Identification of host genes involved in the biotrophic interaction between grapevine and powdery mildew

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Abstract

Grapevine powdery mildew is caused by *Erysiphe necator*, an Ascomycete fungus and an obligate biotroph restricted to growth on its grapevine host. Biotrophic pathogens form a stable association with host cells without directly causing cell death, and take up nutrients from, in the case of powdery mildew (PM), host epidermal cells (Rumbolz et al., 2000). As the fungus grows, its increasing biomass becomes a strong nutrient sink capable of altering assimilate flow and storage in the host.

To identify host genes that may mediate nutrient delivery to powdery mildew infected tissues and therefore may contribute to disease susceptibility, a candidate gene approach using degenerate and RT-PCR, and a non-targeted approach using microarray analysis was instigated. Once identified, "susceptibility genes" could be targeted for manipulation to provide alternative resistance strategies based on reduced susceptibility in the future. In addition to genes encoding pathogenesis and stress related proteins, microarray analysis revealed that transcript levels of a putative metal transporter and a cell wall structural protein were elevated in infected berry skin, while aquaporin water channels and genes associated with photosynthesis were generally repressed. Degenerate PCR was used to isolated new cell wall invertase, monosaccharide and amino acid transporter genes and initial RT-PCR revealed that expression of genes involved in sugar mobilisation were the most significantly modulated by powdery mildew infection.

Previously unreported hexose transporters (HTs), (*VvHT3*, *VvHT4* and *VvHT5*) and a cwINV (*VvcwINV*) had been isolated from cDNA prepared from powdery mildew infected grapevine leaves. Full length clones of grapevine HTs and cwINV were obtained by RACE PCR. Heterologous expression of the three new

HTs in yeast confirmed that VvHT4 and VvHT5 mediated glucose uptake, while VvHT3 did not function in the yeast system. However, transient expression of a translational fusion of the VvHT3 protein with green florescence protein in onion epidermal cells indicated that it is targeted to the plasma membrane of plant cells.

Quantitative RT- PCR analysis of these new genes, together with previously reported grapevine HTs and cytoplasmic and vacuolar invertases, indicated that expression of *VvcwINV* and *VvHT5*, were significantly up-regulated by PM infection, while a vacuolar invertase was strongly down-regulated by PM infection. Invertase activity assays were in agreement with these findings, showing elevated sucrolytic activity in insoluble fractions and reduced sucrolytic activity in soluble fractions. These results suggest that apoplasmic phloem unloading of sucrose in the infected leaf is elevated and that *VvHT5* is induced to recover the additional hexoses from the apoplasm. Basic localisation studies indicated that *VvHT5* and *VvcwINV* are not induced specifically in powdery mildew infected leaf regions, but are induced in a more diffuse distribution within infected leaves.

To determine if induction of *VvHT5* and *VvcwINV* is specific to PM infection or if other stimuli may also mediate these responses, leaves were inoculated with downy mildew or stressed by wounding. Transcript levels of *VvHT5* and *VvcwINV* were elevated by wounding and downy mildew infection, suggesting that the induction of these genes may be part of a general stress response.

To explore the signalling pathways that may underlie these responses, leaves were treated with the plant growth regulators ethylene, jasmonate and abscisic acid. Exogenous application of ethylene and methyl jasmonate only marginally affected the expression of the genes studied, however foliar application of abscisic acid (ABA) induced gene expression changes similar to those observed in response to powdery mildew infection and wounding. Promoter sequences of *VvHT3*, *VvHT4*,

VvHT5 and *VvcwINV* were isolated and analysed for the presence of regulatory elements. Compared with the promoters of VvHTs that were not induced by pathogen infection or wounding, the *VvHT5* and *VvcwINV* promoters contained numerous motifs associated with induction by ABA including ABRE, Myc and Myb binding elements.

The path of sugar loading into the mesocarp of grape berries during ripening is still poorly understood and few molecular components associated with this process have been described. Quantitative RT-PCR was used to monitor the expression of five HTs and *VvcwINV* during Cabernet sauvignon and Shiraz berry development and ripening. Of the three new HTs reported here, the expression of *VvHT3* is most consistent with a potential role in sugar loading, while *VvHT5* is induced late in this process. *VvcwINV* transcript levels were high pre-ripening and also during the later stages of ripening, therefore based on this expression pattern, a role for this enzyme during ripening is not clearly evident. These results are discussed in terms of an apoplasmic step in phloem unloading in ripening grape berries.

This study has provided new insights into the molecular and biochemical processes associated with the formation of carbohydrate sink metabolism in response to stress stimuli, and sugar delivery to grape berries during ripening. ABA-dependant pathways may mediate the stress-associated induction of *VvcwINV* and *VvHT5*, presumably to recruit additional carbohydrates to the affected organ to energise repair and defence responses. At this stage it is unknown if this response is beneficial to pathogen nutrition, however potentially, modification of genes associated with carbohydrate sink metabolism could provide an alternative way to engineer resistance to this pathogen.

Declaration

This work contains no material that 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.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Matthew Hayes

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Abbreviations

ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
BLAST	basic local alignment search tool
bp	base pairs
cDNA	complementary DNA
cwINV	cell wall invertase
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
DPI	days post-inoculation
dsDNA	double-stranded DNA
EDTA	ethylenediamine-*tetra*-acetic acid
ER	endoplasmic reticulum
EST	expressed sequence tag
FD	Faraday(s)
FW	fresh weight
g	gram(s)
g	relative centrifugal force
GFP	green fluorescent protein
h	hour(s)
HT	Hexose (monosaccharide) transporter
IPTG	*Iso*-propyl-β-D-thiogalactopyranoside
kb	kilobase pairs
L	litre(s)

LB	Luria broth
M	molar
min	minute(s)
MOPS	3-N-Morpholinopropanesulfonic acid
mRNA	messenger RNA
MPa	Mega Pascals
nINV	neutral (cytoplasmic) invertase
nt	nucleotide(s)
ORF	open reading frame
PCR	polymerase chain reaction
PM	powdery mildew
RACE	rapid amplification of cDNA ends
RNA	ribonucleic acid
rpm	revolutions per minute
RT-PCR	reverse transcription-PCR
s	second(s)
Ssynth	Sucrose synthase
SUCT	sucrose transporter
SDS	sodium dodecyl sulphate
TBE	tris-borate-EDTA
Tm	temperature of DNA disassociation (melt)
Tris	tris(hydroxymethyl)aminomethane
V	volt(s)
vINV	vacuolar invertase

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