

Ecological function of a water-sensing molecular fossil (Ref IAP2-I8-I03)

Heriot-Watt University Institute of Life and Earth Sciences
In partnership with **Centre for Ecology and Hydrology**

Supervisory Team

- [Peter Morris](#), Heriot-Watt University
- [Ross Alexander](#), Heriot-Watt University
- [Jill Thompson](#), Centre for Ecology & Hydrology

Key Words

Drought stress, salinity, germination, osmosensor, cereals

Overview Abiotic stress tolerance in plants is a critical attribute and a key objective for plant breeders. The world is experiencing unprecedented increases in average global temperatures and extreme weather events leading to intermittent periods of drought and waterlogging (water stress) in all likelihood due to anthropogenic climate change. In addition, over-irrigation of agricultural land has resulted in increased salination of once productive arable soils. This has resulted in a situation in which farmers world-wide face problems of ensuring sufficient agronomic productivity for the future, because of the negative effects of climate change and salination on crop growth and development. In order to mitigate these problems, it is essential that we better understand how it is that plants respond to and adapt to abiotic stresses such as drought and salinity. Central to both water and salinity stresses is how plants go about regulating and optimising their use of water, and one of the most sensitive stages to water stress during a plant life cycle is the transition from seed to seedling: germination. This project will investigate the ecological variability in molecular mechanisms by which drought-tolerant and drought-susceptible barley land races respond to water or salinity stress particularly during germination but also during vegetative growth and reproduction. This will be contrasted with the same mechanisms in drought tolerant tropical cereals such as millet or sorghum. We have recently identified a molecular sensor, and an associated MAP kinase signalling pathway that is critical for how plants respond to water stress. QQ21 is an osmosensor that is conserved in sequence all the way back to the first land plants (liverworts). This plasma membrane protein functionally complements mutations in the yeast osmosensor Sho1 (Fig. 1). In plants, QQ21 is highly

expressed during seed development and germination and is upregulated by dormancy and abscisic acid. Mutations in the QQ21 gene results in plants with enhanced salt sensitivity during germination (Fig. 2). Thus, variability in the levels of QQ21 expression or variability in the QQ21 gene and protein sequence may well underlie the differences in drought and salt tolerance of plants during germination and later growth, and this is the hypothesis that will be explored in this project.

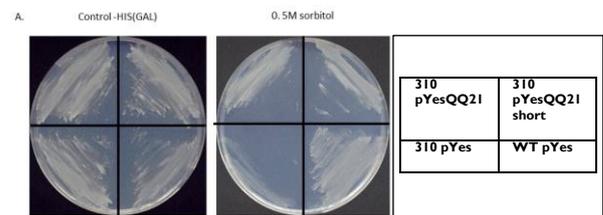


Fig. 1. QQ21 complements the yeast osmosensor mutant Sho1 (310).

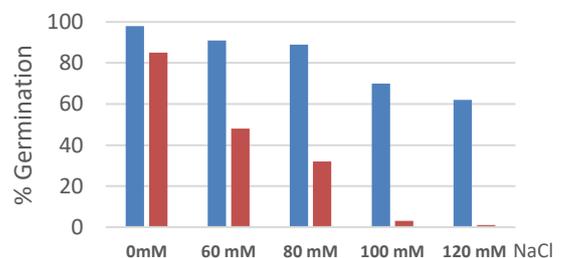


Fig. 2. Germination sensitivity to salt of QQ21 mutant (red bars) and wildtype (blue bars)

Methodology

The key aims of this project will be to: (i) identify allelic variation for barley in a novel plant osmosensing

pathway leading to abiotic stress tolerance. (ii) test the robustness of this tolerance in the field and by genetic complementation of stress compromised mutants. (iii) carry out a comparison with stress tolerance mechanisms in tropical cereals.

We have phenotyped over 150 European barley landraces (including both two and six-rowed types) and 20 elite cultivars in respect of germination under osmotic stress and have identified striking differences in a number of different lines. Some are very tolerant to osmotic stress, others are very intolerant. In this project we will take selected barley varieties and further characterise them in terms of drought tolerance and we will look at the gene expression patterns of the osmosensor gene *QQ21*, and its associated downstream pathway components. We will also look at functionality of variability in the protein sequence of these lines. Additionally, we will compare the expression patterns and sequences of the highly conserved gene *QQ21* from tropical cereals such as millet and sorghum with known drought tolerance traits.

Of the 150 barley cultivars that have been phenotypically characterised with respect to drought, 5 sensitive lines and 5 resistant lines will be taken and grown to maturity under control conditions (greenhouse and hydroponics) and exposed to drought and salinity to monitor the abiotic stress susceptibility of the mature plant. Fresh weight, dry weight, relative water content, reactive oxygen species and stress gene expression will be monitored in these plants. RNA will be prepared from germinating seeds and seedlings under control and stress conditions and qPCR will be used to measure *QQ21* gene expression, as well as other genes such as MAP kinase kinase 3, known to interact with *QQ21* and be involved in the transduction of water stress. In addition, expression of stress-induced genes such as catalase, ascorbate oxidase, and transcription factors such as *MYB1* will be studied. This will yield a thorough description of the role and importance of osmosensor systems in drought tolerance. Gene sequencing will be used to characterise those barley alleles of *QQ21* associated with abiotic stress resistance, and molecular variants will be functionally tested by cloning into a binary vector harbouring the native *QQ21* promoter and complementation of the *Arabidopsis* *QQ21* mutant. In addition, we will grow drought-tolerant tropical cereal species such as millet and sorghum and compare the expression patterns of *QQ21* and associated proteins in these plants under control and stress conditions, in order to seek and confirm similarities in osmosensor associated gene expression.

Overlapping this molecular-based approach will be consideration of the geographic background and field

performance of each sub-selected barley landrace. This will explore the possibility that robust abiotic stress resistance comes with the associated cost of a trade-off for other factors that impact on plant growth. In order to evaluate this, field trials will be carried out over two years in order to monitor important aspects of agronomic performance such as yield and disease resistance of the abiotic stress tolerant and non-tolerant barley varieties.

These experiments will shed further light on how plants have adapted to challenging abiotic stress conditions throughout evolution and in our present world, and will provide valuable information on plant breeding strategies for resilient crops for the future.

Timeline

Year One

Growth of barley varieties under control conditions, initiate physiological and molecular analysis of stress tolerant/intolerant barley varieties including sequence analysis of *QQ21* alleles.

Year Two

Continue with analysis of barley varieties. Comparisons of gene expression patterns and sequences of key genes with stress tolerant tropical cereal species. Field trials of stress tolerant barley varieties. Genetic complementation of *Arabidopsis* mutants with variant alleles of *QQ21*.

Year Three

Field trials of stress tolerant varieties, analysis of data from barley field trials (disease scores, yield). Preparation of the thesis and journal papers

Submission of thesis aimed at 3.5 years after commencement of project.

Training & Skills

The student will have access to the full variety of the extensive IAPETUS2-cohort training, including workshops and cohort meetings, and it is anticipated that they will make full use of these opportunities in order to develop broader transferable skills and knowledge. Furthermore, Heriot-Watt University offers a broad pallet of training opportunities relevant to postgraduate students. Course sessions are typically delivered in three hour sessions facilitated by experts, and mapping onto the four domains of the Researchers Development Framework: A, Knowledge and intellectual abilities: B, Personal effectiveness: C,

Research governance and organisation: D,
Engagement, influence and impact (www.vitae.ac.uk).

In addition to the more generic training, the student will be trained in a range of scientific methods: molecular biological methods (e.g. Q-PCR, bioinformatics) Plant physiology (e.g. abiotic stress biology), data analysis (including appropriate statistical analysis). All methodologies are well established in the applicants' laboratories.

The student will join a strong set of laboratories working on a variety of aspects of plant stress. They will use a range of innovative techniques in molecular biology, plant physiology and environmental studies on drought and stress. The project provides for excellent multidisciplinary training and an exciting opportunity to interact between the fields of molecular biology, ecology of environmental stress and plant physiology.

We anticipate that the PhD student will present their results in at least two appropriate meetings in the UK (for example Society for Experimental Biology) and at least one international symposium (for example International conference for Plant Physiology). The student will also have opportunities to network with project partners at Heriot-Watt and the Centre for Ecology & Hydrology and to become a member of the broader scientific community working on plant stress.

References & Further Reading

Abass, M. and Morris, P. C. (2013). The *Hordeum vulgare* signalling protein MAP kinase 4 is a regulator of biotic and abiotic stress responses. *J. Plant Physiol.* 170, 1353-1359.

Qiu, J. L., Zhou, L., Yun, B. W., Nielsen, H. B., Fiil, B. K., Petersen, K., Mackinlay, J., Loake, G. J., Mundy, J.

and Morris, P. C. (2008). Arabidopsis mitogen-activated protein kinase kinases MKK1 and MKK2 have overlapping functions in defense signaling mediated by MEKK1, MPK4, and MKS1. *Plant Physiol.* 148, 212-222.

Rae, S. J., Macaulay, M., Ramsay, L., Leigh, F., Matthews, D., O'sullivan, D. M., Donini, P., Morris, P. C., Powell, W. and Marshall, D. F. (2007). Molecular barley breeding. *Euphytica* 158, 295-303.

Wendelboe-Nelson, C. and Morris, P. C. (2012). Proteins linked to drought tolerance revealed by DIGE analysis of drought resistant and susceptible barley varieties. *Proteomics* 12, 3374-3385.

Further Information

Peter Morris, Institute of Life and Earth Sciences
Heriot-Watt University, Riccarton
Edinburgh, EH14 4AS
Tel +44 (0)131 451 3452
p.c.morris@hw.ac.uk

Ross Alexander, Institute of Life and Earth Sciences
Heriot-Watt University, Riccarton
Edinburgh, EH14 4AS
Tel +44 (0)131 451 3181
r.alexander@hw.ac.uk

Jill Thompson
Centre for Ecology & Hydrology
Bush Estate, Penicuik
EH26 0QB
Tel +44 (0)131 445 8518
jjom@ceh.ac.uk