Molybdenum transport in plants

A thesis submitted for the Degree of the Doctor of Philosophy

at

The University of Adelaide

Discipline of Wine and Horticulture,

School of Agriculture, Food and Wine.

Submitted by

Kate Louise Fitzpatrick

April, 2008

Table of Contents

List of Figures

List of Tables

Abstract

Molybdenum (Mo) is an essential micronutrient required by plants. It is biologically inactive until bound in a pterin compound named the molybdenum cofactor (Moco) that binds to apoproteins used in both reductive and oxidative reactions such as nitrate reductase (NR), xanthine dehydrogenase (XDH), aldehyde oxidase (AO) and sulfite oxidase (SOX). In *Vitis vinifera* cv. Merlot, molybdenum deficiency is common amongst vines grown on own roots in acidic soils often resulting in yield reductions. Foliar application of molybdenum sprays increases yield and remedies deficiency indicating that Merlot grown on own roots has a reduced capacity for molybdenum uptake from the soil.

Molybdenum generally occurs as molybdate (MoO_4^{2-}) within the soil solution. The mechanism(s) involved in molybdenum transport have recently been discovered in plants, although are well characterised within prokaryotic systems. Unfortunately, no homologues of prokaryotic genes involved in molybdate transport exist within eukaryotes. It has been suggested that molybdenum transport in plants may occur through other systems including sulfate transporters due to chemical similarities between sulfate and molybdate.

A yeast functional complementation approach using a sulfate transport mutant was initially used to identify novel putative plant molybdenum transport proteins. A cDNA library derived from Pinot noir roots starved of molybdenum was screened for transporters. Unfortunately, no cDNAs were identified that met the requirements of a molybdenum transporter when screened on media containing low amounts of molybdenum. However, a number of putative cDNA's partially complemented the yeast mutant YSD1, however none of these could be validated in second round screens.

A candidate gene approach was then initiated to identify pre-characterised genes that may also have capacity to transport molybdenum. The plant sulfate transporter, SHST1, restored growth of YSD1 on media containing low amounts of molybdenum. Kinetic analysis using $^{99}MoO_4^{2-}$ to trace molybdenum transport in yeast cells demonstrated that SHST1 enhanced the uptake of molybdenum at nM concentrations. The uptake was not inhibited by sulfate, but the transport of sulfate was reduced with molybdenum. Further analysis demonstrated that SHST1 did prefer sulfate as the substrate but molybdenum could compete at higher concentrations. This result is the first measurement of molybdenum being transported through a pre characterised sulfate transport protein.

Whole plant experiments using rooted grapevine cuttings and $^{99}MoO_4^{2-}$ to trace molybdenum movement into plants indicated that Merlot did not have reduced capacity to uptake molybdenum compared to other varieties that do not suffer from molybdenum deficiencies such as Chardonnay. When plants were grown with molybdenum, Merlot accumulated more molybdenum than Chardonnay, with the reverse being true when plants were grown without molybdenum. Similar experiments were performed on symbiosomes isolated from *Glycine max* grown with and without molybdenum. Symbiosomes absorbed more molybdenum when plants were grown without molybdenum.

A field site was established to look at the molybdenum profiles within petioles against yield responses over a 3-year period. Molybdenum application did not increase the yield amongst vines despite all vines initially being deficient in molybdenum. There were no cumulative effects of molybdenum application over the trial, however, molybdenum did have limited translocation ability within the vine system.

Declaration

The research presented in this thesis is my own work unless otherwise stated. The project was completed at The University of Adelaide, within the School of Agriculture, Food and Wine. This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution except where it is related to the scope of this project and, to the best of my knowledge and belief, contains no material previously published or written by another person, except were due reference has been made in the text.

Work on various aspects of the project were analysed by individuals including Mrs. Teresa Fowles and Mr Lyndon Palmer of Waite Analytical Services who were both involved in the ICP-MS analysis. The Bureau of Meteorology also kindly provided the climatic data.

I give consent to this copy of my thesis being made available in the University Library.

I acknowledge that copyright of published works contained within this thesis (as listed within the bibliography) resides with the copyright holder(s) of those works.

Kate L. Fitzpatrick
April, 2008

Acknowledgments

Firstly, I would like to thank my supervisors, Dr Brent Kaiser and Prof. Steve Tyerman. Thankyou to Brent who has continued to support me in my career and provide endless encouragement in an often difficult and challenging project. I appreciate all the time that he has helped me with experiments, advice, feedback and showing me the ropes in the world of molecular biology. I have been very lucky to have such a supportive a supervisor who was always willing to fit in an uptake experiment!

Also thankyou to Steve, who provided me with my initial stepping stone into plant science. Steve and has supported me throughout my Honours and now my PhD. I appreciate all of his feedback, ideas and encouragement through out my career. I look forward to collaborating with both Brent and Steve in the future.

The CRC for Viticulture and the McLaren Vale Vine Improvement Society have funded my PhD research and I am grateful for the support and continual interest in my work from both organizations and the opportunities that have arisen.

Thankyou to all the people that have helped me in this journey…there are so many! I would like to thank the fabulous lab in which I have spent the last 4 years working in, those include Megan Shelden, Patrick Louglin, Scott Cater, Dr. Sunita Ramesh and Rebecca Vandeleur. Thanks to Meg who taught me so much about molecular biology and always was willing to answer my many, many questions (especially when things didn't work!). Special thanks should be given to Pat and Scotty for helping me with what seemed like endless uptakes, lifting cumbersome hydroponic equipment and lead. Both Pat and Scott have been great to work with in the lab. I also appreciate all the times that Sunita helped me, she was never too busy to offer advice and assistance. You are all not only colleagues, but also life long friends.

Thankyou to all the techies that have helped me along the way, especially Wendy Sullivan who I first met while on work experience in Year 10 at Flinders University. I must have liked it to stick around for so long! Thankyou to all the lab techs that have been with Team Kaiser over the years including Steve Choimes, Jess Parker, Jana Koleski and Nenah MacKenzie. Your help was always appreciated.

Thankyou to my parents and grandparents who have always supported me. They were always interested in my work and listed to me ramble on, often I am sure, not knowing what I was talking about! I appreciate all the times that I practiced my seminars in front of Mum who always nodded approvingly and though I always asked her to stop me if she didn't understand anything, she never did!

My friends, Felicity and Anthony Cox, Kimberley and Matthew Whittle and Pete and Claire Wirth have also been a great support and a stress relief during this time. Thanks for putting up with me during this time and I am sorry for my standard response of my thesis being finished in a month!

Lastly, thankyou to my fiancé, Matthew Fitzpatrick. Thankyou so much for supporting me, loving me, helping me, and giving me the occasional, but not un-due, reality check! I don't think I would have made it through without your encouragement.

Publications

Kaiser, B. N., **Gridley. K.L**., Ngaire Brady, J., Phillips. T, Tyerman, S. D. (2006) The role of molybdenum in agricultural plant production. *Annals of Botany (London)*. 96(5) 745 – 754.

Fitzpatrick, Kate L., Tyerman, Stephen D., Kaiser, Brent N. (2008) Molybdate transport through the plant sulfate transporter SHST1. *FEBS Letters*, in press.

Abbreviations

Å	Angstrom
AMP	Ampicillin
AO	Aldehyde oxidase
BLAST	Basic local alignment search tool
BNF	Biological nitrogen fixation
Bp	Base pairs
BSA	Bovine serum albumin
cDNA	Complementary deoxyribonucleic acid
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol, threo-2-,3-dihydroxy-1,4-dithiolbutane
dH_2O	Distilled water
EDTA	Ethylene diamine tetracetic acid
EGTA	Ethylene glycerol tetraacetic acid
EMS	Ethyl methane sulfonate
Gal	Galactose
Glu	Glucose
Kb	Kilobase
kD	Kilo Daltons
K_m	Michaelis constant
LB	Luria-Bertani media
Low Mo	Low molybdenum media
Low S	Low sulfate media
MSD	Membrane spanning domain
MES	2-(N-Morpholino)ethanesulfonic acid
Moco	Molybdenum cofactor
MPT	Molybdopterin
MSD	Membrane spanning domain
mRNA	Messenger ribonucleic acid
NCBI	National Centre for Biotechnology Information
NRA	Nitrate reductase activity
NR	Nitrate reductase
OD	Optical density
PBM	Peribacteroid membrane

PBS	Peribacteroid space
PCR	Polymerase chain reaction
PVP	Polyvinypolyprrolidone
RNA	Ribonucleic acid
Rnase	Ribonuclease
SBP	Sulfur binding protein
SC	Synthetic complete media
SDS	sodium dodecyl sulfate
SHST1	*Stylosanthes hamata* sulfate transporter 1
SOX	Sulfite oxidase
STAS	Sulfate transporters and antisigma-factor antagonists
TCA	Trichloroacetic acid
TMD	Transmembrane domain
v/v	volume/volume
V_{max}	maximum velocity of reaction
w/v	weight/volume
XDH	Xanthine dehydrogenase
XO	Xanthine oxidase
YEM	Yeast extract mannitol media
YPAD	Yeast extract peptone dextrose medium with adenine
YSD1	Yeast sulfate deletion mutant 1
2xTL	2 x Homocysteine thiolactone media