Investigating the role of tetrapyrrole biosynthesis under drought stress in cereal transgenics

A thesis submitted in fulfilment of the requirement for the degree of

Doctor of Philosophy

By

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Thesis Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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List of Abbreviations

$^1O_2$	singlet oxygen
ABA	abscisic acid
ABCG2	ATP-binding cassette, subfamily G, member 2
ACTTAG	*Arabidopsis* activation tagging
ALA	aminolevulinic acid
AREB/ABF	ABA Responsive Element Binding protein/ABRE-binding factor
ATP	adenosine triphosphate
CAB	C-terminal chlorophyll a/b binding
CAPS	cleaved amplified polymorphic sequence
CDPK	calcium-dependent protein kinase
CE	carboxylation efficiency
Coprogen III	coproporphyrinogen III
CPO	coprogen III oxidase
FC	ferrochelatase
FLU	fluorescent protein
FLVCR	feline leukemia virus subgroup C cellular receptor
GluTR	glutamyl-tRNA-reductase
GluTRBP	GluTR binding protein
GP	golden promise
GPX	glutathione peroxidase
g_s	stomatal conductance
GSA	glutamate-1-semialdehyde aminotransferase
GUN4	genomes Uncoupled 4

H_2O_2	hydrogen peroxide
HAP	heme activated protein
HBP	heme binding protein
HEMA	*hemin deficient A*
HO	heme oxygenase
HO^-	hydroxyl radicals
hy1	*long hypocotyl*
Lhcb	light harvesting chlorophyll a/b binding
MEcPP	methylerythritol cyclodiphosphate
Mg-Proto IX	Mg-protoporphyrin IX
Mg-Proto IX ME	Mg-protoporphyrin IX monomethylester
NCBI	national center for biotechnology information
NF	norflurazon
NF-Y	nuclear factor Y
NOS	*nopaline synthase*
O_2^-	superoxide radicals
PAP – 3’	phosphoadenosine 5’-phosphate
Pchlide	protochlorophyllide
PGR7	proton gradient regulation7
PhANG	photosynthesis associated nuclear genes
PPO	protoporphyrinogen IX oxidoreductase
PQ	plastquinone
Proto IX	protoporphyrin IX
PSI and PSII	photosystems I and II

PYR/PYL/RCARs	pyrabactin Resistance 1/PYR1-Like/Regulatory Component of ABA Response 1
ROS	reactive oxygen species
Rubisco	ribulose-1,5-bisphosphate carboxylase/oxygenase
RWC	relative water content
sig2	sigma factor2
sig6	sigma factor6
SOD	superoxide dismutase
Sro9	suppressor of RHO3 protein 9
STN7	state transition 7
TSPO	tryptophan-rich sensory protein
UROD	urogen III decarboxylase
Urogen III	uroporphyrinogen III
WUE	water use efficiency
Ydj1	yeast dnaJ

Thesis Abstract

The tetrapyrrole biosynthesis pathway leads to chlorophyll and heme production and plays a key role in primary physiological processes such as photosynthesis and respiration. Recent studies have shed light on heme as a potential candidate molecule for triggering stress defence responses. However, detailed investigations are yet to be conducted to elucidate the potential role of heme in regulating responses to complex abiotic stress conditions such as drought. The terminal enzyme of heme biosynthesis is Ferrochelatase (FC), for which there are two isoforms encoded by separate genes (*FC1* and *FC2*). Previous studies propose that the two *FC*s synthesize two physiologically distinct heme pools with different cellular functions. The overall scientific goal of this thesis was to investigate the roles of the two *FC*s in photosynthesis, drought and oxidative stress tolerance. In this study, barley (*Hordeum vulgare*) was used as both a major cereal crop and also as a model plant for other commercially relevant rain-fed cereal crops. Two *FC*s in barley (*HvFC1* and *HvFC2*) were identified and their tissue-specific and stress-responsive expression patterns were investigated. These genes were cloned from the cultivar Golden Promise (GP) and transgenic lines ectopically overexpressing either *HvFC1* or *HvFC2* were generated. From 29 independent T_0 transgenic lines obtained for each *FC* construct, three single-copy transgenic lines ectopically overexpressing either *HvFC1* or *HvFC2* were evaluated for photosynthetic performance, oxidative and drought stress tolerance.

The two HvFC isoforms share a common catalytic FC domain, while HvFC2 additionally contains C-terminal chlorophyll a/b binding (CAB) domain. The two genes are differentially expressed in photosynthetic and non-photosynthetic tissues and have distinct stress responsive expression profiles, implying that they may have distinct roles. Transgenic plants ectopically overexpressing either *HvFC1* or *HvFC2* exhibited significantly higher chlorophyll content, stomatal conductance (g_s), carboxylation efficiency (CE) and photosynthetic rate relative to controls under both non-stressed and drought stress conditions. Furthermore, these transgenics, showed wilting avoidance and maintained higher leaf water content and water use efficiency relative to control plants when subjected to drought stress. Overexpression of *HvFC*s significantly up-regulated nuclear genes associated with ROS detoxification under drought stress. It also reduced photo-oxidative damage caused by perturbation of tetrapyrrole biosynthesis in *tigrina*^{d12} mutants.

Taken together, this study indicates that both *HvFC*s play roles in photosynthesis and improving oxidative and drought stress tolerance. The results reported in this thesis suggest that both HvFC derived heme pools are likely to be involved in chloroplast-to-nuclear retrograde signaling to trigger drought and oxidative stress tolerance. This study also highlights the tetrapyrrole pathway as an important target for engineering improved crop performance in both non-stressed and stressed environments.

Keywords

Barley, Tetrapyrrole, Heme, Ferrochelatase, Chlorophyll, Drought stress, Photosynthesis, Photo-oxidation, Transcriptional regulation, Post-translational regulation, Stomatal conductance, Reactive oxygen species, Carboxylation efficiency

Outcomes arising from this thesis

The following is a list of Patent and publications that have been prepared in conjunction with this thesis.

Patent

Nagahatenna DSK, Whitford R (2015) Ferrochelatase compositions and methods to increase agronomic performance of plants United States Patent (In process)

Publications

Nagahatenna DSK, Langridge P, Whitford R (2015) Review-Tetrapyrrole-based drought stress signaling Plant Biotechnology Journal, 1-13

Nagahatenna DSK, Tiong J, Edwards EJ, Langridge P, Whitford R Altering tetrapyrrole biosynthesis by overexpressing *Ferrochelatases* (*FC1* and *FC2*), improves photosynthesis in transgenic barley Plant Molecular Biology (In preparation)

Nagahatenna DSK, Parent B, Edwards EJ, Langridge P, Whitford R Barley transgenics overexpressing *Ferrochelatase*s (*HvFC1* and *HvFC2*) maintain higher photosynthesis and reduce photo-oxidative damage under drought stress New Phytologist (In preparation)

List of Abstracts and Conference Presentations

Conference: 1

Name : International Association of Plant Biotechnology (2014)

Location : Melbourne, Australia

Authorship : Nagahatenna DSK, Langridge P, Whitford, R.

Abstract Title : Overexpression of barley *Ferrochelatase I* improves photosynthetic performance under drought stress conditions

Type : Oral presentation

Conference: 2

Name : ComBio (2014)

Location : Canberra, Australia

Authorship : Nagahatenna DSK, Langridge P, Whitford, R.

Abstract Title : Overexpression of barley *Ferrochelatase*s *I* and *II* improves photosynthetic performance under drought stress conditions

Type : Poster