Melbourne-Potsdam PhD Programme (MelPoPP)
Joint projects

Application round 2020/2021

Participating Institutions

University of Melbourne, Australia

Max Planck Institute of Molecular Plant Physiology, Germany

University of Potsdam, Germany

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TABLE: Overview of joint projects | Melbourne-Potsdam PhD Programme | project start: 2021

Legend:
UoM, University of Melbourne; MPI, Max Planck Institute of Molecular Plant Physiology; UP, University of Potsdam;
"Home base" indicates the institution where projects are designed to start and where the candidate will spend more time. At least one year is spent at the "host" institution.
* The columns "Year 1, 2, 3 spent at" indicate approximate timing; exact times may need to be adjusted depending on the project needs.

Project code ID	**Project title**	**PIs / Supervisors MELBOURNE**	**PIs / Supervisors POTSDAM**	**Home base**	Year 1 spent at *	Year 2 spent at *	Year 3 spent at *	**Methods**
P1_BE+JH+JL+MS_1220	Understanding how plants mobilize carbon to build trees	Berit Ebert, Joshua Heazlewood	John Lunn, Mark Stitt	**UoM**	UoM	MPI	UoM	Experimental: Nucleotide sugar, cell wall metabolite + Tre6P analyses via LC-MS/MS, $^{13}CO_2$ labelling, co-IP, confocal microscopy, molecular biology
P2_HV+UR+HMC+ZN_1220	How are phytoplankton metabolism and composition affected by seawater chemistry?	Heroen Verbruggen, Ute Roessner, Harry McClelland	Zoran Nikoloski	**MPI**	MPI	UoM	MPI	Interdisciplinary - experimental & mathematical modelling: phytoplankton growth experiments, transcriptomic + metabolomic profiling, metabolic network construction + modelling
P3_JG+MH+JL_1220	Role of sugar signalling in the control of seed size	John Golz, Mike Haydon	John Lunn	**MPI**	MPI	UoM	MPI	Experimental: Physiology, molecular biology, work with Arabildopsis and *Marchantia polymorph*a (liverwort), Tre6P analyses via LC-MS/MS, pharmacological screen for compounds affecting seed size, *in vitro* activity studies on TPS, TPP and SnRK1 *Marchantia*
P4_MP+DW+ML_1220	Reclaiming the lost genetic diversity in barley	Mohammad Pourkheirandish	Dirk Walther, Michael Lenhard	**UoM**	UoM	MPI /UP	UoM	Interdisciplinary - experimental & genomics: Combination of physiology and phenotyping, genetics, genomics / bioinformatic large-scale data analysis (GWAS), molecular biology
P5_MW+ZN_1220	How do plant roots sense and adapt to soil water? An experimental and modelling study using root cap cells	Michelle Watt	Zoran Nikoloski	**UoM**	UoM	MPI /UP	UoM	Interdisciplinary - experimental & computational analysis and modelling: Combination of physiology, image-based phenotyping, metabolome and transcriptome data acquisition and modelling; isolation of root cap cells, biochemistry, molecular biology, genetics , computational analysis and metabolic modelling
P6_UR+JK+JH_1220	How translational mechanisms contribute to low temperature acclimation in plants	Ute Roessner, Joshua Heazlewood	Joachim Kopka	**MPI**	MPI	UoM	MPI	Experimental, with Omics data analyses: Proteome, transcriptome and translatome analyses, LC-MS, Waters Vion QTOF LC/MS, molecular biology, physiology

Project code: **P1_BE+JH+JL+MS_1220**

Understanding how plants mobilize carbon to build trees

Supervisors:
Dr John Lunn and Prof Mark Stitt - Max-Planck Institute of Molecular Plant Physiology
Dr Berit Ebert and A/Prof Joshua Heazlewood - University of Melbourne

Links to supervisor websites:
Joshua Heazlewood - http://www.heazleome.org/
Berit Ebert - https://blogs.unimelb.edu.au/ebert-lab/
Mark Stitt - https://www.mpimp-golm.mpg.de/9333/Mark_Stitt

Background:

The plant cell wall constitutes the most abundant renewable bioresource on the planet with about 160 billion tonnes produced each year. While representing a major sink for global CO_2, cell walls are a vital source of materials for construction, textiles and feedstocks for the chemical industry, as well as a globally important renewable fuel source. Cell wall biosynthesis is a highly intricate process that involves many hundreds of enzymes and an array of metabolic reactions, intracellular transport of proteins and wall precursors, to assemble the different cell wall polymers. Growing cells are surrounded by the primary cell wall, which provides mechanical strength but can also expand. The much thicker and stronger secondary wall is deposited after the cell has stopped growing, and accounts for most of the carbohydrate in plant biomass.

This transition from a flexible primary cell wall to a rigid secondary cell wall represents a major developmental change requiring extensive metabolic re-programming. This includes changes in the patterns of cellulose deposition, a reduction in pectin biosynthesis, changes to hemicellulose composition (increased xylan) and the inclusion of lignin (Fig. 1). Such a transition is also likely to have major impacts on photosynthesis, sucrose and starch synthesis, amino acid biosynthesis, glycolysis and the TCA cycle. While studying this transition in plants has been difficult, in recent years a simple experimental system has been developed that enables the controlled induction of secondary cell wall development through a transcription factor (VND7). Thus, VND7 inducible plants provide a unique system to examine the controlled transition from primary to secondary cell walls.

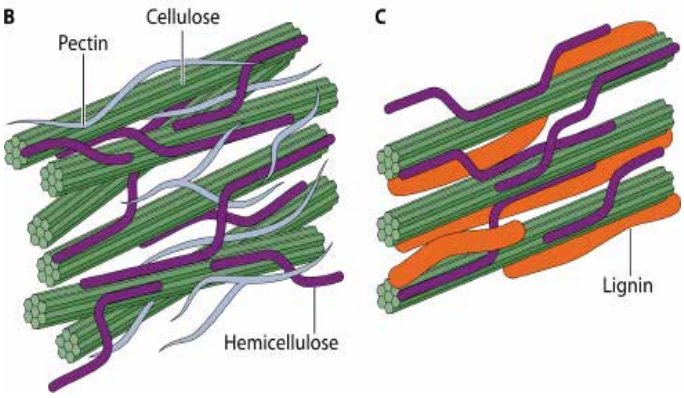


Fig 1. Primary (left) and Secondary (right) Cell Walls

This project will involve carbon isotope labelling studies to provide information about the rates of synthesis of cell wall components and primary metabolism during the transition from primary to secondary cell walls. The project will employ photosynthetic fixation of $^{13}CO_2$ using stable (^{13}C) carbon isotopes (Fig. 2) followed by liquid and gas chromatography coupled to mass spectrometry to determine ^{13}C changes in both nucleotide sugars (LC-MS/MS), the composition of assembled cell wall polymers using HPAEC-PAD / GC-MS and a range of primary metabolites by mass spectrometry.

The project will also address the role of sugar signalling, especially the sucrose-signal trehalose 6-phosphate (Tre6P), during the transition to secondary cell walls. Our unpublished work indicates that TPS1 – the main Tre6P-synthesizing enzyme – interacts with the primary cell wall biosynthetic apparatus. The project will exploit the VND induction system to perform co-immunoprecipitation (co-IP) experiments with TPS1 during the induced transition

to secondary cell wall synthesis, to test whether TPS1 also interacts with the secondary cell wall machinery, and to investigate levels of Tre6P and other central metabolites during the transition to secondary cell walls.

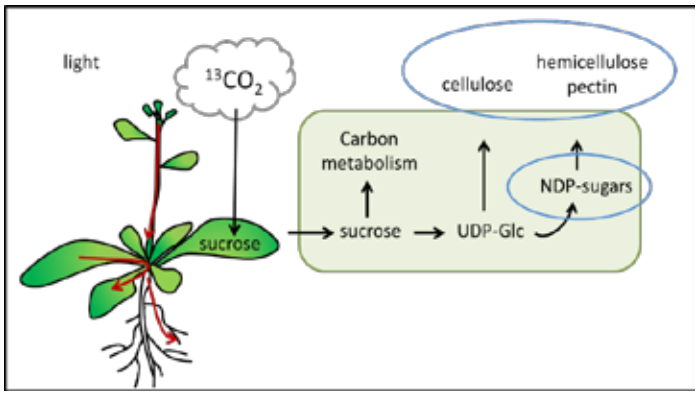


Fig 2. Schematic of $^{13}CO_2$ labelling experiments modified from Verbančič et al., 2017

Depending on initial results, we will also cross VND expressing lines into mutants disturbed in Tre6P synthesis and signalling to learn if Tre6P has an important role in coordinating metabolic reconfiguration during secondary cell wall induction. A major component of secondary cell walls is the hemicellulose xylan, which contains a xylose backbone derived from UDP-xylose. UDP-xylose biosynthetic and transport mutants will be used in combination with ^{13}C labelling to elucidate the pathway and subcellular compartmentation of xylan biosynthesis. Xylan appears to originate from a cytosolic source of UDP-xylose (UDP-Xyl) that is transported into the Golgi. Curiously, plants also biosynthesise a Golgi source of UDP-Xyl but this seems to be a precursor for UDP-arabinose biosynthesis within the Golgi. We have generated mutants that are unable to biosynthesize the cytosolic or lumenal forms of UDP-Xyl and these will be employed to understand how these metabolic pools are used during the transition to secondary cell walls.

This project will examine how the transition to secondary cell walls impacts carbon metabolism and cell wall biosynthesis. Outcomes should provide significant insight into how carbon is being mobilized to form the secondary cell wall –one of the most important renewable resources on the planet.

Proposed timeline:

First year: UoM – (i) generate inducible-VND7 mutants; (ii) optimize induction conditions and perform time course induction experiment; (iii) prepare soluble extracts from samples to measure nucleotide sugars (LC-MS/MS), and send samples to MPIMP for Tre6P analysis; (iv) hydrolyse insoluble (cell wall) material and send samples to MPIMP for sugar analysis (LC-MS/MS); (v) depending on the result, initiate crossing of inducible-VND7 lines with Tre6P mutants; (vi) selection and validation of cell wall (xylan biosynthesis) mutants.

Second year: MPIMP – (i) $^{13}CO_2$ labelling of Arabidopsis wildtype, inducible-VND7 and UDP-Xyl mutants; (ii) isotopomer analysis of soluble metabolites (nucleotide sugar analysis at UoM) and sugar monomers from cell wall hydrolysis using LC-MS/MS; (iii) co-IP experiments on induced and non-induced VND7 lines; (iv) immunoblotting and proteomic analysis of co-IP samples; (v) if (iv) indicates interaction of TPS1 with secondary cell wall synthesis machinery, cross established GFP-tagged TPS1 lines with RFP-tagged CesA4/CesA7/CesA8 lines to investigate interaction *in vivo*.

Third year: UoM – (i) ^{13}C data analysis; (ii) confocal microscopy of GFP/RFP-tagged lines; (iii) write thesis.

Selected publications:

1. **Ebert B**, Rautengarten C, Guo X, Xiong G, Solomon PS, Smith-Moritz AM, Herter T, Chan LJG, Adams PD, Petzold CJ, Pauly M, Willats WGT, **Heazlewood JL**, Scheller HV (2015) Identification and characterization of a Golgi localized UDP-xylose transporter family from Arabidopsis. *Plant Cell* 27: 1218-1227
2. Verbančič J, **Lunn JE**, **Stitt M** and Persson S (2018) Carbon Supply and the Regulation of Cell Wall Synthesis. *Molecular Plant* 11: 75-94
3. Figueroa CM, **Lunn JE** (2016) A tale of two sugars - trehalose 6-phosphate and sucrose. *Plant Physiology* 172; 7–27

Project code: **P2_HV+UR+HMC+ZN_1220**

How are phytoplankton metabolism and composition affected by seawater chemistry?

Supervision team

Potsdam

Prof. Dr. Zoran Nikoloski (primary, UP / MPIMP)
Web: https://www.uni-potsdam.de/en/ibb-bioinformatik/index / https://scholar.google.com/citations?user=N4oI0yQAAAAJ&hl=en&oi=ao

Melbourne

Prof. Dr. Heroen Verbruggen (primary, UoM)
Web: http://phycoweb.net/ / https://scholar.google.com/citations?user=wrySKuIAAAAJ&hl=en

Prof Dr. Ute Roessner (UoM)
Web: https://roessnerlab.science.unimelb.edu.au/ / https://scholar.google.com/citations?user=aTmdqpYAAAAJ&hl=en&oi=ao

Dr. Harry McClelland (UoM)
Web: https://scholar.google.com/citations?user=V-2oxskAAAAJ&hl=en

Introduction

Phytoplankton carbon fixation in the surface ocean is a sink of atmospheric CO_2, and is affected by seawater chemistry via its effect on phytoplankton composition (Falkowski et al. 2004). Studies have shown substantial differences in the types of algae dominating phytoplankton assemblages through time, with green algae being dominant in the Paleozoic and diatoms in present-day conditions. The causes of these changes may include interspecific differences in fitness as a function of seawater chemistry (Giordano et al., 2018), but many questions remain about the nature of phytoplankton metabolic responses to seawater chemistry at the cellular level. The project aims to evaluate how phytoplankton physiology is affected by alterations of seawater conditions using a combination of computational metabolic modelling and experimental work. A series of simple but elegant experiments mimicking different paleo-oceanic conditions will be carried out, with a specific focus on CO_2 concentrations at different times in the last 500 million years, to evaluate their physiological and metabolic effects. The project will rely on complementary techniques and methods from metabolic modelling (Kim et al., 2017) along with transcriptional and metabolic profiling of cultures grown in different conditions. We will study representatives of different phytoplankton groups (cyanobacteria, diatoms, green algae, haptophytes, dinoflagellates) exposed to different seawater regimes. Once an understanding of metabolic responses of individual species has been gained, it becomes possible to study how different organisms act as part of a community and contribute to explaining the relative dominance of different phytoplankton groups in the ocean at different times.

Key questions

1. What are the genes and metabolites that show the largest changes between different sea water chemistry regimes? Are there cellular processes and molecular functions enriched in differentially expressed genes and metabolites common to all species or exclusive to a species?
2. What are the differences in the physiology and metabolic responses of representatives of the main phytoplankton groups to different sea water chemistry?

3. Can computational models be developed to mimic phytoplankton growth on different seawater chemistry?
4. Do the predictions of the computational models match the observations from the omics profiling? What physiological constraints should be imposed to match the observations from omics data?

Hypothesis

Changes in metabolism as a result of altered seawater chemistry alter the excretion and uptake of particular molecules, which in turn affect phytoplankton metabolism and composition.

Scope of work

This project will be based at University of Potsdam / Max Planck Institute of Molecular Plant Physiology with a minimum of 12 month stay at The University of Melbourne after the first year.

You will carry out an extensive literature review on algal nutrient stoichiometry and the influence of ocean chemistry on plankton physiology. Based on this knowledge, you will design a project that spans computational and experimental approaches. From the experimental perspective, you will design phytoplankton culturing experiments to measure growth of different species in media representing relevant present-day and paleo-ocean conditions, differing in critical nutrients like CO_2, sulphate and trace elements. In addition to growth, you will prepare cultures for transcriptomics and metabolomics profiling to determine responses to these conditions at the cell physiological level. You will run experiments on individual species and on mixtures of species to evaluate competition by monitoring community dynamics, again at the cell count and physiological levels. The computational component of the project consists of metabolic modelling in two phases: (1) Metabolic networks of the cyanobacterium *Synechocystis spp.* (Knoop et al., 2013) and the diatom *Phaeodactylum tricornutum* (Smith et al., 2019) as well as several green algae are available and can be used to make predictions about changes in physiology due to altering inputs from the environment. (2) Metabolic networks will be created for the remaining species of interest to facilitate community modelling following well-established protocols.

References

Falkowski *et al.* (2004) The evolution of modern eukaryotic phytoplankton. *Science* 305:354-360. https://dx.doi.org/10.1126/science.1095964

Giordano *et al.* (2018) A tale of two eras: Phytoplankton composition influenced by oceanic paleochemistry. *Geobiology* 16:498-506. https://doi.org/10.1111/gbi.12290

Kim *et al.* (2017) Current state and applications of microbial genome-scale metabolic models. *Current Opinion in Systems Biology* 2:10-18. https://doi.org/10.1016/j.coisb.2017.03.001

Knoop *et al.* (2013) Flux Balance Analysis of Cyanobacterial Metabolism: The Metabolic Network of *Synechocystis* sp. PCC 6803. *PLoS Comp. Biol.* 9: e1003081. https://doi.org/10.1371/journal.pcbi.1003081

Smith *et al.* (2019) Evolution and regulation of nitrogen flux through compartmentalized metabolic networks in a marine diatom. *Nature Comm.*10: 4552. https://doi.org/10.1038/s41467-019-12407-y

Project code: **P3_JG+MH+JL_1220**

Role of sugar signalling in the control of seed size

Home institution: Max Planck Institute of Molecular Plant Physiology
Host Institution: University of Melbourne

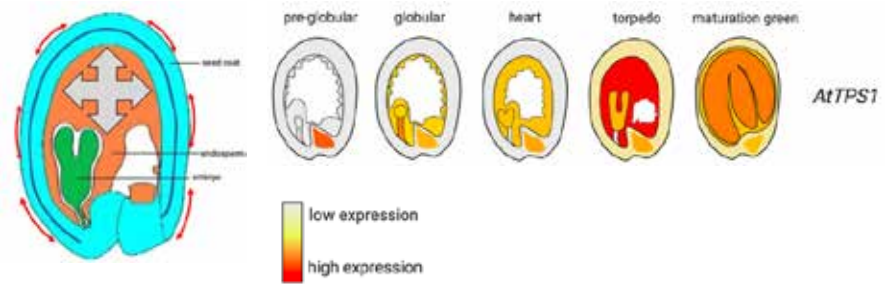
Supervisory team:

- **Dr John Golz** (https://blogs.unimelb.edu.au/golzlab/)
  Dr Golz is a plant developmental geneticist who studies various aspects of seed development including patterning of the embryo, differentiation of the seed coat and the control of seed size. He also uses *Marchantia* as a model system to study the evolution of developmental processes.
- **Dr Mike Haydon** (https://blogs.unimelb.edu.au/haydonlab/)
  Dr Haydon uses genetics, chemical genetics, molecular biology and biochemistry to understand mechanisms that control circadian rhythms with a particular focus on metabolic signalling and nutrient balance
- **Dr John Lunn** (https://www.mpimp-golm.mpg.de/6631/System-Regulation)
  Dr John Lunn is a world leading expert in the field of sugar signaling and Editor-in-Chief of the *Journal of Experimental Botany*.

Project description:

Seed size is a critical trait that affects the long-term survival of plants in the wild and yield potential of our most important crops. Seeds are a unique combination of maternal tissues (the seed coat) with filial tissues derived from double fertilization of the ovules by pollen (the diploid embryo and triploid endosperm). Thus, seed development is a fascinating and complex process that requires coordination between tissues of different origin and ploidy. This collaborative project between the labs of John Golz (UoM), Mike Haydon (UoM) and John Lunn (MPI, Golm) aims to bring together complementary expertise to investigate the role of sugar signaling in coordinating seed development and its impact on the final seed size. Seeds are major sinks for photosynthetically fixed carbon, with the maternal layers of the developing seed providing nourishment to the developing endosperm and embryo. In the model plant *Arabidopsis*, sugars enter the developing seed via the vasculature at the base of the developing seed and subsequently translocate to the endosperm via the multi-layered seed coat. The majority of seed growth occurs during the early phase of development (red arrows, see figure below) and is driven by the rapid expansion of the endosperm, due to a build-up of internal pressure (turgor) (grey arrows, see figure below).

Recent studies have shown that the seed coat plays an important role in regulating seed size with various models being proposed to explain how this is achieved. One intriguing possibility is that seed growth is modulated by trehalose 6-phosphate (T6P) – a signal metabolite that provides information about the availability of sugar supplies from the mother plant. In *Arabidopsis* seeds, the enzyme that is mainly responsible for T6P synthesis, TREHALOSE-6-PHOSPHATE SYNTHASE1 (AtTPS1), is expressed in most parts of developing seeds, including the seed coat, especially in the basal part where nutrients are supplied to the developing embryo and endosperm (see figure below). T6P also regulates the expression of the *FLOWERING LOCUS T* (*FT*) gene, which encodes a mobile protein that triggers flowering ("florigen"). FT protein also plays a role in regulating the onset of dormancy in the mature seed, including structural and metabolic changes to the seed coat. Thus, we hypothesize that T6P coordinates seed growth with sugar supply *via* the seed coat and is involved in triggering the developmental switch to dormancy that marks the end of seed growth, thereby playing a major role in control of final seed size.



Objectives:

Year 1 (MPI-MP):

1. Develop a genetic system to modulate sugar signaling in different layers of the seed coat

Using promoters that are available from the Golz lab, the student will generate a collection of plant lines in which T6P signaling and targets of this signaling pathway (SnRK1) are either elevated or reduced in the outer or inner layers of the Arabidopsis seed coat. All transgene construction and transformations will be done at the MPI.

2. Using a pharmacological approach to disrupt sugar signaling in Arabidopsis seeds

Under the guidance of Dr Lunn, the student will develop a protocol to deliver small synthetic molecules known to disrupt plant sugar signaling to developing Arabidopsis seeds. Once established, the student will then use this protocol to screen a library of small synthetic molecules to identify compounds that affect seed size.

Year 2 (UoM):

1. Phenotypic analysis of transgenic lines

Seeds produced by the transgenic lines generated at the MPI in year 1 will be thoroughly assessed for changes in seed size. This will involve microscopic analysis, to monitor changes in cell size, number and identity as well as transcriptomic approaches to monitor molecular responses to altered sugar signaling in the seed coat.

2. Comparative studies of T6P signaling in land plants

One of the key targets of T6P is SnRK1 (SUCROSE-NON-FERMENTING1-RELATED KINASE1). This protein kinase belongs to an ancient family of proteins found in all eukaryotes, which serve as central hubs for coordinating growth with nutrient and energy status. In flowering plants, SnRK1 is regulated by T6P in several ways, but its homologs in animals and fungi are not regulated by T6P.

The student will investigate when SnRK1 became regulated by T6P during plant evolution, beginning with studies in the liverwort *Marchantia polymorpha*. Liverworts are ideal for evolutionary studies because they represent one of the earliest diverging group of extant land plants which likely retain ancestral functions in key biological pathways. Using protocols established in the Golz lab, the student will generate gain and loss of function mutations in the T6P and SnRK1 signaling pathways. Phenotypes of these lines will be characterized with the aim of understanding the ancestral function of T6P signaling in this basal land plant.

3. Using a pharmacological approach to disrupt sugar signaling in *Marchantia*

Using knowledge arising from the pharmacological screen in year 1, compounds affecting sugar signaling will be applied to *Marchantia* to determine if they cause similar growth defect in this species. Phenotypes arising will then be assessed for disruption to sugar signaling as part of an approach to understand how sugar signaling has evolved in the plant kingdom.

Year 3 (MPI-MP):

Metabolomic profiling of Arabidopsis seeds and *Marchantia* tissues

In year three student will work with Dr Lunn to develop a protocol to characterize metabolites and in particular T6P in Arabidopsis seeds and vegetative tissue of *Marchantia*, based on established methods for metabolite analysis by state-of-the-art mass spectrometry (LC-MS/MS). This protocol will then be applied to transgenic lines generated in the first year of the project. This approach will also be used to characterize well-known seed size mutants where defects in the seed coat result in either increased or decreased seed size e.g. *ap2*, *ttg2*, *ant* and *arf2*.

Marchantia TPS, trehalose-6-phosphate phosphatase (TPP) and SnRK1 proteins will be expressed heterologously in *Escherichia coli*. The purified recombinant proteins will be used in *in-vitro* reconstitution experiments to determine their kinetic and regulatory properties.

Project code: **P4_MP+DW+ML_1220**

Reclaiming the lost genetic diversity in barley

Supervisors:

Dr. Mohammad Pourkheirandish, University of Melbourne, Melbourne, Australia, https://findanexpert.unimelb.edu.au/profile/830516-mohammad-pourkheirandish

Apl. Prof. Dr. Dirk Walther, Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany, https://www.mpimp-golm.mpg.de/9657/Dirk_Walther

Prof. Dr. Michael Lenhard, University of Potsdam, Potsdam, Germany
https://www.uni-potsdam.de/en/ibb-genetik/index

Present day species are the result of evolutionary processes that, over generations, shaped heritable traits to increase fitness in the local environments. Organisms with advantageous characteristics are more likely to survive and reproduce in their habitat, leading to well adapted species, a process referred to as natural selection. By contrast, plant domestication by humans over several millennia has led to an increase in favourable traits based on selection parameters set by humans. Plant domestication is one of the central pillars for the transition of humans from hunter-gatherers to agriculture-based societies. However, domestication has led to a significant decrease in the natural genetic diversity of species due to selection and genetic drift resulting in a loss of allele variations for many traits to keep the gene pool of crops consistent for human benefit.

With early-domesticated landraces having been bred to produce so-called “elite-cultivars”, modern plant breeding has led to further loss of genetic diversity. While the reduction of genetic diversity has advantages for facilitating agriculture by improving crop yield and uniformity, the trend to monocultures has resulted in the loss of alleles that can help crops survive in changing environmental conditions, e.g., tolerance to drought, salinity, disease. With climate change posing multiple biotic and abiotic threats to plant production, there is an increasing need for developing new resilient and productive crop varieties.

Barley (*Hordeum vulgare*) was one of the first crops domesticated by ancient farmers. Cultivated form (*H. vulgare* ssp. *vulgare*) was selected from its wild progenitor (*H. vulgare* ssp. *spontaneum*) during thousands of years of barley domestication. The primary traits identified and selected for in domesticated barley were non-brittle rachis (avoiding grain dispersal), six-rowed spike (increase number of seeds per spike), naked caryopsis (free threshing), reduced seed dormancy (uniform germination), reduced vernalization requirement (spring growing lines), and photoperiod insensitivity (growing suited to diverse geographical regions). All these selections resulted in a relatively narrow gene pool in cultivated barley compared to wild populations; thus, it is believed that wild barley contains many potentially valuable alleles for stress tolerance and pathogen resistance. This project aims at identifying the lost potential diversity for key traits and making it accessible for breeding.

Figure. Wild barley growing in nature at Mount Carmel, Haifa, Israel, compared to the domesticated cultivar on a barley field in Victoria, Australia. Photos by T. Komatsuda (left) and by M. Pourkheirandish (right).

This PhD project will focus on discovering beneficial alleles in wild barley (*H. vulgare* ssp. *spontaneum*), the immediate progenitor of our cultivated barely, and the introduction of these alleles that have been lost during domestication. The goal is to increase genetic variability within the cultivated gene pool and provide opportunities to plant breeders to make significant barley breeding improvements. The plant material to be used in this study is 318 *spontaneum* accessions collected by the International Centre for Agricultural Research in The Dry Areas (ICARDA) from diverse geographical regions in the Fertile Crescent region. The International Wild Barley Sequencing Consortium (IWBSC), led by the University of Minnesota, has performed genotyping in these lines. The seeds and genotyping data are available in Dr. Pourkheirandish's research group at the University of Melbourne as a member of IWBSC.

By developing novel breeding strategies for barley, this project offers the exciting opportunity to contribute to securing the food supply for humankind in dramatically changing climatic conditions, and to work on an interdisciplinary project that includes crop genetics and physiology (*Phase I*), bioinformatics (*Phase II*), and plant biotechnology (*Phase III*).

Timeline:

Phase I, *year 1*: University of Melbourne ("Home" institution) Phenotyping spike density and seed dormancy in wild barley accessions
Dense spikes reduce seed loss before and during the harvest through the reduction of breakage within the spike. Seed dormancy is defined as the temporary inability of a viable seed to germinate under favourable environmental conditions. A high level of seed dormancy is not desirable in agriculture since it will delay seed germination. However, lack of seed dormancy results in pre-harvest sprouting that damages the seed quality.

Phase II, year 2: MPI-MP/ U-Potsdam, Potsdam, Bioinformatic analysis
Analysis of large-scale phenotypic and genotypic datasets, identification of candidate genes that contribute to low spike density and optimal seed dormancy. Association of phenotypic and genotypic data will be undertaken to identify the genomic regions and genes responsible for the target traits. Concepts such as Genome-wide-Association-Studies (GWAS) and Genomic Selection, along with bioinformatics genome annotation and SNP-effect modelling will be applied.

Phase III, *year 3*: University of Melbourne, Validation of candidate genes *in planta*
The candidate genes identified during phase II will be validated using genetic transformation either by complementation tests or through a CRISPR-Cas9 system.

Selected References

Pourkheirandish M, Hensel G, Kilian B, et al (2015) Evolution of the grain dispersal system in barley. **Cell** 162, 527-539.

Pourkheirandish M, Golicz A, Bhalla P, et al (2020) Global Role of Crop Genomics in the Face of Climate Change. **Front Plant Sci** 11, 992.

Perlaza-Jimenez L, Walther D (2018) A genome-wide scan for correlated mutations detects macromolecular and chromatin interactions in *Arabidopsis thaliana*. **Nucleic Acids Res** 46, 8114–8132

Childs LH, Lisec J, Walther D (2012) Matapax: An Online High-Throughput Genome-Wide Association Study Pipeline. **Plant Phys** 158, 1534-1541

Jöst M, Hensel G, Kappel C, Druka A, Sicard A, Hohmann U, Beier S, Himmelbach A, Waugh R, Kumlehn J, Stein N, Lenhard M (2016) The INDETERMINATE DOMAIN Protein BROAD LEAF1 Limits Barley Leaf Width by Restricting Lateral Proliferation. **Curr Biol** 26 , 903-909

Project code: **P5_MW+ZN_1220**

How do plant roots sense and adapt to soil water? An experimental and modelling study using root cap cells

Home Institution: University of Melbourne
Host Institution: Max Planck Institute / University of Potsdam

Supervisors

Melbourne PI: **Prof. Michelle Watt**
Professor Watt is a plant root physiologist who studies the development and function of plant roots for water-efficient agriculture. She uses image-based phenotyping to discover variation.
https://science.unimelb.edu.au/engage/giving-to-science/botany-foundation/impact/professorial-chair

Potsdam PI: **Prof. Zoran Nikoloski**
Professor Nikoloski is a computer scientist and computational biologist focusing on modelling cellular networks. He uses mathematical models to determine principles of complex system functionality by integration of big data from experiments.
https://www.mpimp-golm.mpg.de/8360/nikoloski

Project description

Plant roots are hidden under ground; however, they are the workhorses of agricultural productivity because they access and provide water and nutrients to the shoots. There is still a lot to uncover in how roots sense and respond to soil environments to maintain plant growth and development. This project will discover and dissect the molecular events in root cap cells, the founding cells of plant roots, to identify new solutions to ensure food and resource security. The student will combine image-based phenotyping with biochemistry and genetics (at the University of Melbourne) and feed this data into a mathematical model of root caps cell metabolism (at the Max Planck Institute) to understand and identify how sensing of soil water status alters metabolism. Water is the primary resource required by plants, yet it is not known how root cells sense water; and convert this signal into plant productivity. This project will provide the student with experimental and mathematical modelling skills in the area of plant roots, a much-understudied area of plant sciences.

Central question for the project

What is the plasticity of root cap metabolism and what implication does it have for plant growth under varying soil water conditions?

Objectives and work plans

Objective 1/ Year 1 (University of Melbourne). Isolate root cap cells and establish cell-specific metabolomic and transcriptomic profiles over a time series.

Using previously published protocols, a method will be established to isolate high numbers of root cap cells from primary roots. Part of the project will be to determine the best species that produces large numbers of cells, and from which metabolomics and transcriptomics can be carried out, and for which genome-scale metabolic models are already available. This Objective will be met under well watered conditions, to establish a control metabolic condition of root cap cells over time.

Objective 2/ Year 2 (Max Plank/Potsdam). Map the metabolomic and transcriptomic data from root cap cells into a genome-scale metabolic model to obtain a model specific to root cap cells.

Leveraging the results obtained in Year 1, the student will work with Prof. Nikoloski to map metabolomic and transcriptomic data into genome-scale metabolic models and to determine the biochemical reactions active under the control, well watered conditions. The model will be able to predict the metabolic capacity of root cap cells along with the specific exchanges with the soil and surrounding tissue.

Objective 3/ Year 3 (University of Melbourne). Validate the model predictions about plasticity of root cap cells under varying soil water conditions, and the consequences on root system architecture.

Plants will be grown in fabricated ecosystem under a microscope with defined and varying water conditions. Root cap cell number and location will be mapped to root system architecture, shoot growth and water fluxes. The metabolic model will be validated under different water conditions. The student will answer: What is the plasticity of root cap metabolism and what implication does it have for root architecture and plant water relations under varying soil water?

Timeline of Objectives and Outputs

Objectives	Year 1	Year 2	Year 3	Year 4
1. Root cell isolation and omics				
2. Mathematical model				
3. Prediction and Experimentation				
Writing Outputs	Root cap methodology (publication 1 and thesis chapter)	Mathematical map of root cap cell metabolism (thesis chapter)	Prediction and test of how root cap cells respond to drought (publication 2 and thesis chapter)	Thesis completed (publication 3 of literature review)

Green = Australia; Blue = Germany

Project code: **P6_UR+JK+JH_1220**

How translational mechanisms contribute to low temperature acclimation in plants

Supervisors:

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Links to supervisors' websites:

http://roessnerlab.science.unimelb.edu.au/ | http://www.heazleome.org/
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Introduction:

Low temperature affects plant development and decreases plant growth rate. Our recent research indicates that in order to acclimate to cold temperature, plants not only trigger immediate changes in cellular metabolism but also invest in long-term responses that include a fine-tuning of the protein translation machinery or more specifically, the cytosolic ribosomes. This has led us to hypothesize the existence of specialized cold-induced ribosomes that would selectively translate the proteome that plants need to acclimate to cold temperature (Martinez-Seidel et al., 2020) (**Figure 1**).

The model plant Arabidopsis and the cereal barley halt their growth during the first 5-7 days of cold acclimation (Beine Golovchuk et al., 2018, Martinez-Seidel et al, unpublished). Yet the accumulation of cytosolic ribosomes in acclimating plants exceeds that of plants reared at optimized temperature. Since cytosolic ribosome synthesis is the single most expensive cellular investment, it needs to be essential for acclimation. Proof of this necessity are REIL knock-out mutants in Arabidopsis (Schmidt et al., 2013), which are impaired for ribosome maturation and unable to acclimate to cold (Beine Golovchuk et al., 2018). Cytosolic ribosomes are composed of ribosomal ribonucleic acid (rRNA) and ribosomal proteins (rProteins). In plants there are 80 rProtein families and each of them is composed by 2-7 paralogous genes (Carroll et al., 2008). We have proposed that this inherent heterogeneity has been shaped by plant evolution toward specialized ribosomes (Martinez-Seidel et al., 2020).

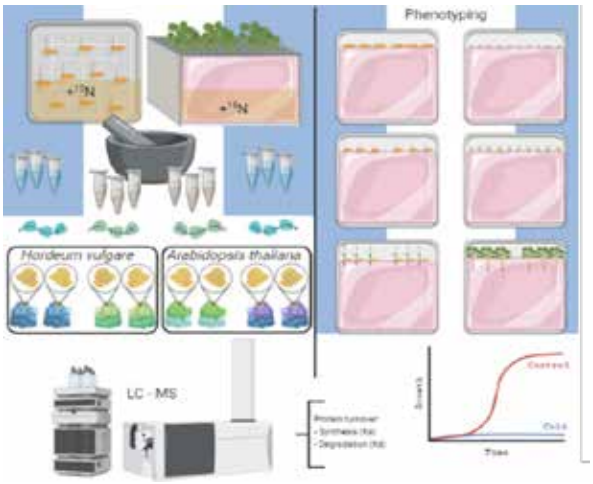

Figure 1. Arabidopsis and Hordeum vulgare (barley) accumulate cytosolic ribosomes during cold acclimation. The accumulated ribosomes have different protein compositions as compared to those from plants reared at optimized (~20°C) temperatures. Suggesting that the plant investment in a new ribosomal population is intrinsically correlated to its capability to adapt to cold. The Figure was exported under a paid license using BioRender.com.

To answer the ribosome heterogeneity and specialization question from a proteomics perspective, this project will use optimized methods that have been developed at the MPIMP and UoM. First the PhD candidate will isolate, separate and characterize the associated proteome of cytosolic ribosomal complexes (Firmino et al., 2020). The proteome profiling includes non-translational and translational ribosomal complexes of Arabidopsis and barley root and shoot material. The project will use top-down ribosomal proteomics in order to distinguish between rProtein paralogs and their posttranslational modification (PTM) status. The PTM status is likely a fingerprint of altered translation in plants (Carroll et al., 2008). Subsequently, a bottom-up proteomics approach will be deployed to sequence digested peptides from the protein peaks and confirm the identity of ribosomal proteins. Additionally, to distinguish between newly synthesized complexes and "old" ribosomes, specific amino acid residues of rProteins can be routinely labelled with ^{15}N stable isotope (Erban et al., 2020). This allows the determination of which rProteins are made *de novo* by the plant to enhance acclimation. Finally, our previous proteomics data has suggested that the appearance of divergent ribosomal complexes is concomitant to protein S-Glutathionylation (Martinez-Seidel et al., n.d.). This observation will be followed using proteomics approaches tailored made for the oxidized proteome. Briefly, free-thiols in plant proteome extracts are alkylated, followed by a selective reduction of S-Glutathione groups using a Glutathione reductase enzymatic step and finally resin affinity binding of the free thiols. This will be coupled

into a bottom-up proteomics pipeline and the outcome will be the identification of proteins that are modified with S-GSH PTM upon cold stimuli.

As a second, equally important research line of ribosome specialization, functional analyses of rProtein coding genes will be performed, which, according to our own data, are of significant relevance during cold acclimation. Specific rProtein paralogs change their abundances during acclimation in plants (Cheong et al., 2020). Those candidates will be selected for reverse genetics approaches and explore the mechanisms that lead to ribosome specialization. Ultimately, biochemical specialization of ribosomes will be evaluated by interrogating the translated proteome in mutant genotypes against a wild-type like plant (Ito et al., 2014). The translated proteome will be profiled through ^{15}N labelling of newly synthesized proteins, calculating their synthesis and degradation rates (**Figure 1**).

This project will study the translational mechanisms that lead to successful low temperature acclimation in plants. We expect to find structurally and functionally divergent ribosomes that are heterogeneous. This heterogeneity would be triggered by cold and can encompass different rProtein paralogs or posttranslational modifications. Functional studies will reveal how the triggered cold heterogeneity relates to the translational status of transcripts in the plant cell.

Project outline:

This project is set up for the candidate to spend two years at MPIMP and one year at UoM (Potsdam-based candidature). Balance between proteomics and molecular biology work needs to be tuned according to the project outline.

First Year: MPIMP; Protein profiling (bottom-up ^{15}N mediated proteomics) of wild type and rProtein mutant genotypes under temperature stress. This includes sucrose gradients and / or cushion centrifugations, protein extraction methods, digestion, LC-MS and data analysis. Transcriptome and translatome analyses, in order to assess the translational bias in mutant and acclimated ribosomes.

Second Year: UoM; Shoot and root rProtein profiling (bottom-up proteomics) under temperature stress, Top-down (Waters Vion QTOF LC/MS) and functional analyses coupled to the other outlined technologies.

Third Year: MPIMP; rProtein mutant genotypes (molecular biology techniques, such as cloning, plant transformation) and experimental rearing system preparation.

Selected publications:

Beine Golovchuk O, Firmino AAP, Dąbrowska A, Schmidt S, Erban A, Walther D, Zuther E, Hincha DK, **Kopka J**. 2018. Plant temperature acclimation and growth rely on cytosolic ribosome biogenesis factor homologs. Plant Physiol 176:2251–2276. doi:10.1104/pp.17.01448

Carroll AJ, **Heazlewood JL**, Ito J, Millar AH. 2008. Analysis of the Arabidopsis cytosolic ribosome proteome provides detailed insights into its components and their post-translational modification. Mol Cell Proteomics 7:347–369. doi:10.1074/mcp.M700052-MCP200

Cheong BE, Beine-Golovchuk O, Gorka M, Ho WWH, Martinez-Seidel F, Firmino AAP, Skirycz A, **Roessner U**, **Kopka J**. 2020. Arabidopsis REI-LIKE proteins activate ribosome biogenesis during cold acclimation. bioRxiv 2020.02.18.954396. doi:10.1101/2020.02.18.954396

Erban A, Martinez-Seidel F, Rajarathinam Y, Dethloff F, Orf I, Fehrle I, Alpers J, Beine-Golovchuk O**, Kopka J**. 2020. Multiplexed Profiling and Data Processing Methods to Identify Temperature-Regulated Primary Metabolites Using Gas Chromatography Coupled to Mass Spectrometry In: Hincha DK, Zuther E, editors. Methods in Molecular Biology. New York, NY: Springer US. pp. 203–239. doi:10.1007/978-1-0716-0660-5_15

Firmino AAP, Gorka M, Graf A, Skirycz A, Martinez-Seidel F, Zander K, **Kopka J**, Beine-Golovchuk O. 2020. Separation and paired proteome profiling of plant chloroplast and cytoplasmic ribosomes. Plants 9:1–29. doi:10.3390/plants9070892

Ito J, Parsons HT, **Heazlewood JL**. 2014. The Arabidopsis cytosolic proteome: The metabolic heart of the cell. Front Plant Sci. doi:10.3389/fpls.2014.00021

Martinez-Seidel F, Beine-Golovchuk O, Hsieh YC, **Kopka J**. 2020. Systematic review of plant ribosome heterogeneity and specialization. Front Plant Sci 11:948. doi:10.3389/fpls.2020.00948

Martinez-Seidel F, Suwanchaikasem P, Nie S, Leeming MG, Pereira Firmino AA, Williamson NA, **Kopka J**, **Roessner U**, Boughton BA. n.d. Membrane-enriched proteomics link cold translational reprogramming to potential S-glutathionalytion mediated priming in barley roots. [unpublished data].

Schmidt S, Dethloff F, Beine-Golovchuk O, **Kopka J**. 2013. The REIL1 and REIL2 proteins of Arabidopsis thaliana are required for leaf growth in the cold. Plant Physiol 163:1623–1639. doi:10.1104/pp.113.223925