Effect of High Temperature Shock During Grain Maturation On Dormancy Of Wheat (*Triticum aestivum* L.) and Analysis of *TaDOG1*

Submitted by,

Cong Rong Cheng

This thesis is submitted in fulfilment of the requirements for the degree

Doctor of Philosophy

School of Agriculture, Food and Wine

Faculty of Sciences

University of Adelaide, Waite Campus

Glen Osmond, South Australia, 5064, Australia

June 2015

Statement of authorship

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.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

June 2015

Acknowledgements

This thesis remains one of the most challenging tasks I have undertaken in my academic life.

Associate Professor Daryl Mares is crucial to the completion of the thesis and I am proud to be under his guidance and supervision. I am grateful for the trust, patience and the continuous explanations. Seeing Daryl working hard under unfavourable weather conditions in the field has been motivating and inspiring.

I would like to thank Dr Klaus Oldach for the challenge, motivation, and supervision. I appreciate the conversations we have from time to time.

I would like to thank both my supervisors not only for the academic guidance, but also for listening to my personal troubles and thoughts. Life was not a smooth sailing these four years, especially the final year. Coping with personal and health issues were difficult. I am grateful to those who listened and helped.

I would like to thank Dr Kolumbina Mrva for her help with the work throughout my four years being in the lab. In addition, her morning greetings have been both cheerful and encouraging.

Thank you to Dr Judy Cheong, Dr John Harris, Dr Yusuf Genc, Arezou, Rosemary Hsu, Hai Yunn Law, Cameron Mares and Jay Rongala for their assistance and friendship.

I am highly indebted.

List of tables and figures

Figure 9 Changes in germination index of proximal-half grains incubated in water (blue) and 25 µM ABA (red) between 30 dpa and 110 dpa for wheat genotypes (a) Gladius, (b) B14 and (c) SUN325B.

Figure 10 Germination index for genotypes, Gladius (blue), B14 (red) and SUN325B (green) harvested at 60 dpa following different temperature shock treatment at different stages between 25 to 55 dpa. Zero germination was recorded for control SUN325B and plants shocked at 35, 40, 45, 50 or 55 dpa. Error bars represent standard deviation.

Figure 11 The release of dormancy of SUN325B following high temperature shock of 5 days at 25 (blue), 30 (red), 35 dpa (green) and control (purple). GI values for control at 35 dpa and 55-65 dpa were recorded as nil. Error bars represent standard deviation.

Figure 12 Germination index of high temperature shock-treated SUN325B at 60 dpa (a) and 65 dpa (b). GI values for control were recorded as nil. Error bars represent standard deviation.

Figure 13 (a) Germination index comparisons of dormant genotype SUN325B for experiment 1 (glasshouse/blue), 2 (glasshouse/green) and 3 (field/red). (b) There was a lack of correlation between the temperature shock time point for maximum effect on dormancy and (i) temperature (GDD) and (ii) humidity (Bars), between anthesis and 25 dpa.

Figure 14 Abscisic acid concentration changes in embryo of glasshouse-grown SUN325B when treated with 5 days temperature shock in a growth chamber at 25 dpa (red), 30 dpa (purple), 35 dpa (green) and control (blue). All temperature-treated plants were returned to the glasshouse for maturation. Error bars represent standard deviation.

Figure 15 Abscisic acid concentration changes in de-embryonated grain of glasshouse-grown SUN325B when treated with 5 days temperature shock in the growth chamber at 25 dpa (red), 30 dpa (purple), 35 dpa (green) and control (blue). All temperature-treated plants were returned to the glasshouse for maturation. Error bars represent standard deviation.

Figure 16 The release of dormancy of glasshouse-grown SUN325B following 5 days growth chamber high temperature shock treatments of 40°C (yellow), 35°C at 25 dpa (red), 30 dpa (green), 35 dpa (purple) and control (blue). All temperature-treated plants were returned to the glasshouse for maturation.

Figure 17 GI of proximal-half grains imbibed with water (blue) or ABA (ABA sensitivity) (red) of SUN325B, B14 and Gladius following a temperature shock at 25 or 30 dpa compared with non-treated controls.

Figure 18 a and b Change in GI of proximal-half grains imbibed in water (a) and ABA (b) of SUN325B following a temperature shock at 25 dpa (red), 30 dpa (green) or 35 dpa (purple) compared with a non-treated control (blue).

Figure 19 a and b Rate of SUN325B grain moisture loss following high temperature shock at 25 dpa (red), 30 dpa (blue) or control (green) in the field (a) and in the glasshouse (b).

Figure 20 a and b SUN325B grain weight at 60 dpa following high temperature shock at 25 dpa (red), 30 dpa (green), 35 dpa (purple) or control (blue) in the field (a) and in the glasshouse (b). Error bars represent standard devation.

Figure 27 Comparative analysis between four reported rice dormancy QTL and a wheat dormancy QTL on 3B (Li et al. 2011; Gu et al. 2005; Xie et al. 2011; Dong et al. 2003; Mares et al. 2009).

Figure 28 Flanking markers of the dormancy QTL on 4A in wheat and their adjacent markers mostly mapped on wheat deletion bins 4AL13-0.59-0.66 (Mares et al. 2005).

Figure 29 Colinearity shown between ESTs mapped in 4A deletion bin 4AL13-0.59-0.66 with rice genes on chromosome 3 (Sorrells et al. 2003).

Figure 30 Comparative analysis showed rice dormancy QTL on chromosome 3 did not correspond to wheat dormancy QTL on chromosome 4A (Mares et al. 2005; Hori et al. 2010).

Figure 31 Nearest marker to the dormancy QTL on 2B in wheat, WMC474 mapped on wheat deletion bin 2BS1-0.53-0.75 and a comparative analysis with rice dormancy QTL on chromosome 7 (Sorrells et al. 2003). Position of TaDOG1L5-1 or AK330559 was not verified in the deletion bin.

Figure 32 Colinearity shown between ESTs mapped in chromosome 1A deletion bin 1AL1-0.17-0.61 with rice genes on chromosome 5 and 10 (Sorrells et al. 2003).

Figure 33 Nearest marker to the dormancy QTL on 1A in wheat, CFA2129, its adjacent markers and a comparative analysis with rice dormancy QTL on chromosome 5. Os05g0537400 (putative ortholog of HvABI1b) and Os05g0560200 (putative ortholog of TaDOG1L2 or AK332921) seem to collocate with the dormancy QTL on 1A (Munkvold et al. 2009).

Figure 34 Expression levels of *TaDOG1L1* in embryo tissues of 5 days 40°C temperature-shocked (red) SUN325B at 30 dpa relative to control (blue) and. No detectable expression levels at later time points and also in the de-embryonated grain tissues. One way ANOVA was performed to determine statistical significance. P = 0.17.

Figure 35 Expression levels of *TaDOG1L2* in embryo tissues of 5 days 40°C temperature-shocked (red) SUN325B at 30 dpa relative to embryo tissues of control (blue). No detectable expressions at later time points and also in the de-embryonated grain tissues. Temperature shock downregulates *TaDOG1L2* by 10-fold. One way ANOVA was performed to determine statistical significance. P = 0.007.

Abstract

Pre-harvest sprouting (PHS) is the germination of seed under wet environmental conditions whilst still on the mother plant prior to harvest. In wheat, PHS causes farmers substantial economic losses due to quality downgrading. A high level of dormancy is regarded as an important mechanism of resistance to PHS in cereal species, such as wheat (*Triticum aestivum* L.). Many dormancy quantitative trait loci (QTL) have been identified and the corresponding genes that impart tolerance to PHS are actively being sought. Besides genetic factors, environmental conditions during grain maturation have been shown to have profound effects on dormancy. These environmental factors include temperature, light, drought and nutrients.

This project aimed to determine the role of high temperature shock during wheat seed maturation on its dormancy at harvest-ripeness. The results of these experiments showed that seed of dormant or intermediate dormant wheat genotypes may have lower dormancy levels after experiencing a high temperature shock (5 days of 40°C). The highest sensitivity towards high temperature shock is limited to a short "window" of approximately five days starting around 25 dpa. The sensitivity increases and reaches a peak at 25 to 30 days post anthesis (dpa), causing an effective and quicker release of dormancy. The sensitivity later decreases and high temperature becomes less influential on dormancy. For the dormant genotype SUN325B, release from dormancy occurred 35 days earlier if treated with temperature shock. There was no significant correlation between the timing of the peak of sensitivity and factors such as daily temperatures before temperature shock, humidity or subsequent grain moisture losses. However, the stronger the intensity (40°C *versus* 35°C) and longer duration (5 days *versus* <5 days) of temperature shock seem to influence dormancy more significantly. Changes in embryonic and endospermic abscisic acid (ABA) concentration following temperature shock could not explain the change in dormancy levels in genotype SUN325B. Instead, the change in

dormancy levels following the temperature shock could potentially be ascribed to the loss of ABA sensitivity.

A comparison of the dormancy response to temperature shock of 23 genotypes that harbour different combinations of known dormancy QTL was performed. Strongly dormant genotypes, P07.683, 50213/Cunn798 and DM1073 #31, did not show an increased germination index at 65 dpa. Other genotypes, ranging from dormant to intermediate dormant phenotype under control conditions, i.e. displaying germination index less than 0.6 at 65 dpa, responded strongly to temperature shock. Dormancy levels significantly decreased, reflected by increases of germination index ranging from 0.4 to 0.9. It is highly likely that a temperature-shock-induced decrease in dormancy is a common response of these genotypes, and a higher number of combinations of QTL are required to resist dormancy changes.

The identification of *Delay of Germination 1* (*DOG1*) as the gene underlying a major dormancy QTL in *Arabidopsis thaliana* has led to stronger understanding of the mechanism of dormancy. Based on results of studies of the *AtDOG1* and *TaDOG1-like* genes in *Arabidopsis* and cereal species respectively, and their functional conservation and responsiveness towards temperature changes during seed development, it seems likely that the *TaDOG1-like* genes in wheat might play a role in temperature shock-regulated dormancy levels.

A search of publicly available bioinformatics databases revealed three expressed sequence tags (ESTs) with accession numbers *AK336217*, *X56782* and *D12921* that could be *TaDOG1-like* genes in wheat additional to the five previously reported by Ashikawa *et al*. (2010, 2014). Protein motif analysis showed that the orthologs of *DOG1* possess combinations of protein motifs specific to each clade. Promoter analysis revealed RY repeats (CATGCAT) and (ACGTG)-core-containing ABA-responsive elements-like sequences in the promoters of these genes. Comparative mapping analysis also showed that one *TaDOG1-like* gene (accession:

X56782, could reside in dormancy QTL regions in wheat and similarly, two rice putative orthologs (*Os01g0159000* and *Os05g0560200*) reside in dormancy QTL regions in rice.

In a reverse transcription quantitative PCR (RT-qPCR) analysis, no significant expression of two *TaDOG1-like* genes (accession: *AB555729* and *AK332921* or *TaDOG1-L1* and *TaDOG1-L2* respectively) were detected in either embryos or de-embryonated wheat grains of SUN325B at 45 and 60 dpa. However, both genes were detected to be expressed at 25 dpa and 30 dpa in the excised embryo tissues, signifying the mRNA to be short-lived. *TaDOG1-L1* showed a non-significant increase following temperature shock, hence failed to explain the increase in germination index. *TaDOG1-L2* was significantly down-regulated at 30 dpa following temperature shock, which could cause a lower protein accumulation towards 65 dpa. Further investigation could focus on the role of *TaDOG1-L2* in dormancy in white wheat genotypes to better explain the effect of temperature shock during the sensitive “window” on dormancy in general.