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

A thesis presented in partial fulfilment of the requirements for the degree of Master of Science (with Honours) in Plant Biology at Massey University

Samuel James Gregory
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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 (0.2 mM AlCl₃) 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 (0.2 mM
AlCl₃) 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.
ACKNOWLEDGEMENTS

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>i</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>iii</td>
</tr>
<tr>
<td>Table of contents</td>
<td>iv</td>
</tr>
<tr>
<td>List of figures</td>
<td>vii</td>
</tr>
<tr>
<td>List of tables</td>
<td>ix</td>
</tr>
<tr>
<td>Abbreviations</td>
<td>x</td>
</tr>
<tr>
<td>1. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>1.1 Overview</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Aluminium Toxicity and the Acid Soil Syndrome</td>
<td>2</td>
</tr>
<tr>
<td>1.2.1 Formation of Acidic Soils</td>
<td>2</td>
</tr>
<tr>
<td>1.2.2 Aluminium and Acidic Soils</td>
<td>3</td>
</tr>
<tr>
<td>1.2.3 Aluminium Toxicity</td>
<td>4</td>
</tr>
<tr>
<td>1.3 Formation of ROS and Oxidative Stress</td>
<td>8</td>
</tr>
<tr>
<td>1.3.1 The Antioxidant Pathway</td>
<td>10</td>
</tr>
<tr>
<td>1.3.1.1 Antioxidant Pathway – The non-enzymic system</td>
<td>10</td>
</tr>
<tr>
<td>1.3.1.2 Antioxidant Pathway – The enzymic system</td>
<td>10</td>
</tr>
<tr>
<td>1.4 Superoxide Dismutase (SOD)</td>
<td>14</td>
</tr>
<tr>
<td>1.4.1 Aluminium Toxicity and SOD Expression</td>
<td>16</td>
</tr>
<tr>
<td>1.5 Genetic Engineering of plants with SOD genes</td>
<td>18</td>
</tr>
<tr>
<td>1.6 Lolium perenne cv. Nui</td>
<td>19</td>
</tr>
<tr>
<td>1.7 Aim (s) and Hypothesis of Current Research</td>
<td>20</td>
</tr>
<tr>
<td>1.8 Note on nomenclature</td>
<td>21</td>
</tr>
<tr>
<td>2. Materials and Methods</td>
<td>22</td>
</tr>
<tr>
<td>2.1 Propagation</td>
<td>22</td>
</tr>
<tr>
<td>2.1.0 Germination and Establishment of Ryegrass Seedlings</td>
<td>22</td>
</tr>
<tr>
<td>2.2 Hydroponics and Aluminium Treatment</td>
<td>23</td>
</tr>
<tr>
<td>2.2.1 Establishment of the hydroponic growth system</td>
<td>23</td>
</tr>
<tr>
<td>2.2.2 Aluminium Treatment</td>
<td>26</td>
</tr>
<tr>
<td>2.2.3 Harvesting of Plant Material</td>
<td>26</td>
</tr>
</tbody>
</table>
2.3 Molecular Methods ................................................................. 27
  2.3.0 RNA Extraction ................................................................. 27
  2.3.1 RNA Quantification ............................................................ 28
  2.3.2 Reverse transcriptase – Polymerase chain reaction ............ 28
  2.3.3 3’ RACE (Rapid Amplification of cDNA ends) .................. 29
  2.3.4 Semi-quantitative RT-PCR expression analysis ................. 31
  2.3.5 Purification of PCR Products ............................................. 31
  2.3.6 DNA Ligation ................................................................. 32
  2.3.7 Preparation of _E. coli_ (strain DH5α) Competent Cells ........... 32
  2.3.8 Plasmid DNA Transformation Into _E. coli_ (DH5α) ............ 33
  2.3.9 Selection of Transformants .............................................. 33
2.4 Preparation of Plasmid DNA .................................................. 35
  2.4.1.0 Alkaline lysis/PEG precipitation mini prep method ......... 35
  2.4.1.1 Autosequencing Reaction – BigDye Terminator v3.1 .... 36
  2.4.1 Precipitation of terminator DNA and automated DNA sequencing 37
  2.4.2 Agarose gel electrophoresis ............................................. 37
2.5 Sequence Analysis and Primer Design ................................... 38
3. Results ..................................................................................... 40
  3.1 RT-PCR Amplification of SOD Isoforms in _L. perenne_ ........ 40
      3.1.1 Sequence analysis of clones generated using degenerate Cu/ZnSOD primers ................................................. 41
      3.1.2 Sequence analysis of degenerate FeSOD primer sequences .... 45
      3.1.3 Sequence analysis of clones amplified using the degenerate MnSOD primer sequences ........................................... 49
      3.1.4 Sequence alignment of the three isoform sequences ........ 49
  3.2 Amplification of the 3’-UTR corresponding to the SOD isoenzymes of _L. perenne_ ................................................................. 53
      3.2.1 Screening of putative inserts using SOD gene-specific primers 53
      3.2.2 Analysis of 3’ UTR sequences corresponding to the FeSOD gene isolated using degenerate primers .......................... 54
      3.2.3 Analysis of 3’ UTR sequences corresponding to the MnSOD gene isolated using degenerate primers ....................... 60
      3.2.4 Analysis of 3’ UTR sequences corresponding to the CuSOD
gene isolated using degenerate primers ................................................................. 65
3.2.5 Clustal analysis alignment of 3’UTR sequences and full length sequences obtained in this trial ................................................................. 65
3.3 Growth Analysis of L. perenne in Hydroponic Media ................................. 69
  3.3.1 Growth Analysis of the First Hydroponic Trial (Experiment I). .......... 69
  3.3.2 Growth Analysis for the Second Hydroponic Trial ............................. 73
3.4 Expression studies on isolated SOD isoforms in L. perenne .................... 77
  3.4.1 Analysis of FeSOD gene expression in L. perenne in response to 0.2 mM AlCl$_3$ ................................................................. 77
  3.4.2 Analysis of MnSOD gene expression in L. perenne in response to 0.2 mM AlCl$_3$ ................................................................. 79
  3.4.3 Analysis of total CuSOD activity in L. perenne in response to 0.2 mM AlCl$_3$ ................................................................. 79
4. Discussion ........................................................................................................ 82
  4.1 Aluminium treatment does not negatively affect root and shoot growth of L. perenne over a short treatment course ........................................... 82
    4.1.1 Affect of aluminium on root growth in L. perenne ......................... 82
      4.1.1.1 Affect of aluminium on root morphology in L. perenne .......... 84
    4.1.2 Affect of aluminium on shoot growth in L. perenne ..................... 85
    4.1.3 Affect of aluminium treatment on total biomass in L. perenne .... 87
4.2 Sequencing and Isolation of Genes Encoding the SOD Enzymatic System in L. perenne ......................................................................................... 88
    4.2.1 Evolutionary Nature of the SOD enzymatic system and L. perenne ......................................................................................... 88
    4.2.2 Gene relationships amongst SOD isoforms in L. perenne ............ 91
4.3 Expression analysis of the three genes coding for isoforms of the SOD enzymatic system ......................................................................................... 93
    4.3.1 Possible explanation of observed expression results .................... 94
      4.3.1.1 Organic Acids ..................................................................... 94
      4.3.1.2 SOD activity in response to oxidative stress ....................... 95
4.4 Future Work .............................................................................................. 98
5. References ...................................................................................................... 99
**LIST OF FIGURES**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.1</td>
<td>Flow diagram of how Al-toxicity can result in oxidative stress</td>
<td>7</td>
</tr>
<tr>
<td>Figure 1.2</td>
<td>The Halliwell-Asada pathway</td>
<td>11</td>
</tr>
<tr>
<td>Figure 1.3</td>
<td>Inactivation of the superoxide anion by antioxidant components</td>
<td>12</td>
</tr>
<tr>
<td>Figure 1.4</td>
<td>Diagrammatic representation of how the antioxidant system in plants detoxify ROS</td>
<td>13</td>
</tr>
<tr>
<td>Figure 2.1</td>
<td><em>L. perenne</em> germinated seedlings under sterile conditions</td>
<td>23</td>
</tr>
<tr>
<td>Figure 2.2</td>
<td><em>L. perenne</em> in control and Al-treated containers</td>
<td>24</td>
</tr>
<tr>
<td>Figure 2.3</td>
<td>Diagrammatic approach to the capture of 3’ UTR sequences using the 3’ RACE protocol</td>
<td>30</td>
</tr>
<tr>
<td>Figure 2.4</td>
<td>Structure of a typical plant protein coding mRNA including the untranslated regions (UTRs)</td>
<td>30</td>
</tr>
<tr>
<td>Figure 2.5</td>
<td>pGEM-T Easy vector map with sequence reference points</td>
<td>34</td>
</tr>
<tr>
<td>Figure 3.1.0</td>
<td>Examples of PCR generated DNA inserts from the pGEM vector using Cu/ZnSOD degenerate primers (A) and FeSOD degenerate primers (B)</td>
<td>42</td>
</tr>
<tr>
<td>Figure 3.1.1</td>
<td>A Clustal W alignment illustrating the 5 bp insertion (A) of the CuSOD9 sequence used for expression analysis with resulting amino acid change (B) and (C)</td>
<td>43</td>
</tr>
<tr>
<td>Figure 3.1.2</td>
<td>Clustal W alignment of the CuSOD, MnSOD and FeSOD genes sequences isolated in this study</td>
<td>51</td>
</tr>
<tr>
<td>Figure 3.1.3</td>
<td>Clustal W alignment of CuSOD, MnSOD and FeSOD SOD genes for amino acid identity</td>
<td>52</td>
</tr>
<tr>
<td>Figure 3.2.1</td>
<td>Results from the NCBI database BLAST-n analysis of the putative FeSOD reading frame and partial 3’-UTR sequence identifying the STOP (TGA) codon</td>
<td>55</td>
</tr>
<tr>
<td>Figure 3.2.2</td>
<td>Results from the NCBI database BLAST-n analysis of the putative FeSOD 3’-UTR sequence</td>
<td>56</td>
</tr>
<tr>
<td>Figure 3.2.3</td>
<td>The generated 1072 bp FeSOD sequence obtained from <em>L. perenne</em> in this study</td>
<td>58</td>
</tr>
<tr>
<td>Figure 3.2.4</td>
<td>The reference sequence of <em>O.sativa</em> (AB014056) obtained from the</td>
<td></td>
</tr>
</tbody>
</table>
NCBI database and used to predict the start (ATG) of the coding sequence for the FeSOD gene from *L. perenne* generated in this study ........................................................................................................ 59

Figure 3.2.5 Results from the NCBI database BLAST-n analysis of the putative MnSOD sequence identification of STOP codon and subsequent 3’UTR sequence ........................................................................ 61

Figure 3.2.6 The generated 705 bp MnSOD sequence obtained from *L. perenne* in this study ........................................................................................................ 63

Figure 3.2.7 The reference sequence of *T.aestivum* (AF092524) obtained from the NCBI database and used to predict the start (ATG) of the coding sequence for the MnSOD gene from *L. perenne* generated in this study ........................................................................................................ 64

Figure 3.2.8 Clustal W alignment analysis of the FeSOD and MnSOD 3’ UTR sequences along with identity........................................................ 67

Figure 3.2.9 Clustal W alignment analysis of the FeSOD and MnSOD full sequences obtained in this study ........................................................................ 68

Figure 3.3.1 Growth analysis of the first hydroponic trial .................................. 71

Figure 3.3.2 Growth analysis of the first hydroponic trial .................................. 72

Figure 3.3.3 Growth analysis of the second hydroponic trial .............................. 75

Figure 3.3.4 Growth analysis of the second hydroponic trial .............................. 76

Figure 3.4.0 Semi-quantitative RT-PCR (sqRT-PCR) analysis of expression of a specific FeSOD gene .............................................................. 78

Figure 3.4.1 Semi-quantitative RT-PCR (sqRT-PCR) analysis of induction of the MnSOD UTR gene .............................................................. 80

Figure 3.4.2 Semi-quantitative RT-PCR (sqRT-PCR) analysis of induction of total CuSOD gene expression (using degenerate CuSOD primers). 81
LIST OF TABLES

Table 2.1 Composition of the Hoagland solution with one-third strength macro and full strength micronutrients ........................................ 25
Table 2.2 Primer sequences used in this study ................................................ 39
Table 3.1.0 NCBI BLAST-n analysis of the putative CuSOD sequence showing the highest identity sequences from the database ...................... 44
Table 3.1.1 NCBI BLAST-n analysis of the putative FeSOD sequence showing the highest identity sequences from the database ...................... 47
Table 3.1.2 NCBI BLAST-n analysis of the putative MnSOD sequence showing the highest identity sequences from the database ...................... 48
Table 3.1.3 Alignment scores (%) of SOD gene sequences obtained using degenerate primers in this study ........................................ 52
Table 3.2.1 Sequences producing significant alignments to the 500 bp 3'-UTR FeSOD sequence of L. perenne ........................................ 57
Table 3.2.2 Sequences producing significant alignments to the 197 bp 3'-UTR MnSOD sequence of L. perenne ........................................ 62
Table 3.2.3 Results from the NCBI database BLAST-n analysis of the putative CuSOD 3’UTR sequence ........................................ 66
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)
$L.\ perenne$  $Lolium\ perenne$
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>LiCl</td>
<td>Lithium chloride</td>
</tr>
<tr>
<td>M</td>
<td>Molar, moles per litre</td>
</tr>
<tr>
<td>mM</td>
<td>Millimolar</td>
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<tr>
<td>mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>MQ Water</td>
<td>Water purified by a Milli-purification system</td>
</tr>
<tr>
<td>min</td>
<td>Minute</td>
</tr>
<tr>
<td>mL</td>
<td>Millilitre</td>
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<tr>
<td>NaOAc</td>
<td>Sodium acetate</td>
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<tr>
<td>NCBI</td>
<td>National Centre for Biotechnology Information</td>
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<tr>
<td>ng</td>
<td>Nanogram</td>
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<tr>
<td>nm</td>
<td>Nanometre</td>
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<tr>
<td>n</td>
<td>Night</td>
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<tr>
<td>NaCl</td>
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<tr>
<td>NaOH</td>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>NZ</td>
<td>New Zealand</td>
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<tr>
<td>OD</td>
<td>Optical density</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>PCR Water</td>
<td>Filtered, sterile MQ water</td>
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<td>PEG</td>
<td>Polyethylenglycol</td>
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</tr>
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<td>Revolutions per minute</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Reverse transcriptase-polymerase chain reaction</td>
</tr>
<tr>
<td>s</td>
<td>Second</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulphate</td>
</tr>
<tr>
<td>sqRT-PCR</td>
<td>Semi-quantitative RT-PCR</td>
</tr>
<tr>
<td>TAE</td>
<td>Tris-Acetate-EDTA</td>
</tr>
<tr>
<td>Tris</td>
<td>Tris (hydroxymethyl) aminomethane</td>
</tr>
<tr>
<td>µg</td>
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</tr>
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</tr>
<tr>
<td>U</td>
<td>Units</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>UTR</td>
<td>Untranslated region</td>
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<tr>
<td>UV</td>
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</tr>
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</tr>
</tbody>
</table>