SALINITY RESEARCH

ACPFG
AUSTRALIAN CENTRE FOR PLANT FUNCTIONAL GENOMICS PTY LTD

Identification of genes that help wheat and barley maintain yield in high saline environments

Salt Group

PhD Research Areas (2014)

To develop improved varieties of wheat and barley that are better able to grow in saline conditions

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THE PROBLEM:

Salinity is a major abiotic stress affecting crop plants worldwide. It has been estimated that the global cost of irrigated-induced salinity is US$11 billion a year. In Australia the problem will only get worse - already 51% of Western Australian farms are affected in some way by saline soils. In most crop plants, the main toxic component of salinity is the sodium ion (Na^+). This interferes with metabolic processes, such as enzyme activities and protein synthesis, as well as causing osmotic stress through the reduced ability of cells to obtain and retain water. Due to these toxic effects, crops grown on saline soils have significantly reduced yield.

THE SOLUTION:

Plants use three main mechanisms to tolerate salinity stress:

- **Shoot ion independent stress** – the plant's ability to maintain growth during salt stress by various mechanisms, some of which remain largely unknown, and others that may in part be related to long-distance signalling
- **Ion exclusion** – reducing Na^+ & Cl^- accumulation in the shoot by manipulating transport processes to minimise delivery of ions to the shoot
- **Na^+ tissue tolerance** – Compartmentalising ions into organelles within a leaf cells

OUR AIM: To generate salt tolerant cereal crops

OUR STRATEGY:

- Identify genes and cellular processes involved in salinity tolerance in current crops and other tolerant lines
- Understand and manipulate the three salt tolerance mechanisms in Australian crop plants to create varieties that can survive and produce viable yields on saline soils.

THE SALT GROUP RESEARCH TEAMS:

GENE DISCOVERY:

The Gene Discovery Team identifies new and novel genes responsible for increasing a plant's salinity tolerance.

Screening of cultivars of wheat, barley, rice and near wild relatives has revealed major differences in the ability of these plants to grow under salt stress. We have used this variation to identify quantitative trait loci (QTLs) regions in the plant genome containing genes important for salt tolerance.

Fine mapping of these regions allows us to narrow to the candidate gene(s) responsible for the observed phenotypes, which we can then introduce into crop varieties through either breeding and/or genetic engineering.

We are one of a few laboratories in the world using techniques to use non-destructive imaging and analysis to phenotype plant growth during salinity stress. This allows us to screen for a large number of salt tolerance traits.

THE SALT GROUP RESEARCH TEAMS:

GENE CHARACTERISATION:

The Gene Characterisation Team is responsible for the characterisation of genes identified as likely to increase salinity tolerance of plants.

From species such as *Arabidopsis*, barley, wheat, rice, moss and yeast we have identified genes involved in the manipulation of ion transporter, such as the *CIPKs*, *HKTs*, *AVPs*, and *HVPs*.

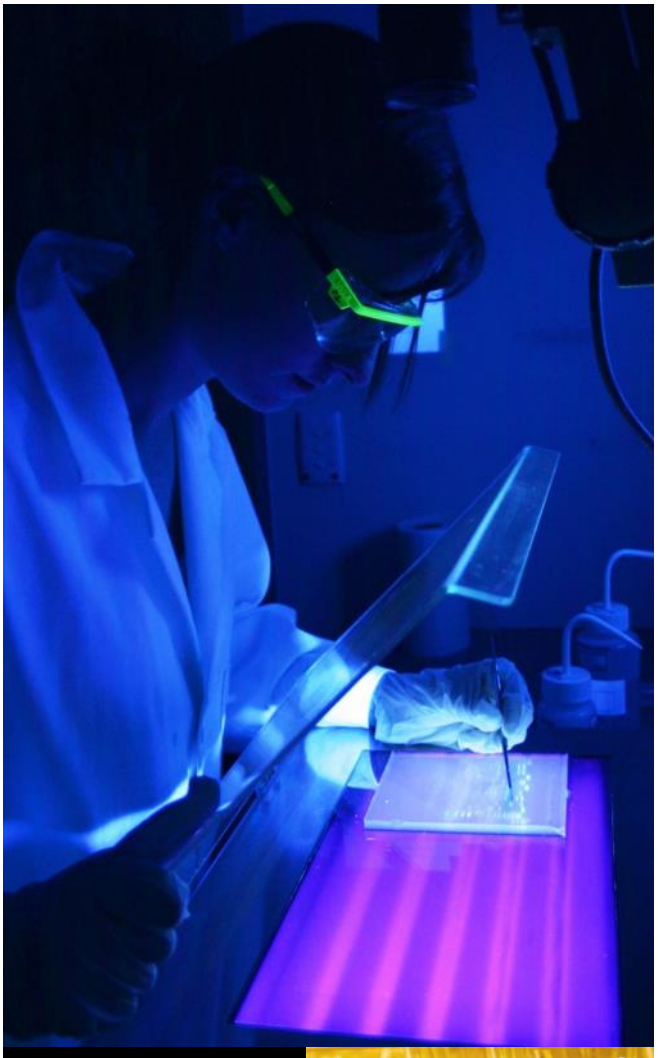
These candidate genes are knocked out or over-expressed in a variety of model and crop species to investigate the effects on salinity tolerance.

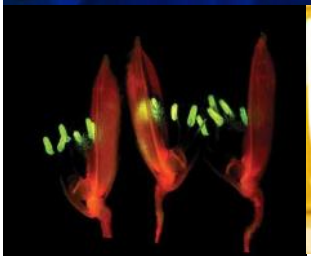
The fusion of the gene's promoter to reporter genes such as green fluorescent protein (GFP) allows us to visualise the tissue and subcellular location of our genes of interest. In the case of novel Na^+ transporters, these genes are additionally expressed in heterologous expression systems, such as yeast and *Xenopus* oocytes, for electrophysiological examination.

THE SALT GROUP RESEARCH TEAMS:

CONTROL OF GENE EXPRESSION:

The Control of Gene Expression Team develops better methods for the control of candidate genes for salinity tolerance.





Many of the genes involved in salinity tolerance transport ions into or out of cells. Constitutive expression of an ion transporter throughout a plant may be detrimental overall as, although the gene's expression in one tissue/cell type may be beneficial, expression in others can be detrimental.

We have generated Arabidopsis, rice and barley lines which express green fluorescent protein in a cell type-specific manner. Using this system we were the first laboratory in the world to express a Na^+ transporter in specific cells of the root of Arabidopsis, greatly enhancing the plant's salt tolerance.

Identification of gene promoters which have a cell type-specific pattern in cereals will also allow us to express our genes in specific cells. We have projects looking for both cell specific and stress inducible promoters in wheat and barley.

THE SALT GROUP RESEARCH TEAMS:

DELIVERY TEAM:

The Delivery Team is focused on combining the knowledge gained from the other research teams to produce a salt tolerant plant.

Conventional breeding practices are used to introduce salinity tolerance traits identified in foreign cultivars of cereals into Australian wheat and barley, which have better grain quality traits. We are developing molecular markers specific to various salt tolerance mechanisms for breeders.

Salinity tolerant transgenic rice, wheat and barley plants, which have been transformed with genes altering ion transport, are currently undergoing yield evaluations in both the glasshouse and field. Several lines are looking promising, having increased salinity tolerance and plant biomass under salt stressed conditions.

THE TECHNIQUES:

Molecular Biology

- DNA and RNA extractions
- PCRs and restriction digests
- QPCR
- Fluorescently activated cell sorting
- Microarrays
- Transcript analysis

Plant Physiology

- Non destructive phenotypic analysis
- Flame photometry
- ICP-AES
- Field work
- Electrophysiology
- Confocal microscopy

Biotechnology

- Cloning
- Generation of transgenic plants

Genetics

- QTL mapping
- Molecular markers
- Marker assisted selection

POTENTIAL PH.D. PROJECTS:

Project title Identification of loci and genes for salinity tolerance in cereals

Supervisors Dr Stuart Roy, Dr Matthew Gilliham and Dr Joanne Tilbrook

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Background

Salinity is a significant problem affecting agriculture worldwide, resulting in substantial losses in crop yield. To address this problem we must first understand the effects of salt (primarily the Na^+ iand Cl^- ions) on plant growth and the mechanisms plants use to survive in saline conditions. Plants use three main mechanisms to survive salinity stress: osmotic tolerance, shoot Na^+ exclusion and Na^+ tissue tolerance. Until recently, however, it has been difficult to separate these three mechanisms from each other, due to the destructive sampling methods involved. We have recently developed techniques for the non-destructive analysis of plant growth and health using image capturing devices in the Plant Accelerator (www.plantaccelerator.org.au/). This allows us to measure a plant's response to salinity over time and to separate the shoot ion independent effects, which occur early in salt stress, from the ionic effects which happen later. The aim of this project will be to use a forward genetics approach to identify genes for Na^+ exclusion, osmotic tolerance and Na^+ tissue tolerance in mapping populations of wheat and barley. As such, the project will make substantial use of the new Plant Accelerator (www.plantaccelerator.org.au/) to rapidly phenotype a variety of parameters for plant health, including shoot and root growth rates, areas of tissue damage, transpiration rates and chlorophyll fluorescence.

Aims & significance

- To identify QTLs on chromosomes linked to osmotic tolerance, shoot Na^+ exclusion, shoot Cl^- exclusion and ion tissue tolerance in wheat and barley.
- Fine mapping of QTL(s) and candidate gene(s) identification
- Cloning and sequencing of candidate gene(s)
- Heterologous expression of candidate gene(s) in yeast and oocytes
- Transformation of Arabidopsis, wheat and barley to produce salt tolerant plants

Techniques to be used

Plant Accelerator, Image analysis, QTL mapping, molecular markers, DNA and RNA extractions, cloning, plant transformation.

References

1. Roy, S.J., Huang, W., Wang, X.J., Evrard, A., Schmöckel, S.M., Zafar, Z.U. and Tester, M. (2013) A novel protein kinase involved in Na^+ exclusion revealed from positional cloning. *Plant Cell Environ*, **36**, 553-568.
2. Rajendran K., Tester M. & Roy S.J. (2009) Quantifying the three main components of salinity tolerance in cereals. *Plant, Cell & Environment* **32**: 237-249.
3. Roy, S.J., Tucker, E.J. and Tester, M. (2011) Genetic analysis of abiotic stress tolerance in crops. *Curr Opin Plant Biol*, **14**, 232-239.

GENE IDENTIFICATION

POSSIBLE PH.D. PROJECTS:

Project title Identification of genes involved in cereal salinity tolerance

Supervisors Dr Stuart Roy, Dr Delphine Fleury

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Background

Research in model plant species, such as Arabidopsis and rice, has now identified a large range of genes encoding proteins which are involved in salinity tolerance. While we know where these genes are in model plants, the location of their homologues in wheat and barley are unknown. Recent advances in the sequencing of the barley and wheat genome allows us to now identify the genomic location of these salinity tolerance genes on the chromosomes of wheat and barley. Such knowledge would be used to determine the best alleles for known salinity tolerance genes, which can then be used in traditional breeding or transgenic approaches to improve crop salinity tolerance. Genes identified by this approach will be correlated with known quantitative trait loci for salinity tolerance in wheat and barley mapping populations which will validated.

Aims & significance

The aim of this project is to identify the wheat genomic location of known genes involved plant salinity tolerance. When identified a range of wheat cultivars will be screened for allelic variation of these genes to identify sources of natural variation in salinity tolerance genes. Molecular markers will be developed for these genes for use by breeders in marker assisted selection

Techniques to be used

- Bioinformatic skills, including sequence analysis, identification of homologous genes
- Common molecular biology techniques including RT-PCR, DNA and RNA extractions as well as DNA sequencing
- Molecular marker design
- Genetic mapping software
- Non-destructive phenotyping of wheat and/or barley

References

1. Roy, S.J., Tucker, E.J. and Tester, M. (2011) Genetic analysis of abiotic stress tolerance in crops. *Curr Opin Plant Biol,* **14**, 232-239.
2. Roy, S.J. and Tester, M. (2012) Increasing salinity tolerance of crops, Avenues and Approaches *In* Encyclopedia of Sustainablility Science and Technology (Meyers, R.A. ed. New York: Springer.

GENE IDENTIFICATION

POTENTIAL PH.D. PROJECTS:

Project title Improving chloride exclusion of barley to improve its salinity tolerance

Supervisors Dr Stuart Roy, Dr Matthew Gilliham

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Background

Soil salinity (high concentrations of soil sodium (Na^+) and Cl^-)) is already a problem for Australian agricultural production, reducing economic returns, and is an issue predicted to significantly increase in the short term due to climate change and water shortages. Whereas Na^+ is not required by most crop plants, Cl^- is a micronutrient, involved in osmoregulation, charge balance, membrane potential and pH regulation, stomatal function and is a co-factor for oxygen evolving enzymes during photosynthesis. Both Na^+ and Cl^- interfere with metabolism when accumulated at high concentrations in shoot cytoplasm and some economically important crop varieties are primarily affected by Cl^- toxicity, not Na^+ toxicity e.g. grapevine, soy, medic, barley and citrus. Furthermore, the magnitude of Cl^- toxicity in salinity stress in general is believed to be underestimated as most salinity research has focussed primarily on Na^+ tolerance. Recently, work on barley has revealed significant natural variation in the ability to exclude Cl^- from the shoot. This project aims to discover the basis of this variation which will be a significant step toward the breeding salt tolerant crop plants.

Aims & significance

This research project will examine the genetic basis for the variation in chloride exclusion seen between different barley varieties by identifying candidate genes and associated transport networks involved in chloride exclusion. Novel and pre-identified candidate proteins will be functionally characterised *in planta* (cell-type and membrane localisation, regulation by salt/osmotic stress, selectivity, effect of knockout, pharmacology) and *in vitro* using heterologous expression in yeast and *Xenopus laevis* oocytes (anion selectivity/regulation). Novel candidate genes will be identified by screening barley mapping populations for chloride content. Transgenic plants will be produced misexpressing the candidate genes in an attempt to improve salt tolerance.

Techniques to be used

RNA and DNA extractions, RT-PCR, restriction digest, cloning, plant transformation, ICP-AES, electrophysiology, yeast expression systems, natural variation

References

1. White PJ & Broadley MR (2001) Chloride in soils and its uptake and movement within the plant: a review. *Annals of Botany* **88:**967-988.
2. Tavakkoli E, Fatehi F, Coventry S, Rengasamy P, McDonald, GK (2011) Additive effects of Na^+ and Cl- ions on barley growth under salinity stress. *Journal of Experimental Botany* **62:**2189-2203.
3. Teakle NL & Tyerman SD (2010) Mechanisms of Cl- transport contributing to salt tolerance. *Plant Cell and Environment* **33:**566-589.
4. Gilliham M & Tester M (2005) The regulation of anion loading to the maize root xylem. *Plant Physiology* **137:** 819-828.

GENE CHARACTERISATION

POTENTIAL PH.D. PROJECTS:

Project title Utilisation of a cell type-specific transgene expression system to express multiple salinity tolerance genes in different but specific cell types.

Supervisors Dr Stuart Roy

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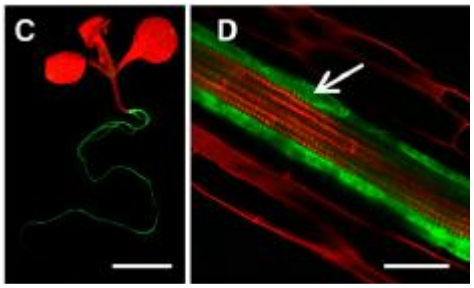
Background

Loss of crop yield due to high concentrations of Na^+ on agricultural land is a significant problem for farmers. One approach to remedy the situation is to generate transgenic plants expressing genes involved in salt transport. Constitutive over-expression of genes involved in the transport of ions, however, is often detrimental with transgenic plants showing a reduced fitness and reduced salt tolerance. We have previously shown that cell specific expression of a Na^+ transporter results in a significant reduction of shoot Na^+ concentrations, resulting in an increase in plant salinity tolerance. Root stelar specific expression of the gene *AtHKT1;1* in Arabidopsis results in a 33-66% reduction in shoot Na^+ concentrations, with similar results obtained for rice. Interestingly, these transgenic plants do not store Na^+ in stelar cells where the gene is expressed, but in root cortical cells, with evidence the plants have upregulated a variety of other Na^+ transport genes to store in the most optimal cell in the root.



The project proposed here will build on these observations, generating transgenic plants with multiple genes expressed in specific, but different, cell types to further improve the salinity tolerance of both rice and barley. The results of this project will guide future transformations of wheat .

Aims & significance

To develop lines of wheat, rice and barley expressing multiple reporter genes in different cell types to test system.
To develop lines of rice and barley expressing multiple genes for salt tolerance but in different cell types.
To characterise transgenic plants to determine if shoot Na^+ concentrations have been altered and salinity tolerance increased

Techniques to be used

RNA and DNA extractions, RT-PCR, restriction digest, cloning, plant transformation, ICP-AES, confocal microscopy

References

1. Moller, I.S., *et al.* (2009) Shoot Na^+ exclusion and increased salinity tolerance engineered by cell type-specific alteration of Na^+ transport in Arabidopsis. *Plant Cell* **21**: 2163-2178
2. Plett, D., Safwat, G., Møller, I., Gilliham, M., Roy, S.J., Shirley, N.J., Jacobs, A., Johnson, A.A.T. and Tester, M. (2010) Improved salinity tolerance of rice through cell type-specific expression of *AtHKT1;1*. *PLOS One*, **5**, e12571.

GENE MANIPULATION

POTENTIAL PH.D. PROJECTS:

Project title Improving abiotic stress tolerance in wheat and barley.

Supervisors Dr Darren Plett and Dr Stuart Roy

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Background

Abiotic stresses, such as salinity and drought stress, negatively affect plant growth and potential yield (see picture below). These stresses are particularly relevant to agriculture in Australia, causing multi-million dollar losses in the potential income of farmers and the agriculture industry. As well, these abiotic stresses are increasing due to the effects of climate change. Improvement of the abiotic stress tolerance of the largest Australian crops, wheat and barley, is crucial to alleviate this problem.

Aims & significance

Barley and wheat varieties have been genetically modified to express a variety of genes which has been shown previously to confer abiotic stress tolerance. These plants were significantly more salinity tolerant than the parental line of barley in preliminary experiments. These promising lines need to be further characterised to confirm their tolerance to salinity, drought and limited phosphorus availability.

Techniques to be used

- Common molecular biology techniques including cloning, sequencing, plasmid isolation and DNA and RNA isolation.
- Wheat transformation via particle bombardment and barley transformation via *Agrobacterium* transformation.
- Genetic analysis of genetically modified plants using Southern blot hybridisation and quantitative reverse-transcriptase PCR.
- Physiological analysis of genetically modified plants using hydroponics to analyse plant sodium transport, $^{22}Na^{+}$ radiotracer analysis of sodium transport and drought tolerance assays.
- Analysis of transgenic plants in field trials.

References

1. Roy, S.J., Huang, W., Wang, X.J., Evrard, A., Schmöckel, S.M., Zafar, Z.U. and Tester, M. (2013) A novel protein kinase involved in Na^{+} exclusion revealed from positional cloning. *Plant Cell Environ*, **36**, 553-568.
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DELIVERY

FURTHER READING:

- Roy, S.J., Tucker, E. & Tester, M. (2011) Genetic analysis of abiotic stress tolerance in crop plants. *Current Opinion in Plant Biology* **14**: 232-239
- Golzarian, M.R., Frick, R.A., Rajendran, K., Berger, B., Roy, S., Tester, M & Lun, D.S. (2011) Accurate inference of shoot biomass from high-throughput images of cereal plants. *Plant Methods* **7**: 2 (11 pp)
- Cotsaftis, O., Plett, D., Johnson, A.A.T., Walia, H., Wilson, C., Ismail, A.M., Close, T.J., Tester, M. & Baumann, U. (2011) Root-specific transcript profiling of contrasting rice genotypes in response to salinity stress. *Molecular Plant* **4**: 25–41
- Rivandi J, Miyazaki J, Hrmova M, Pallotta M, Tester M, Collins NC (2011) A SOS3 homologue maps to *HvNax4*, a barley locus controlling an environmentally-sensitive Na^+ exclusion trait. *Journal of Experimental Botany* **62**: 201–1216
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- Shavrukov Y., Gupta N.K., Miyazaki J., Baho M.N., Chalmers K.J., Tester M., Langridge P., Collins N.C. (2010) *HvNax3*—a locus controlling shoot sodium exclusion derived from wild barley (*Hordeum vulgare* ssp. *spontaneum*). *Functional and Integrative Genomics*. **10**: 277-29
- Plett, S.C., Safwat, G., Gilliham, M., Møller, I.S., Roy, S.J., Shirley, N., Jacobs, A., Johnson, A. and Tester, M. "Improved salinity tolerance of rice through cell type-specific expression of AtHKT1;1" *PLoS ONE* **5**:e12571
- Genc, Y., Oldach, K., Verbyla, A.P., Lott, G., Hassan, M., Tester, M., Wallwork, H. & McDonald, G.K. (2010) Sodium exclusion QTL associated with improved seedling growth in bread wheat under salinity stress. *Theoretical and Applied Genetics* **121**:877–894
- Plett, D.C. and Møller, I.S. (2010) Na^+ transport in glycophytic plants: what we know and would like to know, *Plant Cell and Environment*, **33**: 612-626
- Jha, D, Shirley, N., Tester, M. and Roy, S.J. (2010) Variation in salinity tolerance and shoot sodium accumulation in Arabidopsis ecotypes linked to differences in the natural expression levels of transporters involved in sodium transport, *Plant, Cell and Environment* **33**:793-804
- Shavrukov Y., Langridge P., Tester M. (2009) Salinity tolerance and sodium exclusion in genus *Triticum*. Breeding Science 59: 671–678.
- Møller I.S., Gilliham, M., Jha, D., Mayo, G.M., Roy, S.J., Coates, J.C., Haseloff, J. & Tester, M. (2009) Shoot Na^+ exclusion and increased salinity tolerance engineered by cell type-specific alteration of Na^+ transport in Arabidopsis. *Plant Cell* **21**: 2163-2178.
- Rajendran K., Tester M. & Roy S.J. (2009) Quantifying the three main components of salinity tolerance in cereals. *Plant, Cell & Environment* **32**: 237-249.
- Munns R. & Tester M. (2008) Mechanisms of salinity tolerance. *Annual Reviews of Plant Biology*, **59**: 651-681.

WE ARE LOOKING FOR:

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