Long- and short-term nitrate uptake regulation in maize

By

Luke Reid Holtham

Thesis submitted in fulfilment of the requirements for the degree of

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The University of Adelaide

Australian Centre for Plant Functional Genomics, Adelaide

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I dedicate this thesis

in loving memory of my dearest Nan

Audine Kay Holtham
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Declaration

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Luke Reid Holtham

December, 2014
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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

Declaration .................................................................................................................................................. iii  
Acknowledgements .................................................................................................................................... iv  
Table of Contents ....................................................................................................................................... vi  
Abstract ......................................................................................................................................................... xii  
List of Abbreviations .................................................................................................................................... xiv

## Chapter 1: Literature review ..................................................................................................................... 1  
1.1 The importance of cereals ..................................................................................................................... 2  
1.2 Meeting global demand ......................................................................................................................... 2  
1.3 Nitrogen in agriculture ........................................................................................................................... 3  
1.3.1 A brief history .................................................................................................................................... 3  
1.3.2 Economics ......................................................................................................................................... 4  
1.3.3 Environmental impact ....................................................................................................................... 5  
1.4 Nitrogen use efficiency .......................................................................................................................... 5  
1.4.1 Defining NUE ..................................................................................................................................... 5  
1.4.2 Agronomy .......................................................................................................................................... 6  
1.4.3 Improving plant NUE ....................................................................................................................... 6  
1.5 The plant nitrogen management system ............................................................................................... 7  
1.5.1 N in soils ........................................................................................................................................... 7  
1.5.2 Nitrate uptake .................................................................................................................................... 7  
1.5.3 Assimilation and storage .................................................................................................................... 10  
1.5.4 Transport within the plant ............................................................................................................... 10  
1.5.5 Remobilisation .................................................................................................................................. 11  
1.6 The controllers of nitrate uptake ........................................................................................................... 12  
1.6.1 Transcriptional ............................................................................................................................... 13  
1.6.2 Post Transcriptional ....................................................................................................................... 14  
1.6.3 Post translational ............................................................................................................................ 15
Chapter 3: Dynamics of N response depends on N status in maize plants: Comparison between nitrate induction and steady state

ABSTRACT

KEYWORDS

INTRODUCTION

MATERIALS AND METHODS

RESULTS

Biomass

Nitrate

Amino Acids

ACKNOWLEDGEMENTS

LITERATURE CITED

FIGURES

SUPPORTING INFORMATION FIGURES
Chapter 5: General discussion .......................................................... 180

5.1 Advances in knowledge from this thesis ....................................... 180

5.1.1 The HATS – a main contributor to total nitrate uptake .............. 180

5.1.2 NRT levels fluctuate daily in response to N demand .................. 180

5.1.3 NRT changes in response to decreasing nitrate availability .......... 181

5.1.4 Nitrate may be the key signalling molecule for the HATS ............ 181

5.1.5 The energy cost of nitrate uptake may be important ................. 182

5.1.6 A new model ........................................................................... 183

5.1.7 Understanding a complex system requires complex approaches .... 183

5.1.8 NRT2.5 cis-trans regulatory motifs .......................................... 184

5.2 Future directions ......................................................................... 185

5.2.1 Completing the loop – Phloem sap measurements ...................... 185

5.2.2 Relating transcripts to functional protein ................................. 185

5.2.3 Investigating the energy cost of nitrate uptake ......................... 186

5.2.4 Transcriptomics ....................................................................... 186

5.2.5 The generation of cereal NRT mutants ..................................... 187

5.2.6 Extending the comparative study ............................................. 187
5.2.7 Continuing the cis-trans regulation discovery .............................................. 188
5.3 Summary ................................................................................................................ 189
5.4 Literature cited ....................................................................................................... 190
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\textsubscript{3}\textsuperscript{-}) being the main form of N available to cereal crops there has been a significant global research effort to understand plant NO\textsubscript{3}\textsuperscript{-} uptake. Despite this, our understanding of how the NO\textsubscript{3}\textsuperscript{-} uptake system is regulated remains limited.

To advance our understanding of the NO\textsubscript{3}\textsuperscript{-} 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\textsubscript{3}\textsuperscript{-} uptake system and the signalling molecules which modulate it. Secondly, with the literature containing alternative approaches to studying NO\textsubscript{3}\textsuperscript{-} 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\textsubscript{3}\textsuperscript{-} uptake system, new leads were sought for modulating transcription of NO\textsubscript{3}\textsuperscript{-} transporter genes.

To explore these knowledge gaps, dwarf maize (Zea mays L. var. Gaspe Flint) was grown hydroponically with either sufficient or limiting NO\textsubscript{3}\textsuperscript{-} availability. During the vegetative growth period a subset of plants grown were moved from sufficient to limiting NO\textsubscript{3}\textsuperscript{-} conditions and a range of physiological parameters were measured. The results showed: the high affinity NO\textsubscript{3}\textsuperscript{-} uptake system (HATS) appears to contribute a major proportion of total NO\textsubscript{3}\textsuperscript{-} uptake capacity and responds to N demand at external concentrations where it was previously thought to be saturated; NO\textsubscript{3}\textsuperscript{-} itself appears to play a key role in modulating the NO\textsubscript{3}\textsuperscript{-} uptake system, and; temporal variation of NRT transcripts are more variable than previously understood. The observed responses to reduction in NO\textsubscript{3}\textsuperscript{-} revealed a series of responses leading to a new model for the control of the NO\textsubscript{3}\textsuperscript{-} uptake system. Using the same growth system, plants were grown under steady state NO\textsubscript{3}\textsuperscript{-} 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 NO₃⁻ 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

<table>
<thead>
<tr>
<th>Page</th>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
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<td>AA</td>
<td>amino acid</td>
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<tr>
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<td>ANOVA</td>
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</tr>
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<td>282</td>
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<tr>
<td>283</td>
<td>DAE</td>
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<tr>
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<td>DW</td>
<td>dry weight</td>
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<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>286</td>
<td>HATS</td>
<td>high-affinity transport system</td>
</tr>
<tr>
<td>287</td>
<td>LATS</td>
<td>low-affinity transport system</td>
</tr>
<tr>
<td>288</td>
<td>N</td>
<td>nitrogen</td>
</tr>
<tr>
<td>289</td>
<td>NH$_4^+$</td>
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</tr>
<tr>
<td>290</td>
<td>NiR</td>
<td>nitrite reductase</td>
</tr>
<tr>
<td>291</td>
<td>NO$_3^-$</td>
<td>nitrate</td>
</tr>
<tr>
<td>292</td>
<td>NPF</td>
<td>nitrate transporter 1/peptide transporter family</td>
</tr>
<tr>
<td>293</td>
<td>NR</td>
<td>nitrate reductase</td>
</tr>
<tr>
<td>294</td>
<td>NRT</td>
<td>nitrate transporter</td>
</tr>
<tr>
<td>295</td>
<td>NUE</td>
<td>nitrogen use efficiency</td>
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<tr>
<td>296</td>
<td>NUpE</td>
<td>nitrogen uptake efficiency</td>
</tr>
<tr>
<td>297</td>
<td>NUtE</td>
<td>nitrogen utilisation efficiency</td>
</tr>
<tr>
<td>298</td>
<td>R:S</td>
<td>root to shoot biomass ratio</td>
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<td>SEM</td>
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<tr>
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