

**A comparison of water stress-induced xylem  
embolism in two grapevine cultivars,  
Chardonnay and Grenache, and the role of  
aquaporins**

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# TABLE OF CONTENTS

<b>1</b>	<b>INTRODUCTION .....</b>	<b>1-1</b>
1.1	INTRODUCTION .....	1-2
1.1.1	<i>Water Transport in Plants and the Cohesion-Tension (CT) Theory.....</i>	<i>1-3</i>
1.2	XYLEM EMBOLISM .....	1-5
1.2.1	<i>Vulnerability of Xylem Vessels to Cavitation .....</i>	<i>1-5</i>
1.2.2	<i>Water Stress-Induced Embolism.....</i>	<i>1-6</i>
1.2.3	<i>Detection of Cavitation .....</i>	<i>1-8</i>
1.3	MECHANISM OF EMBOLISM REPAIR .....	1-8
1.4	AQUAPORINS .....	1-9
1.4.1	<i>Structural Characteristics.....</i>	<i>1-11</i>
1.4.2	<i>Plant Aquaporins.....</i>	<i>1-14</i>
1.5	THE ROLE OF AQUAPORINS IN RESPONSE TO WATER STRESS .....	1-17
1.5.1	<i>Regulation of Aquaporins in Response to Water Stress.....</i>	<i>1-18</i>
1.5.1.1	<i>Transcriptional and Post-Transcriptional Regulation .....</i>	<i>1-18</i>
1.5.1.2	<i>Post-Translational Modification .....</i>	<i>1-20</i>
1.5.1.3	<i>The Role of Aquaporins in Planta .....</i>	<i>1-21</i>
1.5.2	<i>Involvement of Aquaporins in Embolism Recovery.....</i>	<i>1-22</i>
1.6	CONCLUSION .....	1-22
1.7	SIGNIFICANCE OF THE PROJECT .....	1-23
1.8	PROJECT AIMS .....	1-24
<b>2</b>	<b>WATER-STRESS INDUCED XYLEM EMBOLISM IN TWO GRAPEVINE CULTIVARS, CHARDONNAY AND GRENACHE.....</b>	<b>2-26</b>
2.1	INTRODUCTION .....	2-27
2.2	METHODS.....	2-30
2.2.1	<i>Plant Material and Growth Conditions .....</i>	<i>2-30</i>
2.2.2	<i>Detection of Cavitation by Acoustic Emissions .....</i>	<i>2-31</i>
2.2.3	<i>Data Analysis.....</i>	<i>2-32</i>
2.2.4	<i>Measurement of Leaf Water Potential .....</i>	<i>2-33</i>
2.2.5	<i>Measurement of Petiole Hydraulic Conductance.....</i>	<i>2-35</i>

2.2.6	<i>Xylem Anatomy</i> .....	2-40
2.2.7	<i>Data Analysis</i> .....	2-40
2.3	RESULTS .....	2-41
2.3.1	<i>Correlation of Pressure Chamber with Leaf Psychrometers</i> .....	2-41
2.3.2	<i>Leaf Water Potential</i> .....	2-43
2.3.3	<i>Cavitation</i> .....	2-46
2.3.4	<i>Petiole Hydraulic Conductance</i> .....	2-48
2.3.5	<i>Xylem Anatomy</i> .....	2-50
2.4	DISCUSSION .....	2-52
2.4.1	<i>Water Stress-Induced Xylem Embolism</i> .....	2-52
2.4.2	<i>Hydraulic Properties</i> .....	2-54
<b>3</b>	<b>IDENTIFICATION OF GRAPEVINE AQUAPORINS</b> .....	<b>3-59</b>
3.1	INTRODUCTION .....	3-60
3.2	MATERIALS AND METHODS .....	3-63
3.2.1	<i>Plant Material</i> .....	3-63
3.2.2	<i>Solutions and Media</i> .....	3-63
3.2.3	<i>RNA Solutions</i> .....	3-66
3.2.4	<i>Bacterial Transformation</i> .....	3-66
3.2.5	<i>Agarose Gel Electrophoresis</i> .....	3-66
3.2.6	<i>Total and Poly (A)<sup>+</sup> RNA Isolation</i> .....	3-67
3.2.7	<i>cDNA Library Construction</i> .....	3-67
3.2.8	<i>Macroarray</i> .....	3-68
3.2.9	<i>Fixation of DNA to Membrane</i> .....	3-69
3.2.10	<i>Screening of cDNA Library</i> .....	3-70
3.2.11	<i>Plasmid Purification and Sequencing</i> .....	3-73
3.2.12	<i>Cloning of VvPIP2;1 and VvTIP1;1 by RT-PCR</i> .....	3-73
3.2.13	<i>5' RACE PCR</i> .....	3-74
3.2.14	<i>Cloning Full-length cDNAs</i> .....	3-75
3.2.15	<i>Bioinformatics</i> .....	3-76
3.3	RESULTS .....	3-77

3.3.1	<i>Grapevine cDNA library</i> .....	3-77
3.3.2	<i>Screening of the cDNA Library for Grapevine Aquaporin cDNAs</i> .....	3-77
3.3.3	<i>5' RACE PCR of Partial Aquaporin cDNAs</i> .....	3-84
2.3.6	<i>Cloning of VvPIP2;1 and VvTIP1;1 by RT-PCR</i> .....	3-87
3.3.4	<i>Phylogenetic Analysis of Grapevine PIPs and TIPs</i> .....	3-92
3.3.5	<i>Expression of Grapevine cDNAs</i> .....	3-92
3.3.6	<i>Structural Characteristics and Conserved Motifs</i> .....	3-94
3.4	DISCUSSION .....	3-97
3.4.1	<i>Identification and Phylogenetic Analysis of Grapevine Aquaporins</i> .....	3-97
3.4.2	<i>Structural Characteristics of Grapevine Aquaporins</i> .....	3-99
<b>4</b>	<b>FUNCTIONAL CHARACTERISATION OF GRAPEVINE AQUAPORINS.....</b>	<b>4-103</b>
4.1	INTRODUCTION .....	4-104
4.2	MATERIALS AND METHODS .....	4-106
4.2.1	<i>Conversion of pGEMHE to Gateway Vector</i> .....	4-106
4.2.2	<i>Amplification of attB PCR Products</i> .....	4-107
4.2.3	<i>Cloning of AQP cDNAs into Oocyte Expression Vector</i> .....	4-108
4.2.4	<i>cRNA Transcription</i> .....	4-108
4.2.5	<i>Harvesting Oocytes</i> .....	4-109
4.2.6	<i>Expression of Grapevine Aquaporins</i> .....	4-110
4.2.7	<i>Oocyte Swelling Assay</i> .....	4-110
4.2.8	<i>Acidification of the Cytosol</i> .....	4-111
4.2.9	<i>Modelling of AQP</i> .....	4-111
4.3	RESULTS .....	4-112
4.3.1	<i>Cloning of Full-length Constructs into pGEMHE</i> .....	4-112
4.3.2	<i>Oocyte Permeability in Response to Hypotonic Shock</i> .....	4-114
4.3.3	<i>Effect of Cytosolic pH on Aquaporin Water Permeability</i> .....	4-116
4.4	DISCUSSION .....	4-119
<b>5</b>	<b>EXPRESSION OF AQUAPORIN TRANSCRIPTS IN RESPONSE TO WATER-STRESS IN CHARDONNAY AND GRENACHE VINES .....</b>	<b>5-124</b>
5.1	INTRODUCTION .....	5-125

5.2	MATERIALS AND METHODS .....	5-127
5.2.1	<i>Plant Material</i> .....	5-127
5.2.2	<i>Drought Experiments</i> .....	5-127
5.2.3	<i>RNA Extractions</i> .....	5-128
5.2.4	<i>cDNA Synthesis</i> .....	5-129
5.2.5	<i>Design and Optimisation of Primers for Quantitative Real-Time PCR</i> .....	5-129
5.2.6	<i>Semi-Quantitative Polymerase Chain Reaction</i> .....	5-130
5.2.7	<i>Quantitative Real-Time Reverse Transcriptase Polymerase Chain Reaction</i> .....	5-130
5.2.8	<i>Calculation of Changes in Transcript Abundance</i> .....	5-133
5.3	RESULTS .....	5-134
5.3.1	<i>Optimisation of Quantitative Real-Time RT-PCR Primers</i> .....	5-134
5.3.2	<i>Optimisation of Housekeeping Genes</i> .....	5-135
5.3.3	<i>Tissue specific Expression in Cabernet Sauvignon by Semi-Quantitative PCR</i> .....	5-136
5.3.4	<i>Measurement of Leaf Water Potential</i> .....	5-138
5.3.5	<i>Quantitative Real-Time RT-PCR</i> .....	5-140
5.3.6	<i>Diurnal Regulation of Aquaporin Transcript Abundance</i> .....	5-143
5.3.7	<i>Transcriptional Regulation of Aquaporins in Response to Water Stress</i> .....	5-145
5.4	DISCUSSION .....	5-148
5.4.1	<i>Tissue Specific Expression of Grapevine Aquaporins</i> .....	5-149
5.4.2	<i>Diurnal Regulation of Aquaporin Gene Expression</i> .....	5-150
5.4.3	<i>Transcriptional Regulation of Aquaporins in Response to Water Stress</i> .....	5-151
<b>6</b>	<b>GENERAL DISCUSSION AND FUTURE DIRECTIONS</b> .....	<b>6-155</b>
6.1	WATER STRESS-INDUCED XYLEM EMBOLISM IN GRAPEVINES .....	6-157
6.2	THE IDENTIFICATION AND FUNCTIONAL CHARACTERISATION OF GRAPEVINE AQUAPORINS ....	
	.....	6-158
6.3	THE PHYSIOLOGICAL ROLE OF AQUAPORINS IN GRAPEVINE PETIOLES .....	6-160
6.4	ARE AQUAPORINS INVOLVED IN REFILLING OF EMBOLISED XYLEM VESSELS? .....	6-166
6.5	CONCLUSION .....	6-167
	<b>APPENDIX A</b> .....	<b>168</b>
	<b>REFERENCES</b> .....	<b>191</b>

## ABSTRACT

Aquaporins (AQP) are membrane bound proteins that facilitate the movement of water and other small neutral solutes across cellular membranes. Plant aquaporins belong to a large family of highly conserved proteins called the Membrane Intrinsic Protein (MIP) superfamily. In many plant species the expression of aquaporin genes and their regulation has been linked to water stress. Grapevines respond to water stress with a variety of physiological mechanisms, including the susceptibility to xylem embolism. The formation of embolised vessels can lead to a reduction in hydraulic conductivity of the xylem. Recently, it has been hypothesised that aquaporins may contribute to the water movement required for embolism recovery of xylem vessels thus restoring the hydraulic pathway. Molecular and physiological techniques have been combined to study the putative role of plasma membrane and tonoplast membrane aquaporins in response to water stress induced xylem embolism in two cultivars of grapevine (*Vitis vinifera* cv. Chardonnay and Grenache).

Water-stress induced cavitation was measured in the stems and petioles of pot grown grapevines of a drought tolerant (Grenache) and a drought sensitive variety (Chardonnay) by the detection of ultrasonic acoustic emissions (UAEs) over both a drying and diurnal cycle. Vulnerability curves were generated by correlating the UAEs with the leaf water potential ( $\psi_L$ ). Varietal differences in cavitation vulnerability and hydraulic properties were observed. Grenache was more susceptible to water-stress induced xylem embolism than Chardonnay, and displayed a higher hydraulic capacity (measured by maximum hydraulic conductivity). This is most likely due to anatomical differences of the xylem vessels. Chardonnay displayed vulnerability segmentation, with cavitation occurring first in the petiole and later in the stem, before developing into “runaway” cavitation under severe water stress.

Vulnerability segmentation was not observed in Grenache, with both petioles and stems equally vulnerable to the formation of xylem embolism. Under severe water stress, Grenache did not develop runaway cavitation indicating that they must have some mechanism to prevent the onset of runaway cavitation.

To determine the role of aquaporins, candidate genes were identified, by screening a *Vitis vinifera* cv. Cabernet Sauvignon cDNA library, for aquaporin cDNAs encoding members of the Plasma membrane Intrinsic Protein (PIP) and Tonoplast Intrinsic Protein (TIP) subfamilies. The screen resulted in the identification of 11 full-length and two partial aquaporin cDNAs. Sequence analyses of these cDNAs reveal five are homologous to PIP2 aquaporins, six to PIP1 and two to the TIP aquaporins. Functional expression of the full-length AQP cDNAs in *Xenopus* oocytes showed PIP2 members have significantly higher water permeability compared to PIP1 aquaporins. VvPIP2;1 showed very high water permeability which was reduced by acidic cytosolic pH, as has been reported for other members of the PIP2 family. Transcript analysis of some of these aquaporin genes provides preliminary evidence that aquaporins may contribute to differences in the hydraulic response of these two grapevine varieties to conditions of water stress.

## DECLARATION

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 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.

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Megan Cherie Shelden

2<sup>nd</sup> of October, 2007



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I would like to dedicate this thesis to my late grandmother, Valmai Shelden and Grandfather, Ronald Henderson.

## ABBREVIATIONS

$\psi$	Water Potential
Amp	Ampicillin
AQP	Aquaporin
BLAST	Basic Local Alignment Sequence Tool
BLASTn	Basic Local Alignment Sequence Tool nucleotide
bp	base pairs
BSA	Bovine Serum Albumin
cDNA	complimentary deoxyribonucleic acid
CRCV	Cooperative Research Centre for Viticulture
cRNA	capped ribonucleic acid
CS	cleavage site
cUAE	cumulative Ultrasonic Acoustic Emission
DEPC	diethylpyrocarbonate
EST	Expressed Sequence Tag
FUE	far upstream element
Kan	Kanamycin
LB	Luria Broth
MCS	multiple cloning site
MIP	major intrinsic protein
MOPS	3-(N-morpholino) propanesulfonic acid
mRNA	messenger ribonucleic acid
NPA motif	asparagine, proline, alanine motif
NUE	near upstream element

PCR	Polymerase Chain Reaction
Pf	Water permeability
PIP	plasma membrane intrinsic protein
QPCR	Quantitative polymerase chain reaction
RACE	Rapid amplification of cDNA ends
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase polymerase chain reaction
TAE	Tris Acetic Acid EDTA
TBE	Tris Boric Acid EDTA
TIP	Tonoplast intrinsic protein
TMD	transmembrane domain
UAE	ultrasonic acoustic emission
UTR	untranslated region
VAC	vessel associated cell