Functional Characterisation
of Plant Cytosolic
Thioredoxins

Submitted by
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This thesis is submitted in fulfilment of the requirements for
the degree of Doctor of Philosophy

Discipline of Plant and Food Science
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Abstract

Thioredoxins are small, ubiquitous, disulfide oxidoreductase proteins characterised by a conserved dicysteine active site. Within the cell, they are believed to maintain the redox environment and participate in a broad range of biochemical processes. Plant thioredoxins are a diverse multigene family, primarily classified according to the system by which they are reduced and their subcellular localization. Thioredoxins located in the cytoplasm (type -h) are usually dependent on NADPH for reduction by NADPH-thioredoxin reductase. There are four cytosolic thioredoxins in grass species, with subclass 4 believed to be the most ancient. The highly conserved nature of thioredoxin-h4, in plant species as diverse as angiosperms and gymnosperms, implies a conservation of gene function. Discovery of thioredoxin-h4 function in barley (*Hordeum vulgare*) was the core focus of the research presented in this thesis.

The characterisation of thioredoxin-h4 was approached from both, genetic, and protein biochemistry perspectives. To commence the research, the transcript profile of barley thioreodoxin-h4 (*HvTrx-h4*) was examined in barley reproductive tissues. As a direct consequence of findings, anther and stigma tissues were used in protein interaction studies employing a mono-cystenic active-site HvTrx-h4 affinity chromatography technique. *HvTrx-h4* was mutated, recombinantly expressed, purified and immobilised in order to isolate and identify proteins with which it interacted. Identification of *HvTrx-h4* protein targets sought to reveal the pathways in which thioredoxin-h4 is involved.

To further characterise the expression of *HvTrx-h4*, the promoter and 5′ untranslated regions of genomic sequence were isolated and used to drive expression of green fluorescent protein in transgenically modified barley. This enabled examination of the temporal and spatial regulation of *HvTrx-h4* under normal growth conditions, as well as in response to abiotic stress and plant hormone treatments. Through these studies it was discovered that *HvTrx-h4* is likely to be the subject of post-transcriptional modifications. Subsequent investigations revealed *HvTrx-h4* is also regulated at the post-translational level through glutathionylation.

Previous studies have ascribed a role for thioredoxins in plant oxidative stress defence. The question of whether modulation of *HvTrx-h4* expression could be manipulated to alter plant oxidative stress tolerance was considered. To investigate, transgenic tobacco plants (*Nicotiana tabacum*) containing altered amounts of thioredoxin-h4 protein were subjected to various stresses; abiotic, biotic and chemical, in nature. Tobacco constitutively over-
expressing thioredoxin-h4 displayed increased tolerance to ultraviolet light B, heat and methyl viologen treatment.

Knowledge acquired by this study and presented in this thesis, suggest a role for barley thioredoxin-h4 in the oxidative stress response. Furthermore, the description of both post-transcriptional and post-translational regulation of HvTrx-h4 constitutes the first report of this level of regulation for a plant cytosolic thioredoxin.
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>A</td>
<td>Absorbance</td>
</tr>
<tr>
<td>ABA</td>
<td>Abscisic acid</td>
</tr>
<tr>
<td>ACPFG</td>
<td>Australian Centre for Plant Functional Genomics</td>
</tr>
<tr>
<td>AGRF</td>
<td>Australian Genome Research Facility</td>
</tr>
<tr>
<td>Amp</td>
<td>Ampicillin</td>
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<td>AOX</td>
<td>Alternative oxidase</td>
</tr>
<tr>
<td>APS</td>
<td>Ammonium persulphate</td>
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<tr>
<td>APX</td>
<td>Ascorbate peroxidase</td>
</tr>
<tr>
<td>BLAST</td>
<td>Basic local alignment search tool</td>
</tr>
<tr>
<td>bp</td>
<td>Base pair(s)</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine Serum Albumin</td>
</tr>
<tr>
<td>cv</td>
<td>Cultivar</td>
</tr>
<tr>
<td>C-terminal</td>
<td>Carboxy terminal</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary DNA</td>
</tr>
<tr>
<td>CDSP32</td>
<td>Chloroplastic drought-induced stress protein</td>
</tr>
<tr>
<td>Da</td>
<td>Dalton(s)</td>
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<td>Dimethylsulphoxide</td>
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<td>Deoxynucleotide triphosphate</td>
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<td>Ethylenediamine tetra-acetic acid</td>
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<td>Ethidium bromide</td>
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<td>Ferredoxin-thioredoxin reductase</td>
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<td>g</td>
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<td>MAPK</td>
<td>Mitogen-activated protein kinase</td>
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<td>Nickel-nitrilotriacetic acid</td>
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<tr>
<td>N-terminal</td>
<td>Amino terminal</td>
</tr>
<tr>
<td>NTR</td>
<td>NADPH-thioredoxin reductase</td>
</tr>
<tr>
<td>ng</td>
<td>Nanogram(s)</td>
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**Note:** This table includes common abbreviations used in biochemistry and molecular biology, along with their full forms and meanings.
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<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Unit</th>
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<tr>
<td>nm</td>
<td>Nanometre(s)</td>
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<tr>
<td>(^1\text{O}_2)</td>
<td>Singlet oxygen</td>
<td></td>
</tr>
<tr>
<td>(\text{O}_2) (^-)</td>
<td>Superoxide</td>
<td></td>
</tr>
<tr>
<td>OD</td>
<td>Optical density</td>
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</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
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</tr>
<tr>
<td>PCD</td>
<td>Programmed cell death</td>
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</tr>
<tr>
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</tr>
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<td>Quantitative PCR</td>
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</tr>
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<td>Rose Bengal diacetate</td>
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</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
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</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen specie(s)</td>
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</tr>
<tr>
<td>rpm</td>
<td>Revolutions per minute</td>
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</tr>
<tr>
<td>s</td>
<td>Second(s)</td>
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</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulphate</td>
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<tr>
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<td>SDS-polyacrylamide gel electrophoresis</td>
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<tr>
<td>SI</td>
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<tr>
<td>SOD</td>
<td>Superoxide dismutase</td>
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<td>TBS</td>
<td>Tris buffered saline</td>
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<td>TEMED</td>
<td>Tetramethylethylenediamine</td>
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</tbody>
</table>

\(\text{OD}\) Optical density, \(\text{rpm}\) Revolutions per minute, \(\text{OD}°\) Degrees Celsius, \(\text{PBS}\) Phosphate buffered saline, \(\text{PCD}\) Programmed cell death, \(\text{PCR}\) Polymerase chain reaction, \(\text{Q-PCR}\) Quantitative PCR, \(\text{RBDA}\) Rose Bengal diacetate, \(\text{RNA}\) Ribonucleic acid, \(\text{ROS}\) Reactive oxygen specie(s), \(\text{rpm}\) Revolutions per minute, \(\text{s}\) Second(s), \(\text{SDS}\) Sodium dodecyl sulphate, \(\text{SDS-PAGE}\) SDS-polyacrylamide gel electrophoresis, \(\text{SI}\) Self-incompatibility, \(\text{SOD}\) Superoxide dismutase, \(\text{TBS}\) Tris buffered saline, \(\text{TEMED}\) Tetramethylethylenediamine, \(\text{Trx-}\(f\)\) Chloroplastic type-\(f\) thioredoxin, \(\text{Trx-}\(h\)\) Cytoplasmic type-\(h\) thioredoxin, \(\text{Trx-}\(m\)\) Chloroplastic type-\(m\) thioredoxin, \(\text{Trx-}\(o\)\) Mitochondrial type-\(o\) thioredoxin, \(\text{Tm}\) Melting temperature, \(\text{Trx}\) Thioredoxin, \(\text{UTR}\) Untranslated region, \(\text{UVB}\) Ultraviolet light B.
Statement of Authorship

This work contains no material that has been accepted for the award of any other degree or diploma in any university or other tertiary institute and to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference being made in the text.

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