

Nitrate and Ammonium Interactions in Maize

By

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Thesis submitted in fulfilment of the requirements for the degree of

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Australian Centre for Plant Functional Genomics, Adelaide June 2014 I dedicate this thesis

in loving memory of my mother

AMMINI GEORGE

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Table of Contents

Abstract	v
Declaration	vii
Acknowledgements	viii
List of abbreviations	X
Chapter 1: Introduction and literature review	1
1.1 INTRODUCTION	1
1.2 LITERATURE REVIEW	2
1.2.1 Nitrogen in the soil	2
1.2.2 N fertilizer use and its environmental and economic impact	4
1.2.3 NO ₃ ⁻ and NH ₄ ⁺ uptake in plants	5
1.2.4 Factors affecting plant preference for different nitrogen sources	13
1.2.5 Inhibition of NO_3^- uptake by NH_4^+	15
1.3 AIM & OBJECTIVES.	16
Chapter 2: Small amounts of ammonium (NH4 ⁺) increase plant growt	h in
maize (Zea mays L.)	18
Chapter 3: Why do small amounts of ammonium (NH ₄ ⁺) increase plan	nt
growth in maize (Zea mays L.)?	46
Chapter 4: Long and short term effect of ammonium (NH ₄ ⁺) on nitrat	e
(NO ₃ ⁻) uptake capacity	104
Chapter 5: Amino acid distribution in different plant tissues of maize	(Zea
mays L.)	140
Chapter 6: General discussion & Future directions	170
6.1 ADVANCES IN KNOWLEDGE FROM THIS STUDY	170
6.2 FUTURE DIRECTIONS.	174
Literature cited (Literature review &General Discussions)	176

Abstract

Nitrogen (N) is one of the major mineral nutrients required by a plant for its growth and development. Nitrate (NO₃⁻) and ammonium (NH₄⁺) are the predominant forms of N available to plants in agricultural soils. Plants have the ability to absorb both these forms efficiently from the soil solutions. With soil solution concentrations of NH₄⁺ being much lower (on average 10%) than NO₃⁻, contribution of these small amounts of NH₄⁺ to the overall N budget of crop plants is often overlooked. This research focussed on the contribution of this NH₄⁺ in the nitrogen economy of maize plants. The study also investigated whether NH₄⁺ has any effect on uptake and utilization of other nutrients, and most importantly, NO₃⁻.

Growth of maize inbred line B73 was increased when one-third of total nitrogen was supplied as NH_4^+ with low NO_3^- , but not for another inbred line Gaspe Flint. Further investigations on B73 found a 20% increase in plant growth when supplied with 10% NH_4^+ along with sufficient NO_3^- . Ammonium being a cheaper N source and the low energy and carbon skeleton requirement for its assimilation has contributed in increased shoot dry matter accumulation in these plants. A corresponding increase in total N, total free amino acids and sugars in the leaves of these plants were observed. A positive correlation was seen between transcript levels of putative high affinity NO_3^- and NH_4^+ transporters. This together with an increased activity of N assimilatory enzymes suggested that small amounts of NH_4^+ can increase the uptake and assimilation of N in these plants. 10% NH_4^+ in the nutrient solution does not inhibit the NO_3^- uptake capacity in plants but when the concentration was increased to 50% there is a reduction in NO_3^- uptake capacity for plants growing in low N. This indicates that high concentration of NH_4^+ limit the absorption of NO_3^- which is an important signalling molecule for various metabolic activities in plants. Reduction in NO_3^-

uptake capacity of plants grown in 10% NH_4^+ at sufficient N was correlated with higher total free amino acids in the roots, particularly glutamine and asparagine. This reduction in $NO_3^$ uptake capacity when grown in small amounts of NH_4^+ is a long term effect caused by the products of N assimilation and could be reversed by moving plants to solely $NO_3^$ treatments. Higher concentrations of amino acids in the roots of these plants suggests that NH_4^+ that enters the root gets first into the assimilatory pathway in the cytosol prior to the assimilation of NH_4^+ formed by the reduction of NO_3^- in the plastids.

This study showed that small amounts of NH_4^+ improve plant growth and lead to major changes in N uptake and assimilation processes. Based on these effects and the fact that plants in the field always have a small amount of N available as NH_4^+ , it is recommended that NH_4^+ be added to the experimental nutrient solutions with maize and the effect be explored in other major plant species.

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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List of Abbreviations

%, percent AMT, ammonium transporters ANOVA, analysis of variance B, boron C, carbon Ca, calcium. CHL, chloride transporter Cu, copper DAE, days after emergence Fe, Iron GHA, γ-glutamyl hydroxamate GOGAT, glutamate synthase GS, glutamine synthetase HATS, high affinity transport system K, potassium. LATS, low affinity transport system Mg, Magnesium Mn, manganese Mo molybdenum N, nitrogen NH₄⁺, ammonium NiR, nitrite reductase NO_2^- , Nitrite NO₃, nitrate NR, nitrate reductase NRT, nitrate transporters

NUE, nitrogen use efficiency

- NUpE, nitrogen uptake efficiency
- NUtE, nitrogen utilization efficiency

P, Phosphorus

- PCR, polymerase chain reaction
- Q-PCR, quantitative real time polymerase chain reaction

S, sulphur

Zn, zinc