

Functional Characterisation of Plant Cytosolic Thioredoxins

Submitted by

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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 thioredoxin-*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

A	Absorbance	GUS	β -glucuronidase
ABA	Abscisic acid	GPX	Glutathione peroxidase
ACPFPG	Australian Centre for Plant Functional Genomics	GRX	Glutaredoxin
AGRF	Australian Genome Research Facility	h	Hour(s)
Amp	Ampicillin	H ₂ O ₂	Hydrogen peroxide
AOX	Alternative oxidase	HO [•]	Hydroxyl radical
APS	Ammonium persulphate	HPLC	High performance liquid chromatography
APX	Ascorbate peroxidase	IPTG	Isopropylthio- β -o- galactopyranoside
BLAST	Basic local alignment search tool	JA	Jasmonic acid
bp	Base pair(s)	Kb	Kilobase(s)
BSA	Bovine Serum Albumin	kDa	KiloDalton(s)
cv	Cultivar	LB	Lauria Broth
C-terminal	Carboxy terminal	M	Molar
cDNA	Complementary DNA	MAPK	Mitogen-activated protein kinase
CDSP32	Chloroplastic drought-induced stress protein	mBBr	Monobromobiamane
Da	Dalton(s)	min	Minute(s)
DMSO	Dimethylsulphoxide	mL	Millilitre(s)
DNA	Deoxyribonucleic acid	mm	Millimetre(s)
DNase	Deoxyribonuclease	mM	Millimolar
dNTP	Deoxynucleotide triphosphate	mg	Milligram(s)
DTT	Dithiothreitol	mRNA	Messenger ribonucleic acid
EDTA	Ethylenediamine tetra-acetic acid	MS	Murashige & Skoog
ER	Endoplasmic reticulum	mV	millivolt(s)
EtBr	Ethidium bromide	MV	Methyl viologen (paraquat)
FTR	Ferredoxin-thioredoxin reductase	N-terminal	Amino terminal
g	Gram(s)	NADPH	Nicotinamide adenine dinucleotide phosphate
		NTR	NADPH-thioredoxin reductase
		ng	Nanogram(s)
		Ni-NTA	Nickel-nitrilotriacetic acid

nm	Nanometre(s)	V	Volt(s)
¹ O ₂	Singlet oxygen	v/v	Volume per volume
O ₂ ^{•-}	Superoxide	w/v	Weight per volume
OD	Optical density	°C	Degrees Celsius
PBS	Phosphate buffered saline	g	Units of relative centrifugal force
PCD	Programmed cell death		
PCR	Polymerase chain reaction	λ	wavelength
Q-PCR	Quantitative PCR	Ω	Ohm
RBDA	Rose Bengal diacetate	μl	Microlitre(s)
RNA	Ribonucleic acid	μg	Microgram(s)
ROS	Reactive oxygen specie(s)	μM	Micromole(s)
rpm	Revolutions per minute		
RT-PCR	Reverse transcriptase PCR		
s	Second(s)		
SDS	Sodium dodecyl sulphate		
SDS-PAGE	SDS-polyacrylamide gel electrophoresis		
SI	Self-incompatibility		
SOD	Superoxide dismutase		
TBS	Tris buffered saline		
TEMED	Tetramethylethylenediamine		
Trx- <i>f</i>	Chloroplastic type- <i>f</i> thioredoxin		
Trx- <i>h</i>	Cytoplasmic type- <i>h</i> thioredoxin		
Trx- <i>m</i>	Chloroplastic type- <i>m</i> thioredoxin		
Trx- <i>o</i>	Mitochondrial type- <i>o</i> thioredoxin		
Tm	Melting temperature		
Trx	Thioredoxin		
UTR	Untranslated region		
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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