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-*h*4, in plant species as diverse as angiosperms and gymnosperms, implies a conservation of gene function. Discovery of thioredoxin-*h*4 function in barley (*Hordeum vulgare*) was the core focus of the research presented in this thesis.

The characterisation of thioredoxin-*h*4 was approached from both, genetic, and protein biochemistry perspectives. To commence the research, the transcript profile of barley thioreodoxin-*h*4 (*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-*h*4 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-*h*4 protein targets sought to reveal the pathways in which thioredoxin-*h*4 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-*h*4 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-*h*4 expression could be manipulated to alter plant oxidative stress tolerance was considered. To investigate, transgenic tobacco plants (*Nicotiana tabacum*) containing altered amounts of thioredoxin-*h*4 protein were subjected to various stresses; abiotic, biotic and chemical, in nature. Tobacco constitutively over-

expressing thioredoxin-*h*4 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-*h*4 in the oxidative stress response. Furthermore, the description of both post-transcriptional and post-translational regulation of HvTrx-*h*4 constitutes the first report of this level of regulation for a plant cytosolic thioredoxin.

Abbreviations

A	Absorbance
ABA	Abscisic acid
ACPFG	Australian Centre for Plant Functional Genomics
AGRF	Australian Genome Research Facility
Amp	Ampicillin
AOX	Alternative oxidase
APS	Ammonium persulphate
APX	Ascorbate peroxidase
BLAST	Basic local alignment search tool
bp	Base pair(s)
BSA	Bovine Serium Albumin
cv	Cultivar
C-terminal	Carboxy terminal
cDNA	Complementary DNA
CDSP32	Chloroplastic drought-induced stress protein
Da	Dalton(s)
DMSO	Dimethylsulphoxise
DNA	Deoxyribonucleic acid
DNase	Deoxyribonulclease
dNTP	Deoxynucleotide triphosphate
DTT	Dithiothreitol
EDTA	Ethylenediamine tetra-acetic acid
ER	Endoplasmic reticulum
EtBr	Ethidium bromide
FTR	Ferredoxin-thioredoxin reductase
g	Gram(s)
GUS	β-glucuronidase
GPX	Glutathione peroxidase
GRX	Glutaredoxin
h	Hour(s)
H_2O_2	Hydrogen peroxide
$HO^{\bullet}$	Hydroxyl radical
HPLC	High performance liquid chromatography
IPTG	Isopropylthio-ß-o-galactopyranoside
JA	Jasmonic acid
Kb	Kilobase(s)
kDa	KiloDalton(s)
LB	Lauria Broth
M	Molar
MAPK	Mitogen-activated protein kinase
mBBr	Monobromobiamane
min	Minute(s)
mL	Millilitre(s)
mm	Millimetre(s)
mM	Millimolar
mg	Milligram(s)
mRNA	Messenger ribonucleic acid
MS	Murashige & Skoog
mV	millivolt(s)
MV	Methyl viologen (paraquot)
N-terminal	Amino terminal
NADPH	Nicotinamide adenine dinucleotide phosphate
NTR	NADPH-thioredoxin reductase
ng	Nanogram(s)
Ni-NTA	Nickel-nitrilotriacetic acid

nm	Nanometre(s)	V	Volt(s)
$^1O_2$	Singlet oxygen	v/v	Volume per volume
$O_2^{\bullet-}$	Superoxide	w/v	Weight per volume
OD	Optical density	^oC	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-*f*	Chloroplastic type-*f* thioredoxin		
Trx-*h*	Cytoplasmic type-*h* thioredoxin		
Trx-*m*	Chloroplastic type-*m* thioredoxin		
Trx-*o*	Mitochondrial type-*o* thioredoxin		
Tm	Melting temperature		
Trx	Thioredoxin		
UTR	Untranslated region		
UVB	Ultraviolet light B		

Statement of Authorship

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