



# Long- and short-term nitrate uptake regulation in maize

By

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*I dedicate this thesis*

*in loving memory of my dearest Nan*

*Audine Kay Holtham*

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48 Luke Reid Holtham

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*"Many of life's failures are people who did not realize how close they were to success when  
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- Thomas A. Edison -

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## 238 **Abstract**

239 Cereal crops supply a major proportion of the world's food and their production capacity is  
240 tightly linked to nitrogen (N) fertiliser use. With on average less than half of the applied N  
241 being captured by crops, there is scope and need to improve N uptake in cereals. With nitrate  
242 ( $\text{NO}_3^-$ ) being the main form of N available to cereal crops there has been a significant global  
243 research effort to understand plant  $\text{NO}_3^-$  uptake. Despite this, our understanding of how the  
244  $\text{NO}_3^-$  uptake system is regulated remains limited.

245 To advance our understanding of the  $\text{NO}_3^-$  uptake system and its regulation, three knowledge  
246 gaps were identified and explored in this thesis. Firstly, there is an identified need to better  
247 understand the  $\text{NO}_3^-$  uptake system and the signalling molecules which modulate it. Secondly,  
248 with the literature containing alternative approaches to studying  $\text{NO}_3^-$  uptake, there is a need  
249 to appreciate how these studies relate to better leverage the existing literature. And finally,  
250 with strong transcriptional control governing the  $\text{NO}_3^-$  uptake system, new leads were sought  
251 for modulating transcription of  $\text{NO}_3^-$  transporter genes.

252 To explore these knowledge gaps, dwarf maize (*Zea mays* L. var. Gaspé Flint) was grown  
253 hydroponically with either sufficient or limiting  $\text{NO}_3^-$  availability. During the vegetative  
254 growth period a subset of plants grown were moved from sufficient to limiting  $\text{NO}_3^-$   
255 conditions and a range of physiological parameters were measured. The results showed: the  
256 high affinity  $\text{NO}_3^-$  uptake system (HATS) appears to contribute a major proportion of total  
257  $\text{NO}_3^-$  uptake capacity and responds to N demand at external concentrations where it was  
258 previously thought to be saturated;  $\text{NO}_3^-$  itself appears to play a key role in modulating the  
259  $\text{NO}_3^-$  uptake system, and; temporal variation of *NRT* transcripts are more variable than  
260 previously understood. The observed responses to reduction in  $\text{NO}_3^-$  revealed a series of  
261 responses leading to a new model for the control of the  $\text{NO}_3^-$  uptake system. Using the same  
262 growth system, plants were grown under steady state  $\text{NO}_3^-$  conditions and a starvation and re-

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

272 The findings in this thesis have identified key time points for future transcriptome analysis,  
273 and revealed putative cis-elements as new leads for discovering novel cis-trans regulatory  
274 elements associated with the regulation of  $\text{NO}_3^-$  uptake. Ultimately, further research may lead  
275 to the identification of key regulatory genes as candidates for the improvement of N uptake  
276 efficiency and overall N use efficiency in cereal crops.



## 277 **List of Abbreviations**

278	AA	amino acid
279	ANOVA	analysis of variance
280	bnt	billion tonnes
281	C	carbon
282	d	days
283	DAE	days after emergence
284	DW	dry weight
285	g	gram
286	HATS	high-affinity transport system
287	LATS	low-affinity transport system
288	N	nitrogen
289	NH <sub>4</sub> <sup>+</sup>	ammonium
290	NiR	nitrite reductase
291	NO <sub>3</sub> <sup>-</sup>	nitrate
292	NPF	nitrate transporter 1/peptide transporter family
293	NR	nitrate reductase
294	NRT	nitrate transporter
295	NUE	nitrogen use efficiency
296	NU <sub>p</sub> E	nitrogen uptake efficiency
297	NU <sub>t</sub> E	nitrogen utilisation efficiency
298	R:S	root to shoot biomass ratio
299	SEM	standard error of the mean
300	TAA	total amino acids
301	TFs	transcription factors