Graduate Research School

RP

Research Proposal Coversheet for Candidates in

Research Higher Degrees

EXAMPLE OF RESEARCH PROPOSAL SUBMISSION

RESEARCH AREA: Plant Biology

DEGREE: PhD

Please note that all identifying information has been removed from this research proposal and replaced with XXX.

PhD Research Proposal

Wheat mitochondrial proteomics: Searching for biomarkers of salinity tolerance

Name (Student Number

Centre

Supervised by XXX and XXX

The effect of salinity on plant growth

Salinity describes soils that contain high concentrations of water-soluble salts, mainly NaCl. Salinity is usually caused by two mechanisms: groundwater salinity and irrigation salinity. Groundwater salinity occurs when saline groundwater is present in the upper layers of the soil. This commonly occurs in areas where native vegetation has been cleared and evaporation rates are high, like the West Australian wheatbelt. Irrigation salinity occurs when irrigation water accumulates in the upper layers of soil. When this water evaporates, the salts remain in the soil. Irrigation salinity is common in areas where soil drainage is poor and low quality water is used for irrigation (Rengasamy, 2006).

Salinity dramatically impedes plant growth, leading to a decrease in crop yield and quality. This occurs due to two mechanisms: osmotic stress and ion toxicity. Osmotic stress occurs because saline soils have high osmotic potential, so plants which grow in saline soils have difficulty taking up water, resulting in low cell turgor and slow shoot growth. Ion toxicity occurs because saline water moves up the transpiration stream, causing Na^+ and Cl^- to accumulate in leaf tissue. Older leaves show higher Na^+ and Cl^- levels than younger leaves, because they have been transpiring for longer. Leaves with high Na^+ and Cl^- levels display premature senescence and death. The mechanism by which ion toxicity occurs is poorly characterised. It is thought that high Na^+ and Cl^- concentrations could disrupt electrochemical gradients across membranes, denature proteins, increase levels of reactive oxygen species (ROS), or displace cellular K^+ - a vital cofactor for many enzymes (Munns and Tester, 2008).

Salinity tolerance is usually defined as: Biomass (salt treatment) / Biomass (control treatment) x 100. This measurement is suited to annual plants, as biomass usually correlates with final grain yield. For perennial species, salinity tolerance is better measured by stating the NaCl concentration which induces injury or death. Plant species differ widely in salinity tolerance. Plants with exceptionally high salinity tolerance are called halophytes, while plants with low salinity tolerance are called glychophytes (Munns, 2002).

Many plant biologists believe that increasing the salinity tolerance of crop plants will enable productive farming on land afflicted by salinity (Witcombe *et al.*, 2008). However, breeding for salinity tolerance has produced poor results, probably because salt affects multiple biochemical and physiological processes, meaning that no "single gene" addition which will dramatically improve salinity tolerance (Genc *et al.*, 2007).

Respiration and plant growth

Mitochondrial respiration extracts chemical energy from carbon-containing molecules, and converts that energy into ATP, the cellular energy currency. The ATP produced by respiration fuels biochemical and physiological processes that enable the plant to survive and grow. Plants assimilate atmospheric carbon through photosynthesis, and allocate most of that carbon to two processes: respiration and biomass accumulation. The sum of these processes is called the "carbon economy" of the plant. Some writers believe that the respiratory component of the carbon economy is central to plant growth rate, ie: that slow respiration enables fast growth. Several

independent studies provide evidence for this hypothesis. Earl and Tollenaar (1998) observed that respiration rate was negatively correlated to biomass in six commercial maize genotypes (Earl and Tollenaar, 1998). Poorter and Lambers (1990) observed a negative correlation between growth rate and respiration rate in 24 plant species (Poorter *et al.*, 1990). Wilson (1982) showed higher biomass can be achieved by selecting ryegrass progeny for slow respiration rates (Wilson, 1982), although this result only occurs when plants are grown at high density (Kraus *et al.*, 1993). However, other studies have found no correlation between respiration rate and crop yield (Volenec *et al.*, 1984, Winzeler *et al.*, 1988).

Table 1 summarises results from the literature which measured the respiratory response to salt stress in intact tissue, while Table 2 summarises results gathered from isolated mitochondria. There is no prevailing theme across these results; in fact, many of them contradict one another. So, further experiments are needed before we can conclusively define the respiratory response to salinity.

The effect of salinity on the plant mitochondrial proteome

Proteins are functionally active molecules that can change the chemical environment of the cell. This contrasts to DNA and RNA, which are relatively inert molecules used to transfer information. So, the study of proteins provides a direct insight into the phenotypic reality of the cell. Proteomics is the broad-scale study of proteins, which has the aim of characterising the function, abundance, and interactions of all the proteins within a cell. To reduce complexity, proteomic studies often focus on one subcellular compartment, such as the mitochondrion. To date, only one study has documented the effect of salt stress on the plant mitochondrial proteome (Chen *et al.*, 2009). So, further experiments are needed to define the mitochondrial proteome response to salinity.

Wheat is a useful organism for proteomic investigations into salt tolerance

To date, most molecular-level investigations of plant biology have utilised the model plant species, Arabidopsis, primarily because its genome sequence was released in 2000 (TAGI, 2000). However, genetic sequence information on wheat is rapidly increasing (Paux *et al.*, 2008), which will greatly increase the power of proteomics applied to wheat. There are several advantages to studying the molecular properties of wheat. First, molecular-level discoveries are more likely to be successfully applied in crop improvement strategies, as there is no “species boundary” to traverse. Second, there is a wide variety of genetic and phenotypic traits distributed across commercial wheat genotypes, as well as wheat landraces adapted to a particular environmental niche, and wild grasses which are related to wheat (Colmer *et al.*, 2006). Third, and most relevant for this project, is the assertion that Arabidopsis displays a different salinity response to most crop species, making it a poor model for salinity tolerance in crops (Moller and Tester, 2007).

A1: Provide a title

Wheat mitochondrial proteomics: Searching for biomarkers of salinity tolerance

A2: Why is this research novel?

This project aims to discover new knowledge by characterising the molecular properties of wheat mitochondria using peptide mass spectrometry. This will complement other molecular-level knowledge of wheat being generated in other laboratories around the world. Furthermore, this project tests a novel theory: that there is a correlative link between respiration rates, mitochondrial proteomes, and salinity tolerance across different wheat cultivars.

B1: Detail the specific aims of the project - the problem(s) it hopes to solve; or particular question(s) it will answer; issues it will explore; and the new knowledge it will create

i) What proteins are targeted to wheat mitochondria, and are there wheat-specific mitochondrial proteins?
ii) How does the wheat mitochondrial proteome respond to salt stress?
iii) Do different wheat cultivars display different mitochondrial proteomes?
iv) Is there a correlation between respiration rates, mitochondrial proteomes, and salinity tolerance across different wheat cultivars?

B2: Detail the methods to be used or the approach to be taken. What similar projects have been undertaken here or elsewhere; have similar methods been used before?

i) What proteins are targeted to wheat mitochondria, and are there wheat-specific mitochondrial proteins?

Wheat seedlings will be grown in the dark for approximately one week, according to Huang *et al*. (2009). Mitochondria will be isolated according to Day *et al*. (1985), with the modifications of Eubel *et al*. (2007). Mitochondrial proteins will be identified by two methods. First, proteins will be separated on 2-D gels, and gel-bound proteins will be digested with trypsin, yielding peptides which can be identified via peptide mass spectrometry, as conducted by Millar *et al*. (2001). Second, mitochondrial proteins will be digested with trypsin, and peptides will be identified using complex mixture mass spectrometry, as conducted by Huang *et al*. (2009). Peptides will be searched against DNA, RNA, and protein databases, similar to methods detailed in Choudhary *et al*. (2001), or Perkins *et al*. (1999). Wheat mitochondrial proteins will then be compared to orthologous proteins in Arabidopsis (the model plant) and rice (the model cereal), in an attempt to identify wheat-specific mitochondrial proteins.

ii) How does the wheat mitochondrial proteome respond to salt stress?

Plants will be grown hydroponically, similar to the method used by Genc *et al*. (2007). Plants will undergo either control treatment or salinity treatment. Mitochondria will be isolated according to Day *et al*. (1985). Quantitative proteomics will be used to identify proteins induced by salt stress, using the DIGE technique employed by Lee *et al*. (2008).

iii) Do different wheat cultivars display different mitochondrial proteomes?

First, a wide range of wheat cultivars will be obtained, from both commercial breeders (XXX), and academic researchers. These cultivars will be selected according to their varying salt tolerance. Next, plants will be grown hydroponically, mitochondria will be isolated, and quantitative proteomics will be used to identify proteins that differ in abundance between the different cultivars.

iv) Is there a correlation between respiration rates, mitochondrial proteomes, and salinity tolerance across different wheat cultivars?

Again, I will obtain different wheat cultivars, and grow the plants hydroponically. Plants will undergo either control treatment or salinity treatment. Plants will be harvested at several time points, and biomass and leaf respiration rate will be measured, according to the method of Kurimoto *et al*. (2004). Mitochondria will be purified from these plants, and mitochondrial proteomes will be compared using quantitative proteomics. I will then analyse the data using ANOVA and regression analyses, to determine whether there is a correlation between respiration rates, mitochondrial proteomes, and salinity tolerance across different wheat cultivars.

B3: What efforts have been made to ensure that the project does not duplicate work already done?

I have reviewed the scientific literature. I have found two papers which use peptide mass spectrometry to describe the wheat leaf proteome (Donnelly *et al.*, 2005, Bahrman *et al.*, 2004). However, neither of these papers enrich samples for mitochondrial proteins, meaning that many wheat mitochondrial proteins remain to be discovered. Nine papers examine the general link between respiration and salt stress (Tables 1 and 2), but no published papers have augmented this data with quantitative mitochondrial proteomics, meaning that the molecular basis that underpins this link is unknown.

C1: The supervisor should assist the student to prepare a framework for the research, with a general timeframe for completion of the various phases and a detailed timeframe for the next 12 months. Each Annual Progress Report will include an update of the general plan and a detailed plan for the next 12 months.

See table 3.

C2: All new PhD students are required within a period of twelve months to complete designated tasks and meet agreed milestones in order for their ongoing candidature to be confirmed. If you are enrolled in a PhD please identify your Confirmation of Candidature tasks and indicate the date at which they will be completed.

Milestones are summarised on page 6 the Graduate Research School coversheet.

The university requires me to complete the academic conduct essential unit (AACE7000). I have completed this unit on WebCT. The school requires a substantial piece of writing at the appropriate conceptual level, and a proposal seminar delivered to the faculty. The piece of writing prefaces this document; it has been approved by my supervisors, and the head of XXX. I delivered a proposal seminar on October 9, which was approved by faculty members from the discipline of XXX. My supervisors require a thorough review of the background literature related to the project, which will eventually be used as the introductory chapter to my thesis. I will submit this review to my supervisors in January 2010.

C3: In partnership with your supervisor(s), please undertake a skills audit to determine if you possess the generic skills required to bring your project to a timely completion. Please identify the special skills your project requires of you, and if you do not possess them map out a strategy for their achievement.

I possess the necessary skills to achieve the tasks outlined in tables 3 and 4.

D: 1. In addition to confirming that proper supervision is available for the project, please comment on any other requirements, for example:
2. Special Equipment - if not already available, how it will be obtained.
3. Special Literature - if not available from the Library, how will access to it be obtained?

Proper supervision is available for this project. Plans for obtaining special equipment are detailed in table 3. No special literature is required.

E: What funds will the School commit to maintain the project? Please include all contributions that the School will make, excluding staff salaries and building/infrastructure costs. Please provide a breakdown of the costs, including, for example, items such as photocopying, telephone, computing and other administrative costs as well as costs specific to the research project.

All laboratory consumables, equipment, and administrative costs will be borne by the XXX.

The annual laboratory consumables budget for this project is $15,000. This can be broken down into: $5,000 for proteomics reagents (fluorescent dyes, acrylamide, trypsin etc.), $5,000 for general laboratory reagents (analytical grade chemicals,

glassware, plasticware etc.) and $5,000 for plant growth costs (glasshouse space, hydroponic systems etc.).

All administrative costs are accounted for by the XXX business unit. This includes stationery, phone and internet access, as well as printing and photocopying. These resources are used by all XXX staff and students, and will therefore not be itemised individually.

F: Are you conducting fieldwork as part of your research?

No.

G: Please provide a list of your supervisors and their role, including percentages, as indicated in the Research Proposal Cover Sheet.

XXX will be the co-ordinating supervisor. He will supervise: experimental design, thesis writing, data analysis, and administrative requirements. He will conduct 25% of the project's supervision.

XXX will be the co-supervisor. He will supervise: experimental design, plant growth, respiration assays, proteomic analysis, mass spectrometry, and data analysis. He will conduct 75% of the project's supervision.

H: If your thesis is likely to contain information of a confidential nature, you must draw this to the attention of the Board of the Graduate Research School from the outset, or as soon as it becomes apparent.

You must also draw the Board's attention to any intellectual property issues that need to be considered, including any existing intellectual property of value that is pertinent to the research and to any agreements that may affect your right to intellectual property arising out of the research.

I do not expect this project to produce confidential information. If intellectual property issues become apparent, I will consult the graduate research office.

I: Safety Approvals

No animal subjects or human subjects will be experimented upon in this project. Any genetically modified plant material will be handled according to the OGTR dealings which are approved for the XXX. Any dangerous chemicals will be handled according to MSDS recommendations, and disposed of according to XXX policy. No ionising radiation will be used.

Tables

Table 1: Summarises reports of respiratory responses to salinity in whole tissue

First author	Year	Species	Tissue and age	Salt treatment	Response (tissue respiration rate)
Malagoli	2008	Rice cv. Pokkali (tolerant)	21 day old roots	25 mM NaCl for 21 days	Increased
		Rice cv. IR29 (sensitive)	21 day old roots	25 mM NaCl for 21 days	Decreased
Kasai	1998	Bread wheat cv. Chinese Spring (sensitive)	14 day old shoots	400 mM NaCl for 7 days	Increased
		Amphiploid of Chinese Spring and Lophopyrum Elongatum (tolerant)	14 day old shoots	400 mM NaCl for 7 days	No change
Ivanova	1993	Climacoptera crassa (salt tolerant)	Shoots of undisclosed age	Between 50 - 800 mM NaCl for an undisclosed time	Decreased
Jolivet	1990	Barley cv. Triumph	7 day old shoots	400 mM NaCl for 3 days	Decreased
Brown	1987	Pea cv. Green Feast	Shoots of undisclosed age	80 mM NaCl for an undisclosed time	Decreased
Livne	1967	Pea cv. Laxton Progress	13 day old shoots	77 mM NaCl for 6 days	Increased
		Pea cv. Laxton Progress	Dark grown shoots of undisclosed age	77 mM NaCl for 14 days	Increased
Nieman	1962	Beetroot cv. Detroit dark red	Shoots of undisclosed age	~91 mM NaCl for an undisclosed time	No change
		Spinach cv. Viroflay	Shoots of undisclosed age	~91 mM NaCl for an undisclosed time	Increased
		Turnip cv. Purple top globe	Shoots of undisclosed age	~91 mM NaCl for an undisclosed time	Increased
		Cabbage cv. Capitala	Shoots of undisclosed age	~91 mM NaCl for an undisclosed time	Increased
		Tomato cv. Mill	Shoots of undisclosed age	~91 mM NaCl for an undisclosed time	Increased
		Mustard (undisclosed cultivar)	Shoots of undisclosed age	~91 mM NaCl for an undisclosed time	Increased
		Lettuce cv. Paris white cos	Shoots of undisclosed age	~91 mM NaCl for an undisclosed time	Increased
		Radish cv. Early scarlet globe	Shoots of undisclosed age	~91 mM NaCl for an undisclosed time	Increased
		Capsicum cv. Grossum	Shoots of undisclosed age	~91 mM NaCl for an undisclosed time	Increased
		Bean cv. Bush stringless green pod	Shoots of undisclosed age	~91 mM NaCl for an undisclosed time	Increased
		Onion (undisclosed cultivar)	Shoots of undisclosed age	~91 mM NaCl for an undisclosed time	No change

Tables

Table 2: Summarises reports of respiratory responses to salinity in isolated mitochondria

First author	Year	Species	Tissue and age	Salt treatment	Response (isolated mitochondrial respiration rate)
Flagella	2006	Durum wheat cv. Ofanto	3 day old shoots	22% seawater for 3 days (~130 mM NaCl)	No change (succinate substrate) Decreased (proline substrate)
			4 day old shoots	37% seawater for 4 days (~233 mM NaCl)	Decreased (succinate substrate) Uncoupled (proline substrate)
Trono	2004	Durum wheat cv. Ofanto	3 day old shoots	125 mM NaCl for 3 days	No change (succinate substrate) Decreased (proline substrate)
			4 day old shoots	210 mM NaCl for 4 days	Decreased (succinate substrate) Decreased (proline substrate)
Jolivet	1990	Barley cv. Triumph	7 day old shoots	400 mM NaCl for 3 days	Decreased (malate substrate)
Livne	1967	Pea cv. Laxton Progress	15 day old shoots	77 mM NaCl for 8 days	Increased (sucrose substrate)

Tables

Table 3: Timeline for the first 12 months of the project.

Task	March	April	May	June	July	August	September	October	November	December	January	February
Comparing mitochondrial proteomes of different wheat genotypes, under control and salinity treatments	Accumulate mitochondria isolated from Wyalkatchem and Janz, under control and salinity treatments →						Use 2-D DIGE to compare Wyalkatchem and Janz, under control and salintiy treatments				Accumulate mitochondria isolated from CS and AMP, under control and salinity treatments →	
Describing the wheat mitochondrial proteome	Isolate highly pure wheat mitochondria via FFE, separate proteins via 2-D gel	Identify gel-bound proteins using mass spectrometry →							Compile preliminary list of wheat mitochondrial proteins		Isolate highly pure wheat (CS) mitochondria via FFE →	
Building hydroponic growth systems	Build prototype hydroponic system		Visit Adelaide University, build a hydroponic system according to their specifications				Test hydroponic system in UWA glasshouse →		Build 3 duplicates of Adelaide hydroponic system →			
Obtaining useful wheat genotypes	Obtain Wyalkatchem and Janz from xxx		Grow Chinese Spring (CS) and Chinese Spring - Lophopyrum Elongatum Amphiploid (AMP) →							Harvest seeds from CS and AMP		

Tables

Table 4: Timeline for the entire project

Date	Outcome
September 2009	Set up prototype hydropnic system, accumulate mitochondria isolated from Wyalkatchem and Janz
March 2010	Complete experiments on Wyalkatchem and Janz, present this work in a manner fit for publication, Set up second hydroponic system, obtain seed for CS and AMP, write a literature review which will serve as the introduction to my thesis
September 2010	Accumulate mitochondria isolated from CS and AMP
March 2011	Complete experiments on CS and AMP, present this work in a manner fit for publication
September 2011	Complete a respiratory and proteomics screen on approximately 20 commercial wheat genotypes
March 2012	Write and submit PhD thesis

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