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

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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 (ψ_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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ABBREVIATIONS

ψ	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