Investigation into the relationship between aluminium treatment and the superoxide dismutase (SOD) enzyme system in *Lolium perenne* (*L. perenne* cv. Nui)

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ABSTRACT

Lolium perenne cv. Nui is a cultivar of ryegrass grown throughout New Zealand in pastures due to favourable traits such as high palatability for livestock and its ability to withstand intensive grazing. However, the productivity of pastures is reduced when levels of aluminium and other metals accumulate in soils to toxic levels, a phenomenon referred to as the 'acid soil syndrome'. In response to this toxicity, plants activate a series of antioxidant reactions, with one catalysed by the superoxide dismutase (SOD) enzymatic system. The enzyme system comprises three isoenzymes, a Cu/ZnSOD, FeSOD and a MnSOD which catalyse the same reaction but differ in amino acid sequence, molecular mass and the metal ion co-factor (hence Cu/ZnSOD, FeSOD and MnSOD). Together these isoenzymes combat the damaging effect of superoxide radicals which accumulate due to metal toxicity. In this thesis, the isolation of genes encoding isoenzymes of the SOD enzyme from *L. perenne* cv. Nui is described. As well, the growth of *L. perenne* cv. Nui and changes in expression of the SOD genes encoding each isoenzyme in response to aluminium treatment (0.2mM $AlCl_3$) is investigated.

A 1072 bp FeSOD gene sequence and a 705 bp MnSOD gene sequence were isolated from shoot tissue of *L. perenne* cv. Nui using a combination of RT-PCR with degenerate primers and 3'-RACE. The FeSOD gene comprised 572 bp of the coding sequence and 500 bp of 3'-UTR while the MnSOD gene comprised 508 bp of coding sequence and a 197 bp 3'-UTR. By alignment of each sequence with the gene from the database with highest identity it was predicted that the translation start codon (ATG) is located a further 196 bp upstream for the FeSOD gene (aligned with an *Oryza sativa* FeSOD sequence as a reference) and a further 152 bp upstream for the MnSOD sequence (aligned with a *Triticum aestivum* MnSOD sequence as a reference). Using RT-PCR with degenerate primers, a 313 bp CuSOD sequence was predominantly cloned from shoot tissue of *L. perenne* cv. Nui, but it was not possible to generate the 3'-UTR using 3'-RACE.

For growth analysis, seedlings of *L. perenne* cv. Nui were germinated and acclimatised in Hoagland's solution, and then subjected to either aluminium treatment (0.2mM

$AlCl_3$) or no treatment to act as a control over a designated time course of 0, 4, 8, or 24 hours. Two growth trials were conducted that differed in the age of seedlings used and plant tissues were separated into root and shoot tissues. Similar growth trends were observed in both trials, but the sampling regime in the second growth trial meant that statistical analysis could be carried out. In this trial, analysis revealed that over a time course of 24 hours exposure to 0.2mM aluminium, both root and shoot tissue fresh weight did not significantly differ when compared to the control (no aluminium). A general trend of an increase in root and shoot fresh weight was observed in plants treated with aluminium, but this trend was not significant at P=0.05. No significant change in fresh weight partitioning from shoot to root, or root to shoot in response to aluminium was also observed.

Using semi-quantitative Reverse Transcriptase-Polymerase Chain Reaction (sqRT-PCR) and primers based around the 3'-UTR with RNA isolated from plants grown in the second hydroponic trial, it was determined that under the conditions used, expression of the FeSOD and MnSOD genes isolated in this study were neither up-regulated or down-regulated in response to aluminium treatment in both shoot and root tissue. Further, using degenerate primers to detect expression of one or more genes encoding the Cu/ZnSOD isoenzyme, total expression of the Cu/ZnSOD isoenzyme was also unresponsive to aluminium treatment.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

A_{260}	Absorbance at 260 nm
Al	Aluminium
$AlCl_3$	Aluminium chloride
Amp	Ampicillin
bp	Base pair
°C	Degrees celcius
$CaCl_2$	Calcium chloride
cDNA	Complementary deoxyribonucleic acid
cm	Centrimetre
cv.	Cultivar
d	Day
DEPC	Diethylpyrocarbonate
DF	Dilution factor
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DTT	Dithiothreitol
E. coli	*Echerichia coli*
EDTA	Ethylenediaminetetraacetic acid
EtBr	Ethidium bromide
FW	Fresh weight
g	g force
g	Grams
h	Hour
HCl	Hydrochloric acid
IPTG	Isopropyl-β-$_D$-thiogalactopyranoside
Kb	Kilo basepair
KCl	Potassium chloride
L	Litre
LB	Luria-Bertani (media or broth)
L. perenne	*Lolium perenne*

LiCl	Lithium chloride
M	Molar, moles per litre
mM	Millimolar
mg	Milligram
MQ Water	Water purified by a Milli-purification system
min	Minute
mL	Millilitre
NaOAc	Sodium acetate
NCBI	National Centre for Biotechnology Information
ng	Nanogram
nm	Nanometre
n	Night
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NZ	New Zealand
OD	Optical density
PCR	Polymerase chain reaction
PCR Water	Filtered, sterile MQ water
PEG	Polyethylenglycol
pH	-Log $[H^+]$
RACE	3´-rapid amplification of cDNA ends
RNA	Ribonucleic acid
RNase	Ribonuclease
rpm	Revolutions per minute
RT-PCR	Reverse transcriptase-polymerase chain reaction
s	Second
SDS	Sodium dodecyl sulphate
sqRT-PCR	Semi-quantitative RT-PCR
TAE	Tris-Acetate-EDTA
Tris	Tris (hydroxymethyl) aminomethane
µg	Microgram
µl	Microlitre
µM	Micromolar
U	Units

UTR	Untranslated region
UV	Ultraviolet light
V	Volts
v/v	Volume per volume
w/v	Weight per volume
w/w	Weight per weight
X-Gal	5-Bromo-4-chloro-3-indolyl-β-$_D$-galactopyranoside