

Genome-wide characterisation of microRNAs and their target
genes in different durum wheat genotypes under water
limiting conditions

By

Haipei Liu

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The University of Adelaide

Faculty of Sciences

School of Agriculture, Food and Wine

Waite Campus,

Adelaide, South Australia

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Abstract

Durum wheat (*Triticum turgidum* L. ssp. *durum*) is a tetraploid wheat species grown primarily in the North American Great Plains, Mediterranean Europe, Northern Africa, Mexico and Australia. An important limiting factor for durum production in Mediterranean environments like South Australia is water deficit immediately prior to and during anthesis, adversely affecting durum productivity and quality. Investigating water deficit response mechanisms and genotypic differences within a crop species is an important strategy for understanding the basis of water-deficit stress response and for selection of elite genotypes with improved stress tolerance. In plants, microRNAs (miRNAs), which are a class of small non coding RNAs, have been identified as important regulators of plant development and abiotic stress responses. While the miRNA transcriptome under water limiting conditions has been investigated in many crop species, it is poorly characterised in durum wheat.

In this study, glasshouse experiments over two years evaluated 20 durum wheat genotypes for their variation in various morphological, physiological and yield responses to pre-anthesis water-deficit stress. Four Australian durum varieties with contrasting stress sensitivities were identified. High-throughput Illumina sequencing of 96 small RNA libraries constructed from the flag leaf and head tissues of these four genotypes detected 110 conserved miRNAs and 159 novel candidate miRNA hairpins. Statistical analysis of sequencing reads revealed the differential expression profiles of durum miRNAs associated with water-deficit stress treatment, tissue type and genotype. Most importantly, several conserved and novel miRNAs showed inverted regulatory profiles between the stress tolerant and sensitive varieties. Subsequent genome-wide *in silico* analysis identified 2055 putative targets for conserved durum miRNAs, and 131 targets for four novel durum miRNAs possibly contributing to genotypic stress tolerance. Predicted mRNA targets of the stress responsive miRNAs encode various transcription factors, binding proteins, and functional enzymes,

which play vital roles in multiple biological pathways such as hormone signalling and metabolic processes, suggesting the extensive involvement of miRNA-target regulatory modules in water-deficit stress adaptation. Quantitative PCR profiling further characterised 50 target genes and 12 miRNAs with stress responsive and/or genotype-dependent expression profiles. A 5' RLM-RACE approach subsequently validated the regulation of nine targets by water-deficit stress responsive miRNAs, providing the first experimental evidence that target mRNAs are genuinely cleaved by miRNAs in durum wheat. Characterisation of the individual miR160/Auxin Response Factors regulatory module further revealed their expression profile over different time points during water-deficit stress.

The present study provides a comprehensive and comparative description of the miRNA transcriptome and their targets in durum wheat varieties with contrasting water-deficit stress tolerance, providing new insights into the functional roles of miRNA-guided RNAi mechanisms. Results derived from this work could contribute to future research on the characterisation of individual miRNA regulatory modules and their specific biological functions, exploiting the potential of *Triticum turgidum* miRNA in developing RNAi-improved crops with stress tolerance.

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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Signature

Haipei Liu

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