

Identification of drought responsive microRNAs and  
functional analysis of a sample microRNA for drought  
tolerance in barley (*Hordeum vulgare* L.)

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## Abstract

Declining water resources coupled with the dwindling size of agricultural land necessitate the development of drought tolerant crop varieties. However, genetic control of drought tolerance is complex because of the involvement of a large number of genes. Studies in plant biotechnology for improved plant stress tolerance mostly concentrate on implementing and manipulating downstream gene, involved in the physiological responses. Recently, the upstream gene regulatory network involving small, non-coding RNAs such as microRNAs (miRNAs) and their target genes has been discovered. However, the drought regulatory relationship between miRNAs and their targets in barley, one of the major cereal crops, is still largely unknown. In this PhD project, potential drought responsive miRNAs and their targets were identified and validated in barley genotypes, and the drought tolerance of transgenic barley over-expressing miR827, a previously reported miRNA that conferred drought tolerance in *Arabidopsis*, was examined.

To identify and validate drought responsive miRNAs and their targets, we conducted expression analysis of several drought responsive miRNAs under drought treatment in four barley genotypes that were reported to perform well in the drought prone areas of Australia. Differences in expression of four miRNAs; *Ath*-miR169b, *Osa*-miR1432, *Hv*-miRx5 and *Hv*-miR166b/c were observed between drought-treated and well-watered barley samples, and this expression varied among the experimental genotypes. Generally, miRNA-mediated cleavage of the target mRNAs was observed at the ideal cleavage site, however we also found miRNA-mediated cleavage not limited to the canonical position. We detected the spatial accumulation of potential drought responsive miRNAs and targets that mostly localized in the mesophyll tissues, and inverse correlation of expression between mature miRNAs and their target mRNAs.

These results suggested a contribution of miRNAs to the molecular control of target gene expression, genotype-specific regulation under drought treatment, and the promise of miRNA: mRNA as biomarkers for genotypic selection in barley.

To examine the drought tolerance of transgenic barley expressing miR827, we monitored the performance under drought of miR827 over-expressing transgenic barley plants under the control of the constitutive promoter *CaMV-35S* and drought-inducible promoter *Zm-Rab17*. We observed that the ectopic overexpression of *Ath-miR827* resulted in unwanted side-effects, that is reduced shoot area, delayed anthesis and reduced whole plant water use efficiency ( $WUE_{wp}$ ) and weight of seeds per plant in the transgenic compared with the wild type counterpart or null plants. In contrast, the drought inducible expression of *Hv-miR827* caused several promising phenotypes; that is, the transgenic barley plants did not show reduced shoot area or delayed anthesis compared to the wild type, while the  $WUE_{wp}$  and leaf relative water content (RWC) were improved. Further, we observed the advantages of drought inducible *Hv-miR827* over-expression in the recovery of transgenic plants after drought stress. These findings suggest that the over-expression of *Hv-miR827* is promising for improving plants' performance under drought and that miRNA-mediated drought tolerance or intolerance also depends on the nature of promoter.

## **Declaration**

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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**Jannatul Ferdous**

**Date** 19/02/2016

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## **List of Publications**

1. Ferdous J., Hussain, S.S. & Shi, B.J. (2015a) Role of microRNAs in plant drought tolerance. *Plant Biotechnology Journal* 13, 293–305.
2. Ferdous J., Li Y., Reid N., Langridge P., Shi B.J. & Tricker P.J. (2015b) Identification of reference genes for quantitative expression analysis of microRNAs and mRNAs in barley under various stress conditions. *PLoS ONE* 10(3), e0118503.
3. Ferdous J., Sanchez-Ferrero J.C., Langridge P., Milne L., Chowdhury J. & Tricker P.J. (2015) Differential expression of microRNAs and potential targets under drought stress in barley (Accepted by *Plant, Cell & Environment*, doi: 10.1111/pce.12764).

## **List of Awards**

### **Best Poster Award (2014)**

Awarded for the best poster presentation at the Research Day, School of Agriculture, Food and Wine, the University of Adelaide.

### **Travel Award (2015)**

Awarded by the Grains Research and Development Corporation, Australia to attend the “Mini-symposium on Cereal Genomics to address Grand Challenges” in Grasmere, Cumbria, England.