The Role of Ethylene and Auxin in Responses of Roots to Phosphate Supply in White Clover (*Trifolium Repens* L.)

Phuong Dinh Thi Yen

2009

The Role of Ethylene and Auxin in Responses of Roots to Phosphate Supply in White Clover (*Trifolium Repens* L.)

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

Phuong Dinh Thi Yen

2009

ABSTRACT

Phosphate (P) supply is one of the major determining factors to plant productivity, since the element affects the growth and the development of plants. In response to P-deficiency treatment, plants display alterations in root system architecture caused by changes in primary root (PR) and lateral root (LR) length and LR density. In this thesis, the root growth of the agronomically important legume, white clover (*Trifolium repens* L.) was found to be slightly stimulated in terms of PR length, LR number and total LR length when plants were grown in a P-deficient media (0.01 mM orthophosphate; Pi) when compared with plants grown in a P-sufficient media (1.00 mM Pi) when using a hydroponic growth system.

When plants are grown in a P-sufficient media, treatment with 100 nM exogenous 1-aminocyclopropane-1-carboxylic acid (ACC) and exogenous auxin (5 nM 1-naphthylacetic acid, NAA) resulted in significant increases in white clover PR length, LR number and LR length. However, when ethylene action or auxin transport were inhibited using 300 ppm 1-methylcyclopropene (1-MCP) and 100 nM 1-N-naphthylphthalamic acid (NPA), respectively, root growth was significantly reduced which suggests roles for ethylene and auxin in mediating white clover root growth.

To examine the effects of these hormones on plants grown in P-deficient media, 100 nM ACC treatment significantly enhanced the stimulatory effects of growth on P-deficient media only, while exposure of plants to P-deficiency alone was sufficient to significantly neutralise the inhibitory effects of 1-MCP on root growth. Hence, exposure to P-deficiency is proposed to increase either ethylene biosynthesis or ethylene sensitivity in white clover roots. In contrast, for plants grown in P-deficient media, treatment with 5 nM NAA significantly abolished the stimulation of white clover root growth observed with P-deficiency so it is proposed that exposure to P-deficiency increases either auxin biosynthesis or auxin sensitivity, but the 5nM NAA concentration used was too high to stimulate root growth. Using *DR5p::GUS* transgenic white clover, auxin activity was found in the root tips and root primordia. Using these plants, it is suggested that P-deficient treatment and ACC treatment influenced white clover root growth through an increase in auxin sensitivity.

Overall, ethylene and auxin are found to be essential in mediating white clover root growth in P-sufficiency, and also in mediating root responses to P-deficiency through changes in terms of the biosynthesis and the sensitivity of these two hormones.

ACKNOWLEDGEMENTS

With the biggest contributions to this thesis, I would like to express my deepest gratitude to my supervisor, Professor Michael. T. McManus, for his enthusiasms, his kindness and his great efforts in guiding me (from the early stage of this research) to pursue my Master degree. I have many thanks to his patience and his valuable times spending in editing my writings. He always encourages me and gives me more confidences to deal with the challenges happened during my experiments.

My sincere thanks also go to Dr. Marissa Roldan, Dr. Sarah Dorling, Dr. Nena Alvarez, Dr. Paul Dijkwel, Miss Susanna Leung, Ms Aluh Nikmatullah, other lab-mates and "group-mates" in AgResearch and in Plant and Food for all of your supports, technical helps, discussions and encouragements. Besides many professional and academic contributions, all of them with good companies, great humours and impressive understanding made my life and my study in New Zealand easier, more enjoyable and more memorials.

Furthermore, I gratefully thank to Institute of Molecular Biosciences (IMBS) for hosting my study, Allan Wilson Centre and Manawatu Microscopy and Imaging Centre for providing me facilities to conduct my experiments. I also appreciate many staffs of IMBS including lectures and secretaries who helped and supported me in various ways during the time I studied in IMBS.

I convey special acknowledgements to New Zealand Agency for International Development for providing the scholarship which gave me chances to expand my studies and complete this thesis. I would like to thank to Massey International Student Support officers, namely: Ms Sylvia Hooker, Ms Susan Flynn and Ms Olive Pimentel who provided me with great cares and supports when I studied in Massey.

I would like to acknowledge the leaders and my colleagues at Southern Horticultural Research Institute in Viet Nam for offering and supporting my study. In addition, I am grateful to have many friends in Palmerston North, especially "Vietnamese group" whom I could not forget in the rest of my life.

TABLE OF CONTENT

LIST OF ABBREVIATIONS

1-MCP	1-methylcyclopropene
6-BAP	6-Benzylaminopurine
ACC	1-aminocyclopropane-1-carboxylate
ACO	1-aminocyclopropane-1-carboxylate oxidase
ACS	1-aminocyclopropane-1-carboxylate synthase
AVG	Aminoethoxyvinylglycine
BCIP	5 bromo-4-chloro-3-indolyl phosphate
BFA	Brefeldin A
bp	base pair
ca.	approximately
Cef^{300}	Cefotaxime (300 $\mu g.mL^{-1}$)
cm	centimetre
CTR	CONSTITUTE TRIPLE RESPONSE
DAT	day after treatment
DMF	Dimethylformamide
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EIN	ETHYLENE INSENSITIVE
ERF	ETHYLENE RESPONSE FACTOR
ERS	ETHYLENE RESPONSE SENSOR
ETR	ETHYLENE TRIPLE RESPONSE
FAA	Formalin acetic acid
g	acceleration due to gravity (9.8 $m.s^{-2}$)
g	gram
GMO	Genetically Modified Organism
GUS	β-glucuronidase
HCl	Hydrochloric acid
IAA	Indole-3-acetic acid
Kan^{150}	Kanamycin sulfate (150 $\mu g.mL^{-1}$)
^{o}C	Degrees in celsius

Kb	kilo base pair
kPa	kilo Pascal
L	Litre
LR(s)	lateral root(s)
M	moles L^{-1}
mg	milligram
$MgSO_4$	Magnesium sulfate
min	minute
mL	millilitre
mM	millimoles L^{-1}
MQ water	water purified by a Milli-purification system
MS	Murashige and Skoog base media
NAA	1-naphthyleneacetic acid
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NBT	Nitro-blue tetrazolium chloride
ng	nanogram
nM	nanomoles L^{-1}
NPA	1-N-naphthylphthalamic acid
P	P value from *t*-test
P	Phosphate
P_{adj}	adjusted P value from Tukey's test
PAT	polar auxin transport
PC2	Physical Containment 2
PCR	Polymerase Chain Reaction
PGU	Plant Growth Unit
pH	- log $[H^+]$
Pi^-	P-deficient treatment
Pi	inorganic phosphate, orthophosphate
Pi^+	P-sufficient treatment
ppm	parts per million
PR(s)	primary root(s)
QC	quiescent centre
RAM	root apical meristem

RH	Relative humidity
RNA	ribonucleic acid
Rnase	Ribonuclease
RO	reverse osmosis
rpm	round per minute
RSA	root system architecture
RT	room temperature
SAM	*S*-adenosyl methionine
SDS	Sodium dodecyl sulfate
SE	standard error of the mean
SSC	Sodium citrate – sodium chloride buffer
TAE	Tris-Acetate-EDTA
TFs	transcription factors
TIBA	2,3,5-triiodobenzoic acid
Tris	Tris(hydroxymethyl)aminomethylamine
Trp	Tryptophan
TY	Tryptone-yeast extract
UV	ultra violet light
V	volts
v/v	volume per volume
w/v	weight per volume
w/w	weight per weight
WT	wild type
X-GLUC	5-Bromo-4-chloro-3-indolyl β-D-glucuronide
μg	microgram
μL	microlitre
μm	micrometre
μM	micromoles L^{-1}

LIST OF FIGURES

LIST OF TABLE