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.

Signature: _______________________________     Date: __________________________
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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>$^{1}O_2$</td>
<td>singlet oxygen</td>
</tr>
<tr>
<td>ABA</td>
<td>abscisic acid</td>
</tr>
<tr>
<td>ABCG2</td>
<td>ATP-binding cassette, subfamily G, member 2</td>
</tr>
<tr>
<td>ACTTAG</td>
<td><em>Arabidopsis</em> activation tagging</td>
</tr>
<tr>
<td>ALA</td>
<td>aminolevulinic acid</td>
</tr>
<tr>
<td>AREB/ABF</td>
<td>ABA Responsive Element Binding protein/ABRE-binding factor</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>CAB</td>
<td>C-terminal chlorophyll a/b binding</td>
</tr>
<tr>
<td>CAPS</td>
<td>cleaved amplified polymorphic sequence</td>
</tr>
<tr>
<td>CDPK</td>
<td>calcium-dependent protein kinase</td>
</tr>
<tr>
<td>CE</td>
<td>carboxylation efficiency</td>
</tr>
<tr>
<td>Coprogen III</td>
<td>coproporphyrinogen III</td>
</tr>
<tr>
<td>CPO</td>
<td>coprogen III oxidase</td>
</tr>
<tr>
<td>FC</td>
<td>ferrochelatase</td>
</tr>
<tr>
<td>FLU</td>
<td>fluorescent protein</td>
</tr>
<tr>
<td>FLVCR</td>
<td>feline leukemia virus subgroup C cellular receptor</td>
</tr>
<tr>
<td>GluTR</td>
<td>glutamyl-tRNA-reductase</td>
</tr>
<tr>
<td>GluTRBP</td>
<td>GluTR binding protein</td>
</tr>
<tr>
<td>GP</td>
<td>golden promise</td>
</tr>
<tr>
<td>GPX</td>
<td>glutathione peroxidase</td>
</tr>
<tr>
<td>$g_s$</td>
<td>stomatal conductance</td>
</tr>
<tr>
<td>GSA</td>
<td>glutamate-1-semialdehyde aminotransferase</td>
</tr>
<tr>
<td>GUN4</td>
<td>genomes Uncoupled 4</td>
</tr>
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</table>
$\text{H}_2\text{O}_2$ 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 methyerythritol 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
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tbody>
<tr>
<td>PYR/PYL/RCARs</td>
<td>pyrabactin Resistance 1/PYR1-Like/Regulatory Component of ABA Response 1</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
</tr>
<tr>
<td>Rubisco</td>
<td>ribulose-1,5-bisphosphate carboxylase/oxygenase</td>
</tr>
<tr>
<td>RWC</td>
<td>relative water content</td>
</tr>
<tr>
<td>sig2</td>
<td>sigma factor2</td>
</tr>
<tr>
<td>sig6</td>
<td>sigma factor6</td>
</tr>
<tr>
<td>SOD</td>
<td>superoxide dismutase</td>
</tr>
<tr>
<td>Sro9</td>
<td>suppressor of RHO3 protein 9</td>
</tr>
<tr>
<td>STN7</td>
<td>state transition 7</td>
</tr>
<tr>
<td>TSPO</td>
<td>tryptophan-rich sensory protein</td>
</tr>
<tr>
<td>UROD</td>
<td>urogen III decarboxylase</td>
</tr>
<tr>
<td>Urogen III</td>
<td>uroporphyrinogen III</td>
</tr>
<tr>
<td>WUE</td>
<td>water use efficiency</td>
</tr>
<tr>
<td>Ydj1</td>
<td>yeast dnaJ</td>
</tr>
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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 FCs 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 FCs 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 FCs 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 T₀ 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 *HvFCs* 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*<sup>412</sup> mutants.

Taken together, this study indicates that both *HvFCs* 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**

**Publications**

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 Ferrochelatases (HvFC1 and HvFC2) maintain higher photosynthesis and reduce photo-oxidative damage under drought stress New Phytologist (In preparation)
List of Abstracts and Conference Presentations

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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 Ferrochelatases I and II improves photosynthetic performance under drought stress conditions
Type: Poster