Functional characterisation of phosphorus uptake pathways in a non-responsive arbuscular mycorrhizal host

by

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Abstract

AM plants acquire P_i via two pathways; the direct uptake pathway via plant roots and the AM pathway via external fungal hyphae and colonised cortical cells. It has been assumed that these two pathways are additive and therefore in non-responsive plants the AM pathway is often considered to be non-functional. However, data from ^{32}P uptake studies indicates that the AM pathway is functional in many non-responsive symbioses and in some instances supplies the majority of plant P. In recent years the high-affinity P_i transporters involved in both direct and AM P_i uptake pathways have been identified. They are expressed at the root epidermis and the symbiotic interface of colonised cortical cells and respond to the P and AM status of the plant. The overall objective of the work described in this thesis was to characterise P_i uptake via the AM pathway in barley, a non-responsive AM host, using an approach which integrated physiological measurements of plant responsiveness and AM contribution with investigations of gene expression and functional characterisation of the plant P_i transporters.

A preliminary survey of field-grown barley demonstrated the persistence of AM colonisation under commercial cropping regimes in southern Australia and highlighted the relevance of AM studies to commercial agriculture. Under glasshouse conditions AM colonisation of barley induced depressions in growth and P uptake compared to NM controls. Growth depressions were unrelated to percent colonisation by two AM fungal species and could not readily be explained by fungal C demand; the strong correlation between growth and P content suggested that P was the limiting factor in these experiments. However, a compartmented pot system incorporating ^{32}P-labelling demonstrated that the AM pathway is functional in colonised barley and, in the interaction with *G. intraradices,* contributed 48% of total P. This suggested that P flux via the direct uptake pathway is decreased in AM barley.

The expression of three P_i transporters, *HvPT1*, *HvPT2* and *HvPT8* was investigated in colonised roots. *HvPT1* and *HvPT2* have previously been localised to the root epidermis and root hairs and are involved in P_i uptake via the direct pathway whilst *HvPT8* is an AM-inducible P_i transporter which was localised by *in-situ* hybridisation to colonised cortical cells. Using promoter::GFP gene fusions the localisation of *HvPT8* to arbuscule-containing cortical cells was confirmed in living roots from transgenic barley. Quantitative real-time PCR analysis of the expression of these three P_i transporters indicated that *HvPT1* and *HvPT2* were expressed constantly, under all conditions regardless of AM colonisation status and indicated that decreased P flux via the direct pathway is

not related to expression of these transporters. *HvPT8* was induced in AM colonised roots. However, the level of expression was not related to flux via the AM pathway or arbuscular colonisation.

The *HvPT8* transporter was further characterised by constitutive over-expression in transgenic barley. ^{32}P uptake assays in excised roots demonstrated increased P_i uptake from low P solution compared to wild-type roots and confirmed that *HvPT8* is a functional P_i transporter with high-affinity transport properties. This is the first report of characterisation of an AM-inducible P_i transporter *in planta*. When these transgenic plants were grown in solution culture there was no increase in growth or P uptake relative to wild-type or transgenic controls and growth in soil and AM colonisation were also unaffected in these transgenic lines.

The data presented in this thesis highlights the importance of combined physiological and molecular approaches to characterising plant AM interactions. The persistence of AM colonisation in barley in the field indicates the importance of improving our understanding of symbiotic function in non-responsive plants. Future efforts should be directed towards understanding the signals which regulate P flux via both the direct and AM pathways with the ultimate aim of enhancing AM responsiveness of non-responsive species. Making the direct and AM pathways additive in non-responsive species should be a key aim of future research.

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.

I give consent for this copy of my thesis, when deposited in the University Library, being made available in all forms of media, now or hereafter known.

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E. Grace

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Date

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Publications arising from this thesis

Book Chapter: Invited Publication

Grace EJ, FA Smith, SE Smith (*submitted*) Deciphering the arbuscular mycorrhizal pathway of P uptake in non-responsive hosts. In: Azcón-Aguilar C, Barea JM, Gianinazzi S, Gianinazzi-Pearson V, Eds. *Mycorrhizas: functional processes and ecological impact*, Springer.

Conference Abstracts: Oral presentations

Grace EJ (2007) Mycorrhizal symbiosis in barley: Secret deals and insider trading, **Royal Society of South Australia**, Adelaide, Australia.

Grace EJ, O Cotsaftis, M Tester, FA Smith, SE Smith (2006) Characterising phosphate transport in barley colonised by arbuscular mycorrhizal fungi, **8th International Congress of Plant Molecular Biology**, Adelaide, Australia.

Grace EJ, O Cotsaftis, M Tester, FA Smith, SE Smith (2006) Phosphorous uptake in a non-responsive host plant: deciphering the mycorrhizal pathway, **5th International Conference on Mycorrhiza**, Granada, Spain.

Conference Abstracts: Poster presentations

Grace EJ, D Glassop, O Cotsaftis, M Tester, FA Smith, SE Smith (2005) Arbuscular mycorrhizal fungi influence phosphorus uptake by barley, **ComBio Conference**, Adelaide, Australia.

Grace EJ, SE Smith, FA Smith, M Tester (2005) The influence of arbuscular mycorrhizal fungi on phosphate uptake by barley, **Genomics in the Barossa Conference**, Adelaide, Australia.

Abbreviations & Symbols

% RLC	percent root length colonised
%	percent
~	approximately
^{o}C	degrees celcius
AM	arbuscular mycorrhiza
ATP, ADP	adenosine triphosphate, adenosine diphosphate
bp	base pairs, of nucleic acids
C	carbon
cDNA	complementary DNA
cv.	cultivar
d, h, min, s	day, hour, minute, second
DNA, RNA	deoxyribo, ribonucleic acid
DTT	dithiothreitol
EC	electrical conductivity
EDTA	ethylenediaminetetraaceticacid
EST	expressed sequence tag
EtOH	ethanol
gDNA	genomic DNA
GDP	gross domestic product
GFP	green fluorescent protein
GOI	gene-of-interest
ha	hectare
kg, mg, µg, ng	kilo, milli, micro, nanogram
K_m	Michaelis constant; affinity of an enzyme for a substrate
L, mL, µL	Litre, millilitre, microlitre
LSCM	laser scanning confocal microscope
m, cm, µm, nm	metre, centimetre, micrometre, nanometre
M	molar
mol	mole
N	nitrogen
NM	non-mycorrhizal
P	phosphorus

P	probability
PCR (RT)	polymerase chain reaction; reverse transcription
P_i	inorganic orthophosphate
Q PCR	quantitative real-time PCR
RE	restriction enzyme
RO	reverse osmosis
rpm	revolutions per minute
RT	room temperature
S/N	supernatant
UV	ultraviolet
V	volt
V_{max}	maximum velocity of an enzyme mediated reaction
W	watt
w/v, w/w	weight per volume; weight per weight