
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

Table of Contents	1
List of Figures	7
List of Tables	13
Abstract	15
Declaration	17
Acknowledgments	18
Publications	20
Abbreviations	21
1. Chapter 1 – Literature review	
1.1 Introduction.....	23
1.2 Molybdenum nutrition in plants.....	24
1.2.1 Soil factors affecting molybdenum uptake in plants.....	24
1.2.2 Molybdenum deficiency symptoms in plants.....	25
1.2.3 Effect of molybdenum deficiency amongst plant species.....	26
1.2.4 Molybdenum deficiency in <i>Vitis vinifera</i> cv. Merlot.....	27
1.3 Biochemical and physiological roles of molybdenum on plants.....	28
1.3.1 The molybdenum cofactor (Moco).....	28
1.4 Molybdenum containing enzymes.....	29
1.4.1 Nitrate reductase (NR)	29
1.4.2 Nitrogenase.....	30
1.4.3 Other molybdenum containing enzymes.....	31
1.5 Molybdenum transport in the model organism, <i>Escherichia coli</i>	32
1.5.1 The <i>Escherichia coli</i> ABC-MoO ₄ ²⁻ transport system (<i>modABCD</i> operon)	33
1.6 Mechanism for molybdenum uptake in plants.....	34
1.6.1 Plant phosphate transporters.....	34
1.6.2 Plant P-type ATPases.....	35
1.7 Plant sulfate transporters.....	36
1.7.1 Sulfur assimilation, reduction and metabolism.....	36
1.7.2 Phylogenetic analysis of plant sulfate transporters.....	36
1.7.3 Topology of plant sulfate transporters.....	37
1.7.4 Regulation of sulfate transporters.....	37

1.8 Sulfate transporters – the new molybdenum transport system?.....	38
1.8.1 Identification of sulfate transporters in <i>Saccharomyces cerevisiae</i> ...	40
1.8.2 Identification of <i>Stylosanthes hamata</i> sulfate transporters.....	41
1.8.3 Identification of putative sulfate transporters on the symbiotic membrane of nodules.....	42
1.9 General aims of thesis.....	42

Chapter 2 – Identification MoO₄²⁻ transport proteins by functional yeast complementation

2.1 Introduction.....	53
2.2 Methods.....	56
2.2.1 Mutant generation.....	56
2.2.1.1 Toxicity determination.....	56
2.2.1.2 EMS mutagenesis of wild type yeast Invsc1.....	56
2.2.2 Targeted gene approach to identify molybdenum transport proteins using the Low Mo assay.....	57
2.2.2.1 Molybdenum removal from media and glassware.....	57
2.2.3 Identification of putative MoO ₄ ²⁻ and SO ₄ ²⁻ transport proteins using functional yeast complementation.....	57
2.2.3.1 <i>Vitis vinifera</i> culture.....	57
2.2.3.2 Total and Poly (A) ⁺ RNA isolation.....	58
2.2.3.3 cDNA library construction.....	59
2.2.3.4 Ligation into pYES3 via Gateway® Technology.....	59
2.2.3.5 cDNA library amplification.....	59
2.2.3.6 Functional complementation of YSD1 with <i>Vitis vinifera</i> cDNA.....	60
2.2.3.7 Yeast plasmid isolation.....	61
2.2.3.8 DNA sequencing.....	61
2.3 Results.....	63
2.3.1 Determination of molybdenum toxicity to yeast.....	63
2.3.2 Identification of putative MoO ₄ ²⁻ and SO ₄ ²⁻ transport proteins using functional yeast complementation.....	63
2.3.2.1 cDNA library construction and ligation into pYES3 via Gateway® Technology.....	63

2.3.2.2 Functional complementation of YSD1 with <i>Vitis vinifera</i> cv. Pinot noir root cDNA's.....	63
2.4 Discussion.....	66

Chapter 3 – Functional yeast complementation and characterisation of *Stylosanthes hamata* root cDNA SHST1 and *Glycine max* nodule cDNA GmNod70

3.1 Introduction.....	76
3.2 Methods.....	76
3.2.1 Gene constructs of SHST1 and GmNod70.....	89
3.2.2 Transformation of YSD1 with SHST1 and GmNod70.....	79
3.2.3 Functional complementation of YSD1 with GmNod70.....	79
3.2.4 Functional complementation of YSD1 with SHST1 on the Low Mo Gal media assay.....	80
3.2.5 Functional expression of SHST1 and GmNod70 in YSD.....	80
3.2.5.1 Yeast cell growth and harvest.....	80
3.2.5.2 ³⁵ SO ₄ ²⁻ and ⁹⁹ MoO ₄ ²⁻ uptake in yeast.....	80
3.3 Results.....	82
3.3.1 GmNod70 analysis.....	82
3.3.2 Functional complementation of YSD1/GmNod70.....	82
3.3.3 ³⁵ SO ₄ ²⁻ accumulation by YSD1 cells containing GmNod70 or pYES3.....	82
3.3.4 Identification of pre-characterised sulfate transporter SHST1 through a targeted gene approach and functional yeast complementation using the Low Mo Gal media assay.....	83
3.3.5 ³⁵ SO ₄ ²⁻ accumulation by YSD1 cells containing SHST1 or pYES3...	83
3.3.6 ⁹⁹ MoO ₄ ²⁻ accumulation by YSD1 cells containing SHST1 or pYES3.	84
3.4 Discussion.....	86
3.4.1 Molybdenum transport through SHST1.....	86
3.4.2 GmNod70 properties.....	90

Chapter 4 – ⁹⁹MoO₄²⁻ uptake in *Vitis vinifera* L. rootlings and *Glycine max* nodule symbiosomes

4.1 Introduction.....	116
4.1.1 Molybdenum uptake in <i>Vitis vinifera</i>	116
4.1.2 Molybdenum uptake in <i>Glycine max</i> symbiosomes.....	117

4.2 Methods.....	119
4.2.1 <i>Vitis vinifera</i> uptake assay.....	119
4.2.1.1 <i>Vitis vinifera</i> cv. Merlot and Chardonnay culture.....	119
4.2.1.2 $^{99}\text{MoO}_4^{2-}$ uptake assay and protocol for <i>Vitis vinifera</i> cv. Merlot and Chardonnay.....	119
4.2.2 <i>Glycine max</i> symbiosome assay.....	119
4.2.2.1 <i>Glycine max</i> culture.....	119
4.2.2.2 Aerobic <i>Glycine max</i> symbiosome isolation.....	120
4.2.2.3 $^{99}\text{MoO}_4^{2-}$ uptake assay and protocol for <i>Glycine max</i> symbiosomes.....	120
4.3 Results.....	122
4.3.1 $^{99}\text{MoO}_4^{2-}$ accumulation of <i>Vitis vinifera</i> cv. Merlot and Chardonnay plants.....	122
4.3.2 Uptake of $^{99}\text{MoO}_4^{2-}$ by <i>Glycine max</i> symbiosomes.....	122
4.4 Discussion.....	124
4.4.1 $^{99}\text{MoO}_4^{2-}$ accumulation of <i>Vitis vinifera</i> cv. Merlot and Chardonnay plants.....	124
4.4.2 Uptake of $^{99}\text{MoO}_4^{2-}$ by <i>Glycine max</i> symbiosomes.....	125

Chapter 5 – Effects of foliar applied molybdenum on yield, yield components and quality parameters in *Vitis vinifera* cv. Merlot

5.1 Introduction.....	135
5.2 Methods.....	138
5.2.1 McLaren Vale Visitor Centre Merlot trial.....	138
5.2.2 Molybdenum application.....	138
5.2.3 Petiole collection and analysis.....	139
5.2.4 Fruit set analysis.....	140
5.2.5 Bunch and berry sampling and analysis.....	140
5.2.6 Grape berry juice extraction.....	141
5.2.7 Total soluble solids (°Brix) determination.....	141
5.2.8 pH determination.....	141
5.2.9 Anthocyanin and total phenolics determination of grape berries.....	141
5.2.10 Climatic data.....	142
5.2.11 Statistical analysis.....	143
5.3 Results.....	144

5.3.1 Fruit set.....	144
5.3.1.1 Regression analysis of flower number and inflorescence length.....	144
5.3.1.2 Fruit set over 3 years.....	144
5.3.2 Yield components.....	144
5.3.2.1 Mean yield per vine.....	144
5.3.2.2 Mean bunches per vine.....	145
5.3.2.3 Mean bunch weight.....	145
5.3.2.4 Mean berries per bunch.....	145
5.3.2.5 Mean berry weight.....	146
5.3.2.6 Mean rachis weight.....	146
5.3.3 Climatic data.....	146
5.3.4 Berry quality.....	146
5.3.4.1 Total soluble solids and pH measurements of grape berry juice from the 2005/2006 growing season (Year 3)	146
5.3.4.2 Anthocyanin and total phenolics determination of grape berry juice from the 2005/2006 growing season (Year 3)	147
5.3.5 Petiole nutrients.....	147
5.3.5.1 Molybdenum petiole concentration.....	147
5.3.5.2 Sulfur petiole concentration.....	147
5.3.5.3 Total nitrogen petiole concentration.....	148
5.3.5.4 Copper petiole concentration.....	148
5.3.5.5 Potassium petiole concentration.....	148
5.3.5.6 Boron petiole concentration.....	149
5.3.5.7 Zinc petiole concentration.....	149
5.4 Discussion.....	150
5.4.1 Molybdenum transport and translocation within <i>Vitis</i> sp.....	150
5.4.2 Petiole nutrient profiles.....	152
5.4.3 Yield and yield component responses in relation to foliar application of molybdenum.....	153
5.4.4 Berry quality parameters.....	154

Chapter 6 – General discussion

6.1 Identification of molybdenum transport proteins.....	194
--	-----

6.2 Functional complementation in yeast to identify molybdenum transport proteins in <i>Vitis vinifera</i>	195
6.3 SHST1 – the new molybdenum transport system.....	196
6.4 Molybdenum transport and translocation in plants.....	198
6.4.1 Molybdenum uptake in <i>Vitis vinifera</i>	198
6.4.2 Molybdenum uptake in <i>Glycine max</i> symbiosomes.....	200
6.5 The effects of foliar applied molybdenum on yield, yield components, quality parameters and petiole nutrient content in <i>Vitis vinifera</i> cv. Merlot.....	201
6.5.1 Molybdenum fertilisation and its effects on productivity in <i>Vitis vinifera</i> cv. Merlot.....	201
6.5.2 Petiole nutrient content.....	202
6.5.3 Yield and yield components responses to molybdenum sprays.....	202
6.5.4 Quality parameters.....	203
6.6 Future research directions.....	203
6.7 Conclusion.....	204
Bibliography	205
Appendix 1	219
Appendix 2	222
Appendix 3	223
Appendix 4	238

List of Figures

Figure 1. Molybdenum deficiency responses in grapevines.....	44
Figure 1.2 Molybdenum deficiency phenotypes in <i>V.vinifera</i> cv. Merlot.....	45
Figure 1.3 The chemical structure of the molybdenum cofactor (Moco).....	46
Figure 1.4 The position in which Moco is found within the 4 plant enzymes (modified from Mendel and Hansch, 2002; Mendel and Bittner, 2006).....	47
Figure 1.5 Transmission electron micrograph showing a symbiosome isolated from a soybean root nodule infected cell. The bacterioids are contained within the peribacteriod space (PBS), which is enclosed by the peribacteriod membrane (PBM).	48
Figure 1.6 Molybdate transport in <i>E.coli</i>	49
Figure 1.7 Phylogenetic analysis of plant sulfate transporters from <i>Arabidopsis thaliana</i> (At), <i>Vitis vinifera</i> EST's (Vv), <i>Oryza sativa</i> (Os) and <i>Stylosanthes hamata</i> (Sh), <i>Glycine max</i> (Gm) <i>Lotus japonicus</i> (Lj) and <i>Zea mays</i> (Zm).	50
Figure 1.8 Alignment of amino acid sequences of SHST1, SHST2 and SHST3.....	52
Figure 2.1 Yeast strain Σ 1278b plated on varying concentrations of molybdenum to determine toxicity levels.....	67
Figure 2.2 Yeast strain S288c plated on varying concentrations of molybdate to determine toxicity levels.....	68
Figure 2.3 Yeast strain Invsc1 plated on varying concentrations of molybdate to determine toxicity levels.....	69
Figure 2.4 A schematic diagram of Gateway® cDNA library construction and Gateway® recombination reactions used in the Gateway cDNA library construction manual.....	70

Figure 2.5 Vector map of pDONR™222 (Invitrogen, 2006).....	72
Figure 2.6 Vector map of pYES3-DEST.....	73
Figure 2.7 Digest of inserts contained within <i>V.vinifera</i> cv. Pinot noir cDNA library.....	74
Figure 2.8 Low S (100 μM) Glu and Low S (100 μM) Gal plates containing putative sulfate transport proteins in YSD1.....	75
Figure 3.1 Kyte/Doolittle hydrophilicity analysis of GmN#70 and GmNod70.....	95
Figure 3.2 Multiple sequence alignment of amino acid sequences of LjSST1, AtSultr1;2, AtSultr1;2, SHST1, GmN#70 and GmNod70.....	96
Figure 3.3 Phylogenetic tree of sulfate transporters from <i>Arabidopsis thaliana</i> , <i>S. cerevisiae</i> , <i>Lotus japonicus</i> , <i>Stylosanthes hamata</i> , <i>Glycine max</i> and <i>Brassica</i> spp. with homology to GmNod70 cloned from this study in addition to GmN#70 identified by Kouchi and Shingo (1993).....	98
Figure 3.4 Growth of YSD1 and Invsc1 cells transformed with either the empty pYES3 vector, or pYES3 vectors containing either SHST1 or GmNOD70. 1=SHST1/pYES3 expressed in YSD1, 2=GmNod70/pYES3 expressed in YSD1, 3=pYES3 expressed in YSD1 4=pYES3 expressed in Invsc1.	99
Figure 3.5. Accumulation of ³⁵ SO ₄ ²⁻ by YSD1 cells containing GmNod70 or pYES3.....	100
Figure 3.6 Growth of YSD1 and Invsc1 cells transformed with either the empty pYES3 vector or the pYES3 vector containing SHST1 on various concentrations of molybdenum.....	101
Figure 3.7. Accumulation of ³⁵ SO ₄ ²⁻ YSD1 cells containing SHST1 or pYES3 grown in 2xTL Gal media.....	102

Figure 3.8 Accumulation of $^{35}\text{SO}_4^{2-}$ by YSD1 cells containing SHST1 or pYES3 grown in Low Mo Gal media.....	103
Figure 3.9 Competition of $^{35}\text{SO}_4^{2-}$ uptake in YSD1 cells containing SHST1 or pYES3 grown in 2xTL Gal.....	104
Figure 3.10 Competition of $^{35}\text{SO}_4^{2-}$ uptake in YSD1 cells containing SHST1 or pYES3 grown in Low Mo Gal.....	105
Figure 3.11 Competitive inhibition of $^{35}\text{SO}_4^{2-}$ uptake by YSD1 cells containing SHST1 grown in 2xTL Gal.....	107
Figure 3.12 Accumulation of $^{99}\text{MoO}_4^{2-}$ in YSD1 cells containing SHST1 or pYES3 grown in Low Mo Gal (10 nM MoO_4^{2-}).....	108
Figure 3.13 Accumulation of $^{99}\text{MoO}_4^{2-}$ in YSD1 cells containing SHST1 or pYES3 grown in Low Mo Gal (80 nM MoO_4^{2-}).....	109
Figure 3.14 Concentration dependent accumulation of $^{99}\text{MoO}_4^{2-}$ in YSD1 cells containing SHST1 or pYES3 grown in Low Mo Gal.....	110
Figure 3.15 Concentration dependent accumulation of $^{35}\text{SO}_4^{2-}$ in YSD1 cells containing SHST1 or pYES3 grown in 2xTL.....	113
Figure 3.16 Substrate competition of $^{99}\text{MoO}_4^{2-}$ uptake in YSD1 cells containing SHST1 or pYES3 grown in Low Mo Gal.....	114
Figure 3.17 Influence of external pH on the uptake of $^{99}\text{MoO}_4^{2-}$ YSD1 cells containing SHST1 or pYES3.	115
Figure 4.1 Accumulation of $^{99}\text{MoO}_4^{2-}$ over time in root tissue of Merlot and Chardonnay rootlings grown without molybdenum.....	128
Figure 4.2 Accumulation of $^{99}\text{MoO}_4^{2-}$ over time in root tissue of Merlot and Chardonnay rootlings grown with molybdenum.....	129

Figure 4.3 Concentration dependent accumulation of $^{99}\text{MoO}_4^{2-}$ in soybean symbiosomes from plants grown with and without molybdenum.....	130
Figure 4.4 A. Concentration dependent accumulation of $^{99}\text{MoO}_4^{2-}$ in soybean symbiosomes from plants grown without molybdenum and D. Concentration dependent accumulation of $^{99}\text{MoO}_4^{2-}$ in soybean symbiosomes from plants grown with molybdenum.....	133
Figure 5.1 Diagram showing grapevine shoot structure.	140
Figure 5.2. Regression analysis to determine number of flowers per inflorescence.	156
Figure 5.3 Mean % fruit set over the 3 year trial period for the 4 clones.....	157
Figure 5.4 Mean yield per vine (kg/vine) over the 3 year trial period for the 4 clones....	158
Figure 5.5 Mean bunch number per vine over the 3 year trial period for the 4 clones.....	159
Figure 5.6 Mean bunch weight (g) over the 3 year trial period for the 4 clones.....	160
Figure 5.7 Mean berry number per bunch over the 3 year trial period for the 4 clones...	161
Figure 5.8 Mean berry weight (g) over the 3 year trial period for the 4 clones.....	162
Figure 5.9 Mean rachis weight (g) over the 3 year trial period for the 4 clones.....	163
Figure 5.10 Mean monthly minimum, maximum and rainfall for years 2003 to 2006.....	164
Figure 5.11 Mean harvest total soluble solids ($^{\circ}\text{Brix}$) from the 2005/2006 growing season.....	165
Figure 5.12 Mean harvest pH from the 2005/2006 growing season.....	166
Figure 5.13 Colour or anthocyanin (malvidin-3-glucose) of berries at harvest in 2005/2006 after 3 years of different molybdenum treatments.	167

Figure 5.14 Total phenolics of berries at harvest in 2005/2006 after 3 years of different molybdenum treatments.	168
Figure 5.15 Mean Mo (mg/kg) concentration in petioles at 50 – 80% flowering for clones D3V14 and Q45-14 over the 3 years of the trial. Suggested deficiency concentrations for molybdenum may occur between 0.05 – 0.09 mg/kg DW (Williams et. al., 2004).	169
Figure 5.16 Mean Mo (mg/kg) concentration in petioles at 50 – 80% flowering for clones 8R and 6R over the 3 years of the trial. Suggested deficiency concentrations for molybdenum may occur between 0.05 – 0.09 mg/kg DW (Williams et. al., 2004).	170
Figure 5.17 Mean S (g/kg DW) concentration in petioles at 50 – 80% flowering for clones D3V14 and Q45-14 over the 3 years of the trial.	175
Figure 5.18 Mean S (g/kg DW) concentration in petioles at 50 – 80% flowering for clones 8R and 6R over the 3 years of the trial.	175
Figure 5.19 Mean total N (% DW) concentration in petioles at 50 – 80% flowering for clones D3V14 and Q45-14 over the 3 years of the trial. Adequate levels of total N occur between 1.8 – 3% DW (Reuter and Robinson, 1997).	177
Figure 5.20 Mean total N (% DW) concentration in petioles at 50 – 80% flowering for clones 8R and 6R over the 3 years of the trial. Adequate levels of total N occur between 1.8 – 3% DW (Reuter and Robinson, 1997).	178
Figure 5.21 Mean Cu (mg/kg DW) concentration in petioles at 50 – 80% flowering for clones 8R and 6R over the 3 years of the trial. Adequate levels of Cu occur between 6 – 11 mg/kg DW (Reuter and Robinson, 1997).	181
Figure 5.22 Mean Cu (mg/kg DW) concentration in petioles at 50 – 80% flowering for clones 8R and 6R over the 3 years of the trial. Adequate levels of Cu occur between 6 – 11 mg/kg DW (Reuter and Robinson, 1997).	182

Figure 5.23 Mean K (% DW) concentration in petioles at 50 – 80% flowering for clones D3V14 and Q45-14 over the 3 years of the trial. Adequate levels of K occur between 1.8 – 3% DW (Reuter and Robinson, 1997).184

Figure 5.24 Mean K (% DW) concentration in petioles at 50 – 80% flowering for clones 8R and 6R over the 3 years of the trial. Adequate levels of K occur between 1.8 – 3% DW (Reuter and Robinson, 1997).185

Figure 5.25 Mean B (mg/kg DW) concentration in petioles at 50 – 80% flowering for clones D3V14 and Q45-14 over the 3 years of the trial. Adequate levels of B occur between 35 – 70 mg/kg DW (Reuter and Robinson, 1997).188

Figure 5.26 Mean B (mg/kg DW) concentration in petioles at 50 – 80% flowering for clones 8R and 6R over the 3 years of the trial. Adequate levels of B occur between 35 – 70 mg/kg DW (Reuter and Robinson, 1997).189

Figure 5.27 Mean Zn (mg/kg DW) concentration in petioles at 50 – 80% flowering for clones D3V14 and Q45-14 over the 3 years of the trial. Adequate levels of Zn occur at >26 mg/kg DW (Reuter and Robinson, 1997).192

Figure 5.28 Mean Zn (mg/kg DW) concentration in petioles at 50 – 80% flowering for clones 8R and 6R over the 3 years of the trial. Adequate levels of Zn occur at >26 mg/kg DW (Reuter and Robinson, 1997).193

List of Tables

Table 3.1 Initial identification of GmN#70 (D13505) homologues through BLAST searches of the public protein databases.	92
Table 4.1 Statistical analyses of the concentrations in concentration dependent accumulation between 0 and 4000 nM of $^{99}\text{MoO}_4^{2-}$ in soybean symbiosomes isolated from plants grown from plants with and without molybdenum.....	131
Table 5.1 Molybdenum foliar spray regime for growing seasons 2003/2004 to 2005/2006.....	138
Table 5.2 Table of vineyard trial site and spray regime imposed.	155
Table 5.3 Treatment differences between the treatments for molybdenum petiole concentrations in years 1, 2 and 3 of the trial.....	171
Table 5.4 Clonal differences between the treatments for molybdenum petiole concentrations in years 1 and 3 of the trial.	173
Table 5.5 Clonal differences between the treatments for sulfur petiole concentrations in year 1, 2 and 3 of the trial.	176
Table 5.6 Treatment differences between the treatments for nitrogen petiole concentrations in years 2 and 3 of the trial.	179
Table 5.7 Clonal differences between the treatments for nitrogen petiole concentrations in year 1, 2 and 3 of the trial.	180
Table 5.8 Treatment differences between the year and clones for copper petiole concentrations in years 1 and 2 of the trial.	183
Table 5.9 Treatment differences between the years and clones for potassium petiole concentrations in years 2 and 3 of the trial.	186

Table 5.10 Treatment differences between the years and clones for potassium petiole concentrations in years 2 and 3 of the trial.	187
Table 5. 11 Treatment differences between the years and clones for boron petiole concentrations in years 2 and 3 of the trial.....	190
Table 5. 12 Clonal differences between the years and treatment for boron petiole concentrations in years 1, 2 and 3 of the trial.....	191

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 $^{99}\text{MoO}_4^{2-}$ 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 $^{99}\text{MoO}_4^{2-}$ 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 where 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 ₂ O	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	<i>Stylosanthes hamata</i> 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