Long- and short-term nitrate uptake regulation in maize

By

Luke Reid Holtham

Thesis submitted in fulfilment of the requirements for the degree of

Doctorate of Philosophy in the Faculty of Sciences at

The University of Adelaide

Australian Centre for Plant Functional Genomics, Adelaide

December 2014

I dedicate this thesis

in loving memory of my dearest Nan

Audine Kay Holtham

Long- and short-term nitrate uptake regulation in maize

By

Luke Holtham

Supervised by:

Associate Prof Sigrid Heuer
Senior Research Scientist
Australian Centre for Plant Functional Genomics
The University of Adelaide

Dr Trevor Garnett
Research Scientist
The Plant Accelerator
The University of Adelaide

Dr Darren Plett
Research Scientist
Australian Centre for Plant Functional Genomics
The University of Adelaide

Dr Mamoru Okamoto
Research Scientist
Australian Centre for Plant Functional Genomics
The University of Adelaide

Prof Mark Tester
Centre for Desert Agriculture
Division of Biological and Environmental Sciences and Engineering
King Abdullah University of Science and Technology
Kingdom of Saudi Arabia

Thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

School of Agriculture, Food and Wine

Faculty of Science,

The University of Adelaide

Waite Research Institute, Glen Osmond, SA 5064

Email: luke.holtham@adelaide.edu.au

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.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1986.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Luke Reid Holtham

December, 2014

Acknowledgements

The successful completion of this dissertation was made possible by the invaluable contribution of a number of people. I would like to profess my deepest gratitude to my supervisors Dr Trevor Garnett, Dr Darren Plett and Dr Mamoru Okamoto for their support, guidance, mentorship, and persistent hard work in shaping my professional growth. Over the many years we worked together I believe in addition to a lasting professional relationship, a lasting friendship also formed. Through all the twists and turns you all made this experience one to remember and that will be treasured. Thank you all for the challenge, the distilled knowledge and the many laughs.

Professor Mark Tester, our time spent engaged in stimulating and insightful discussion further cultivated my entrepreneurial and scientific spirit instilling confidence for my future career, I can't thank you enough for the many wise words. My regards also goes to Associate Professor Sigrid Heuer whose advice and support was invaluable. Dr Ute Baumann, your continual effort to teach and mentor me was truly valued and grew my knowledge significantly, for this I thank you. John Toubia your continued technical support throughout my thesis was a significant factor in helping me to complete this dissertation, I graciously thank you for all your guidance. I would like to take this opportunity to thank all the members of nitrogen use efficiency team for the assistance, care and support they gave me throughout my time with the group. Especially I would like to thank Akiko Enju, Gabriele Fiene and Dr Vanessa Melino for their continual support and laughs.

I gratefully thank the University of Adelaide, the Grains Research and Development Corporation (GRDC) and the Australian Centre for Plant Functional Genomics (ACPFG) for financially supporting me and my research throughout my candidature.

I would like to thank all my friends for their patience and support. This period of my life was extremely important for developing my knowledge and setting up my future career. Those closest to me understood my time constraints and were truly supportive.

My heart goes out to all of my family for their unconditional love and support throughout this period of my life. Without them all I may not have successfully made it to the end of this journey. My beautiful mother Sonya Wyness was there for me the whole way, through all of the ups and downs. She consoled me at important times and enabled me to continually see the vision at the end, when sometimes it seemed to have faded. It is truly a tribute to her, through her support and encouragement over the years that I was able to reach this level of academic accomplishment. Similarly, the love, support and encouragement from my dearest grandmother Elaine Melhuish has been fundamental to my success over the years. She is a role model for hard work which continually gave me strength during tough times and fuelled my perseverance to reach the end of this goal. My beloved father David Holtham continually challenges me to think outside the square and take on life. His energy, optimism and wisdom were key motivators and essential to my success in this journey. Finally I thank my beautiful partner Danica Jeffrey for her patience and love over this tough period. She stuck by my side in times of angst and depression and continually raised my spirits. Without this love and companionship I may not have made it to the finish line.

For anyone reading this, in order to reach your goals and be successful in life I believe you must have a long term vision. Once you have that vision; be tenacious, chase it, and don't give up until you succeed. There is no such word as "can't". In my book, anything is possible.

"*Many of life's failures are people who did not realize how close they were to success when they gave up*"

- Thomas A. Edison -

Table of Contents

NOTE: Statements of authorship appear in the print copy of the thesis held in the University of Adelaide Library.

Abstract

Cereal crops supply a major proportion of the world's food and their production capacity is tightly linked to nitrogen (N) fertiliser use. With on average less than half of the applied N being captured by crops, there is scope and need to improve N uptake in cereals. With nitrate (NO_3^-) being the main form of N available to cereal crops there has been a significant global research effort to understand plant NO_3^- uptake. Despite this, our understanding of how the NO_3^- uptake system is regulated remains limited.

To advance our understanding of the NO_3^- uptake system and its regulation, three knowledge gaps were identified and explored in this thesis. Firstly, there is an identified need to better understand the NO_3^- uptake system and the signalling molecules which modulate it. Secondly, with the literature containing alternative approaches to studying NO_3^- uptake, there is a need to appreciate how these studies relate to better leverage the existing literature. And finally, with strong transcriptional control governing the NO_3^- uptake system, new leads were sought for modulating transcription of NO_3^- transporter genes.

To explore these knowledge gaps, dwarf maize (*Zea mays* L. var. Gaspe Flint) was grown hydroponically with either sufficient or limiting NO_3^- availability. During the vegetative growth period a subset of plants grown were moved from sufficient to limiting NO_3^- conditions and a range of physiological parameters were measured. The results showed: the high affinity NO_3^- uptake system (HATS) appears to contribute a major proportion of total NO_3^- uptake capacity and responds to N demand at external concentrations where it was previously thought to be saturated; NO_3^- itself appears to play a key role in modulating the NO_3^- uptake system, and; temporal variation of *NRT* transcripts are more variable than previously understood. The observed responses to reduction in NO_3^- revealed a series of responses leading to a new model for the control of the NO_3^- uptake system. Using the same growth system, plants were grown under steady state NO_3^- conditions and a starvation and re-

supply (primary nitrate response – PNR) response was explored in parallel. The information generated provided data to relate the PNR literature to longer term steady state studies. The *ZmNRT2.5* gene was highlighted as an interesting candidate for revealing cis-trans regulatory elements associated with low N responses. To explore this, a combined phylogenomics and co-expressed gene promoter analysis was undertaken. A number of evolutionarily and functionally conserved regions were identified in the *ZmNRT2.5* promoter with six regions showing no resemblance to known transcription factor binding sites. These sequences provide a new resource for the discovery of cis-trans regulatory mechanisms associated with the low N expression of *ZmNRT2.5*.

The findings in this thesis have identified key time points for future transcriptome analysis, and revealed putative cis-elements as new leads for discovering novel cis-trans regulatory elements associated with the regulation of NO_3^- uptake. Ultimately, further research may lead to the identification of key regulatory genes as candidates for the improvement of N uptake efficiency and overall N use efficiency in cereal crops.

List of Abbreviations

AA	amino acid
ANOVA	analysis of variance
bnt	billion tonnes
C	carbon
d	days
DAE	days after emergence
DW	dry weight
g	gram
HATS	high-affinity transport system
LATS	low-affinity transport system
N	nitrogen
NH_4^+	ammonium
NiR	nitrite reductase
NO_3^-	nitrate
NPF	nitrate transporter 1/peptide transporter family
NR	nitrate reductase
NRT	nitrate transporter
NUE	nitrogen use efficiency
NUpE	nitrogen uptake efficiency
NUtE	nitrogen utilisation efficiency
R:S	root to shoot biomass ratio
SEM	standard error of the mean
TAA	total amino acids
TFs	transcription factors