf level to the global scale. If the measurements put into the models aren't accurate, model outputs and any conclusions about what the plant is doing may also be inaccurate. Therefore, my summer project aimed to determine what the leak function looked like graphically, how this leak can be corrected for, and whether having a leak significantly affects model outputs.



Katie's experimental Li-Cor set-up

To look at this, my supervisor and I took various measurements with the Li-Cor, including with and without plants, and with dead leaf material, to separate structural leaf properties from its biochemical properties. We then plotted the measurements, fitted equations to what we had observed (to be able to quantify the effect of the leak), and then corrected live plant measurements of carbon assimilation. Our results demonstrated that the shape of the leak function is quadratic, with its exact shape differing depending on how the measurements were taken. Results suggested that the nature of the function is due to CO_2 absorption in the tubing of the Li-Cor. Our results also indicated that leak correction is important in accurately estimating model parameters, as there was a distinct difference between corrected and uncorrected data

estimations.

I really enjoyed the entire summer scholarship experience, but one of the highlights was being mentored by my supervisor. I find that I learn a lot better one-on-one, and there were so many opportunities for this to occur. I got to ask a lot of questions and have aspects of my project, the lab's work, and important background concepts explained to me. I also loved living on campus and getting to explore Canberra with the other summer students.

RIESKE-Y BUSINESS: OVEREXPRESSING THE RIESKE FES PROTEIN TO INCREASE CYTOCHROME B6/F COMPLEX IN *SETARIA VIRIDIS*.

Tegan Norley, ANU

Supervisor: Masha Ermakova, Von Caemmerer lab.

Objective

To genetically engineer overexpression of the Rieske FeS protein in Setaria viridis to determine whether this would cause an increase in cytochrome b6/f complex, and if so, if this overexpression would increase biomass, yield and photosynthetic efficiency.

Importance

Previous antisense and overexpression studies on C3 crop species have shown positive correlation between decreasing/increasing Rieske FeS and cytochrome b6/f complex content. We wanted to see whether this same correlation exists in species that adopt the C4 photosynthetic pathway.

Research approach

Setaria viridis plants were transformed to increase Rieske FeS. One line was targeted in both bundle sheath and mesophyll cells, while two other lines were targeted in only the mesophyll cells. Plants were screened with the multispeQ device to analyse non-photochemical quenching (NPQ) as a proxy to determine successful transformation. Plants were further analysed using fluorescence and P700 measurements on the Dual-PAM-100, gas exchange with the LiCOR, and blotting for protein content.

Findings

From each line several overexpression mutants were found and analysed, all with reduced NPQ. It is still unknown why NPQ is lower in both overexpression and antisense mutants. We now know it is possible to increase cytochrome b6/f in C4, but the study must be continued to discover whether it significantly improves yield.

INVESTIGATING A POTENTIAL INTERSECTION BETWEEN HORMONAL AND CHLOROPLASTIC SIGNALING IN PLANTS DURING DROUGHT STRESS.

Suyan Yee, *ANU*

Supervisor: Kai Xun Chan, *Pogson lab.*

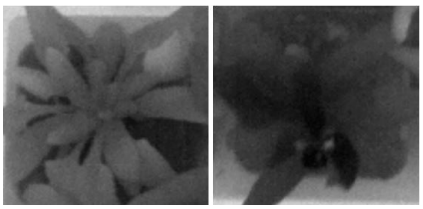
Changing climatic conditions have exacerbated periods of drought stress, which affects plants negatively, and have resulted in large economic and agricultural (crop yield) losses. Thus, improving drought tolerance in plants is of particular interest to scientists, so as to ensure future food security.

One way to achieve this is to investigate the relationship between the hormonal (abscisic acid; ABA) signaling and chloroplast stress signaling pathways under drought. Understanding the molecular pathways and interactions involved in drought stress signaling will enable us to manipulate and engineer plants that are more drought tolerant, ultimately increasing plant survivability under drought, and maintaining crop yields.

To accomplish this goal, one of the things I did was measure the leaf temperatures of several ABA signaling mutants that were placed under drought stress, using an IR camera. In this case, leaf temperature was a proxy for stomatal closure, as plants with open stomata are generally cooler than plants with closed stomata, due to transpirational cooling (water loss) occurring in the former. Ultimately, a clear temperature increase was seen in the wild types (WTs) under drought, which indicates functional stress signaling pathways, resulting in stomatal closure.

Being part of the ANU Summer Scholars & Interns cohort for Biology enabled me to network with, and meet budding scientists from other biological science disciplines, universities, and countries.

The same Arabidopsis plants (wild types and mutants) on Day 0 (left) and Day 11 (right) of drought.



Infra Red images of Wild Type Arabidopsis (left) vs a drought sensitive mutant under drought stress (right). Lighter areas indicate higher temperatures whereas darker areas represent lower temperatures.