CHARACTERISATION OF NUTRITION RESPONSES IN THREE GENOTYPES OF WHITE CLOVER (*Trifolium repens* L.) SELECTED FOR TOLERANCE TO LOW PHOSPHORUS

A thesis presentation in partial fulfilment of the requirements for the degree of
Master of Science in Plant Molecular Biology
At Massey University, Palmerston North, New Zealand

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2013

ABSTRACT

This study focused on the differences in root system architecture (RSA) of three white clover (*Trifolium repens* L.) genotypes, designated 43-7, 45-14 and 47-9 that were isolated from breeding lines selected to tolerate low phosphorus (P). In this study, genetically identical white clover stolons were cut from stock plants at the fourth node, and primary roots emerged (post excision) from either of the two primordia at nodes three or four. The main objective was to establish if differences in RSA were observed in response to a limited P-supply (10 μM KH_2PO_4) compared with a sufficient P-supply (1 mM KH_2PO_4). Each of the cut stolons had a dominant primary root that was nominated to be used for the analysis of root growth in terms of elongation and lateral root emergence. The analysis was standardised by designating the branching zone as the region of the primary root that contained visible lateral roots. The remaining region was designated the elongation zone, and did not contain visible lateral roots. In P-sufficient media, the branching zone was approximately 50% of the primary root length by 15 days post excision and approximately 80% by 25 days in the three genotypes. After 30 days post excision, the branching zone was maintained between 85% and 90% of the primary root length. The response to low P was measured after the emergence of visible lateral roots in experiment I and prior to the emergence of visible lateral roots in experiment II. A third treatment group with a reduced sulfur supply (in experiment II) tested the specificity of the P-stress response. In summary, the morphological responses to P-stress were characteristic for each genotype; the changes to the primary and/or lateral roots occurred within seven days from the reduction in P-supply; and the timing of the reduction in P-supply influenced the degree of the response that was observed with respect to the branching zone. The responses to P-stress included a stimulation of the primary and lateral roots in 47-9 with a reduced root biomass under S-stress; a decrease in the number of visible lateral roots in 45-14; and the decreased elongation of lateral roots in 43-7, which reduced the initiation of tertiary roots. The branching zone decreased in 45-14 only when the P-supply was changed after lateral root emergence. In contrast, the branching zone increased in 47-9 only when the P-supply was changed before lateral root emergence. The results suggest that the P-stress response in the roots is specific and utilisation of P and S may differ in the three genotypes.

ACKNOWLEDGEMENTS

Firstly I would like to say a big thank you to my supervisor Michael McManus for providing me with the opportunity to investigate such an interesting project, and for the great support and patience during the course of the study. Thank you to Susanna Leung for the help with watering the stock plants and the qPCR analysis. Also, thanks to Paul Dijkwel, Warren Williams, Xiao Song, Jay Jayaraman and Nick Albert for being a part of the staff support network in our lab. Thanks to the present and past members of our lab for being excellent colleagues Caleb, Sam, Afsana, Srishti, Jennifer, Alvina, Matt, Jibran, Rubina, Muhammad, Jun, Daniel, Ihsan, Sashini and Lydia. I would like to thank Jollanda Effendy (2007) for her work in the breeding line and genotype screens and selection of the three genotypes used in this study. Other background research that contributed to this study were the effects of auxin and ethylene treatments and changes in the root system architecture in response to low P by Phong Ding (2009) and Marissa Roldan (2008), and the acid phosphatase studies carried out by Celia Zhang (1999) and Don Hunter (1998). Secondly, I would like to thank Barry Scott, Pat Munro, Kathryn Stowell, Ann Truter, Cynthia Cresswell in the Institute of Fundamental Sciences (formally the Institute of Molecular Biosciences) for the opportunity to study in the department. Also to Kathy Hamilton and Agnes Dzang for assistance with administration and Rosie Bradshaw, Paul Stock, Rose Motion, and Katrina Ross for the opportunity to be a demonstrator for first year Biology of Cells and Plant Biology labs. Thirdly, thank you to Ipelx pipelines New Zealand for kindly donating the pipes and caps used in this study. I am very grateful for the financial support of the L.A Alexander Trust and the New Zealand Plant Biology Society (NZSPB) for the contribution to attend the conference ‘Ecology in the Heartland’ conference in Rotorua in 2011. Thanks to Steven Ray and the staff of the Massey University Plant Growth Unit, Lesley Taylor, Lindsay Sylva, Scott Avery for the help with the growth cabinet during the course of the experiments. Also, thanks to Jianyu Chen at the Manawatu Microscopic Imaging Center (MMIC) for the help with the microscopic images and the staff of the Engineering workshop for cutting the PVC pipes and foam lids. Finally, thanks so much to my wonderful family, friends and the good folk in the Manawatu Camera Club for keeping my work/life balance in check during this project.

ABBREVIATIONS

Abbreviation	Description
°C	Degrees Celsius
3PGA	3' phosphoglycerate
Al	Aluminium
APase	Acid phosphatase
At	*Arabidopsis thaliana*
ADP	Adenosine diphosphate
ATP	Adenosine-5'-triphosphate
BCIP	5-bromo-4-chloro-3-indolyl-phosphate
BL	Breeding line
BZ	Branching zone
Ca	Calcium
Col-1, Col-4	*Arabidopsis* ecotype Columbia
cv	Cultivar
CW	Cell wall fraction of the roots, tested for acid phosphatase activity
DPR	Dominant primary root
DTT	Dithiothreitol
DW	Dry weight
Fe	Iron
FFEL	First fully expanded leaf (also referred to as L1)
FW	Fresh weight
GFP	Green fluorescent protein
GUS	β-glucuronidase
K	Potassium
kDa	Kilodaltons
L1, L2, L3	Leaf 1, leaf 2, leaf 3 (up to leaf 7) numbered from the apex
Low P, –P or P-stress(ed)	Low phosphate media. Hoagland's media with half strength macronutrients and full strength micronutrients with 10 μM potassium phosphate (K_2PO_4) (Appendix I)
Low S, –S, or S-stress(ed)	Hoagland's media with half strength macronutrients and full strength micronutrients with 28 μM magnesium sulphate ($MgSO_4$) (Appendix I)
lpi	Low phosphate insensitive mutant
LPR1 and LPR2	Low Phosphate Root 1 and 2
LRD3	Lateral Root Development 3
Mg	Magnesium

Abbreviation	Description
Mo	Molybdenum
MYB	A transcription factor belonging to the **my**elo**b**lastosis family
N	Nitrogen
NZ	New Zealand
P	Phosphorus, Inorganic P (P_i), Organic P (P_o)
p-NPP	*p*-nitrophenol-1-phosphate
P-sufficient, +P or Complete	Hoagland's media with half strength macronutrients and full strength micronutrients (Appendix I)
PAE	Phosphorus Acquisition Efficiency
PAP	Purple Acid Phosphatase
PDR2	Phosphate Deficiency Response 2
PEP	Phosphoenol pyruvate
PHL-1	PHR1-like
PHR1	Phosphate Starvation Response 1
Pht	Phosphate transporter
PNP	*p*-nitrophenol
PUE	Phosphate Use Efficiency
Pv	*Phaseolus vulgaris*
QTL	Quantitative Trait Loci
RGR	Relative Growth Rate
RSA	Root System Architecture
S	Sulfur
SF	Soluble fraction of the representing the cytosol and vacuoles of the roots, tested for acid phosphatase activity
SQD1	UDP-sulfquinovose synthase
SRL	Specific Root Length (cm/mg^{-1})
SUMO	Small ubiquitin-like modifier (E3 ligase siz1)
TPR(s)	Total primary roots representing the dominant primary root and the supporting primary roots

SYMBOLS

Symbol	Description
*	Significant at <0.05 confidence level between P-sufficient and low phosphorus and complete and low sulfur media.
**	Significant at <0.01 confidence level between P-sufficient and low phosphorus (red) and complete media and low sulfur media (olive).
★	Significant at <0.05 confidence level between total relative growth of total primary root length, with significant differences in the dominant primary root indicated by an asterisks (*).
★★	Significant at <0.01 confidence level between total relative growth of primary roots, with significant differences in the dominant primary root indicated by an asterisks (**).
†	Significant at <0.05 confidence level between low phosphorus and low sulfur media.
††	Significant at <0.01 confidence level between low phosphorus and low sulfur media.
3^{o}	Emergence of tertiary roots on the longest lateral root.

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